EFFECTS OF ENDOTOXIN ON THE HEPATIC VENOUS SYSTEM

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by

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ABSTRACT

Endotoxin shock is characterized by irreversible cardiovascular insufficiency associated with low cardiac output. Experiments were carried out in order to determine if endotoxin produced effects on the venous system which could account for some of the observed cardiovascular effects. Further experiments were performed to assess the usefulness of two pharmacological agents, nifedipine and isoproterenol, in reversing any effects of endotoxin on the venous system.

In cats anesthetized with pentobarbital, hepatic venous responses to noradrenaline, angiotensin II and hepatic nerve stimulation were measured before and after endotoxin infusion. Hepatic blood volume (by plethysmography), arterial, hepatic vein and portal vein pressures and cardiac output were measured. Before endotoxin, noradrenaline and angiotensin II infusions and nerve stimulations produced decreases in liver volume. After endotoxin, these hepatic venous responses were reduced markedly and basal hepatic blood volume increased. In other experiments, the smooth muscle relaxant nifedipine was found to cause a decrease in hepatic blood volume in spite of an increase in portal pressure, indicating a venoconstrictor effect. A similar hepatic venoconstrictor effect was confirmed with the smooth muscle relaxant isoproterenol. Neither of these agents was able to reverse the hepatic venodilatory effects of endotoxin.

It is concluded that during endotoxin shock in cats, hepatic venous smooth muscle relaxes and loses its responsiveness to circulating catecholamines and angiotensin II, to nerve stimulations and to the pharmacological agents isoproterenol and nifedipine. These effects of endotoxin may contribute to the circulatory collapse observed during the late stages of endotoxin shock.

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LIST OF ABBREVIATIONS

ASA --- acetylsalicylic acid

C ----- Centigrade

cm ---- centimetre(s)

EDTA -- ethylenediaminetetraacetic acid

g ----- gram(s)

Hz ---- Hertz (cycles per second)

i.a. -- intra-arterial

i.v. -- intra-venous

kg ---- kilogram(s)

l ----- litre(s)

M ----- molar

mM ---- millimolar

mg ---- milligram(s)

ml ---- millilitre(s)

mm Hg - millimeter(s) of Mercury

msec - millisecond(s)

mV -- millivolt(s)

sec -- second(s)

LIST OF DRUGS USED

ANGIOTENSIN II ------- Sigma Chemical Co.

ENDOTOXIN ---------- Bacto-lipopolysaccharide B from *Salmonella enteritidis*, Difco Laboratories

ISOPROTERENOL HCl ---- Winthrop Laboratories

NIFEDIPINE ---------- Adalat, Miles Pharmaceuticals

NORADRENALINE -------- Sigma Chemical Co.

This thesis is dedicated to

the memory of my father,

Michael M. Seaman.

INTRODUCTION

A. Hepatic Venous System

1. Anatomy of the Hepatic Circulation

The liver is a highly perfused organ, receiving an estimated 25-29% of the total cardiac output (126, 135). This is a large flow rate considering that the weight of the liver, amounts to only approximately 3% of total body weight. Blood flow through the liver is unique in that the liver is connected in series with the spleen and gastro-intestinal tract via the portal vein and in parallel with the other body organs via the hepatic artery (187). Of total hepatic blood flow, 67-80% enters through the portal vein. The portal vein is formed by the union of the splenic vein with the superior mesenteric vein posterior to the pancreas. Thus the portal vein drains blood from the spleen, small intestine, large intestine, colon, stomach and pancreas. The portal vein, before entering the liver, branches into main vessels each of which supply an individual lobe of the liver (317). The remaining 20-33% of total liver flow is supplied by the hepatic artery. The hepatic artery is a branch of the common hepatic artery, which in turn is a branch of the celiac artery. The gastroduodenal artery is a branch of the common hepatic artery and supplies portions of the stomach and pancreas.

The main branches of the hepatic artery and portal vein give rise to smaller branches within the liver whereby

two or three arterial branches are in close association with one portal vein. Branching of these vessels continues until sinusoids are formed (37). Some anastomising connections exist between portal and arterial vessels (180).

The histology of the liver has been well described (136, 150, 281). The liver can be subdivided into thousands of lobules or acini. The concept of a lobule is different from that of an acinus and will be described here. The lobule is considered to consist of a central vein which is encircled by 6 parallel portal tracts which form a hexagon around the central vein. Each portal tract consists of a hepatic artery and portal vein. Sinusoids pass at right angles from the portal tracts to the central vein. An acinus, on the other hand, has the same basic structure as the lobule except that another dimension is added to the picture. Where a lobule has sinusoids coming off the portal tract at right angles, the acinus has a small branch of the portal tract coming off. It is from this small branch that eventually sinusoids branch off of at right angles. In the acinus then, the sinusoids are at right angles to <u>both</u> the portal tract and to the small branch.

Microscopy shows that the hepatocytes are arranged as trabeculae which pass from terminal portal tracts to central veins. Trabeculae are at two cell layers thick with each layer being in contact with a hepatic sinusoid. Trabeculae cannot be more than 2 cells thick because each cell must be

in contact with a sinusoid. Between each two layers of cells of the trabeculae run small bile canaliculi which collect bile produced by the hepatic cells. The sinusoids are lined with endothelial and also with reticuloendothelial cells which are referred to as Kupffer cells. The Kupffer cells have a different cell lineage than the endothelial cells and are in fact considered to be macrophages. Kupffer cells are known to phagocytose foreign material and other waste material. The endothelial cells are separated from the hepatocytes by reticular fibers, lipocytes and microvilli on the sinusoidal surfaces of the hepatocytes. The space between the endothelial cells and hepatocytes is referred to as the space of Disse. The endothelial cells of the sinusoids are scattered with large pores of approximately 0.1 to 1 micron in diameter, thus making them relatively permeable to plasma. Because of the sinusoidal permeability to plasma the hydrostatic pressure within the space of Disse should be equal to sinusoidal pressure. Blood flowing through the sinusoids is collected in the central vein and eventually flows into the large hepatic veins.

2. Physical Factors Influencing Hepatic Blood Volume

The splanchnic circulation has been shown to contain approximately 38% of the total body blood volume. Of all the splanchnic organs, the liver contains the largest volume of blood. It has been estimated that the liver contains about

50% of the blood in the splanchnic circulation which amounts to approximately 19% of the total body blood volume (111).

Exactly where in the hepatic vasculature that most of the blood is located is yet a matter of investigation. It can be assumed however, that most of the blood within any body organ is contained within its venous system. In fact, from various studies, it has been estimated that of all the blood in the peripheral circulation, 75% is in the veins with only 19% and 6% being contained in the arterial and capillary systems, respectively (134). Secondly, it is assumed that veins distend under pressure and that the venous system distends far more than the arterial system for a given increase in pressure. These assumptions are based on data from experiments which show that the compliance of the venous system is about 24 times as great as the compliance of the arterial system (137).

From the above assumptions it can be concluded that the volume of blood within an organ and any changes in its blood volume are dependent mostly on the factors which determine the volume of blood contained within the venous system of that organ. This conclusion would apply to the hepatic venous system although, in the liver a significant amount of blood may be contained within the sinusoids. The exact volume of blood contained within the venous system of any organ is a function of the magnitude of the circumference of the veins, i.e., the degree of distension of the walls of

the veins. A change in blood volume, then, can be translated in terms of a change in the degree of vein wall distension. Any change in the degree of distension of the walls of the veins within an organ can be due to either of two mechanisms: 1) a stretch or recoil of the vein wall due to a respective relative increase or decrease in hydrostatic pressure within the lumen, or, 2) a stretch or recoil of the vein wall due to a respective decrease or increase in resting smooth muscle tone. It is only through either or both of the above mechanisms that a change in blood volume of a venous bed can occur.

From the above discussion, it can be stated that the blood content of the liver is dependent on the pressure within hepatic venous system. Pressure within any vessel has been described in terms of two other factors, namely, rate of blood flow through the vessel and resistance to flow. Simply, for a given and constant flow, an increase in resistance would be accompanied by an increase in pressure behind (upstream from) the point of resistance and a decrease in pressure in front of (downstream from) the point of resistance. Thus, sites of resistance can theoretically affect the blood content of an organ by affecting the intra-vascular hydrostatic pressures within the organ. Furthermore, the exact location of a resistance site within the vasculature of an organ can determine whether that resistance site contributes to an increase in blood volume or

to a decrease in blood volume within that organ. If a resistance site was located downstream from the venous system, then this site of resistance would serve to increase venous pressure and, therefore, increase blood volume. Conversely, if a resistance site was located upstream from the venous system, it would act to decrease venous pressure and decrease blood volume. Therefore, in a study of the mechanisms of control of blood volume within the hepatic venous system, it would be necessary to consider any possible sites of resistance and their specific locations.

Many anatomical studies have been produced which were concerned with the possible existence, locations and functions of sphincters located at the sinusoids and capillaries. Transillumination studies have shown that sinusoids may have sphincters at three locations (37). These sphincters have been termed according to their proposed locations, namely, "inlet sphincters", "outlet sphincters" and "intersinusoidal sphincters". Generally however, it can be stated that the existence of sinusoidal sphincters has been disputed (258) and the contribution, if any, of sinusoidal sphincters to blood flow or capacitance regulation has not been demonstrated.

The following discussion presents strong evidence that the major hepatic resistance sites are located is still debatable. By investigating transsinusoidal fluid dynamics, Laine et al. have shown that, in cats and dogs, the pressure

within the sinusoids is close to portal pressure (211). Also, it has been shown that in cats and dogs the pressure within the hepatic vein is close to the pressure within the portal vein (108, 124). If the pressures on both the inlet and outlet sides of the sinusoids are approximately equal, as indicated by the above experiments, then it is very difficult to conceive of how any significant resistance to sinusoidal blood flow could exist. From these data, it must be concluded that the sinusoids do not offer any significant resistance to hepatic blood flow and therefore the greatest resistance site to hepatic blood flow is located at the junction of the hepatic veins and the inferior vena cava. Since this site of resistance is located downstream from the bulk of the venous system, an increase or decrease in the magnitude of this resistance would repectively increase or decrease hepatic venous pressure and therefore would respectively increase or decrease hepatic blood volume. A corollary to this discussion is that since portal sinusoidal and hepatic vein pressures are similar in value, then by measuring portal pressure a fairly accurate measure of hepatic venous pressure can be obtained.

As stated above, rate of flow through a vessel can theoretically affect the hydrostatic pressure within the vessel. If this assumption holds true for the hepatic circulation, then portal pressure and blood volume would be expected to decrease in response to a reduction in hepatic

blood flow. This prediction has been tested in experiments with dogs and has proven to hold true (24). The local mechanical reduction in portal flow in these experiments led to a significant decrease in both portal pressure and hepatic blood volume. Thus hepatic blood volume changes in response to flow changes occur as would be predicted.

3. Hormonal Control of Hepatic Blood Volume

Hepatic blood volume has been found to be influenced by hormones. Angiotensin II, a hormone normally found circulating in the blood, has been shown to cause a dose dependent decrease in hepatic venous blood volume when infused intra-venously into cats (116). The highest dose of angiotensin II infused in the above experiments was 0.5 ug/kg/min. Previous studies had estimated that endogenous angiotensin II could be produced in the amount of 1.5 ug/kg/min in dogs (181, 284), thus, the doses used in this study were within physiological range. It was concluded from this study that doses of angiotensin II within the probable range of endogenous production decreased hepatic blood volume by up to 20%. The possibility that the effects of angiotensin II were centrally mediated was tested by sectioning the hepatic nerves. After the hepatic nerves were sectioned, angiotensin II produced a similar blood volume response as when the nerves were intact. Therefore angiotensin II was acting directly on the smooth muscle of the hepatic

vasculature. As described above, a decrease in hepatic blood volume can occur due to either or both of two possibilities; a decrease in distending pressure, or, an increase in smooth muscle tone of the hepatic venous system. Angiotensin II has been found to cause a reduction in hepatic blood flow (48, 309) and this could account for at least part of the decrease in hepatic blood volume. In fact however, although blood flow does decrease, pressure within the venous system increases in response to angiotensin. Presumably angiotensin II causes this increase in pressure by increasing resitance to outflow from the liver. Since the angiotensin II mediated decrease in blood volume is associated with an increase in hepatic venous pressure, the volume change must be also mediated by a increase in smooth muscle tone.

Other studies have been carried out which examined the effects of angiotensin on capacitance sites within other organs. Jarhult examined the effects of intra-arterial angiotensin II in a sympathectomized skeletal muscle region in the cat (196). Although angiotensin was found to be a potent constrictor of skeletal muscle arterioles, very little effect was observed on the capacitance vessels. Angiotensin has also been demonstrated to have little effect on pulmonary veins (21). It appears then that angiotensin has a relatively selective effect on the hepatic venous system.

Adrenaline and noradrenaline are two hormones which have direct effects on the hepatic blood volume. When infused

intra-venously into cats at 2 ug/kg/min, both noradrenaline and adrenaline were found to decrease hepatic blood volume by 40% (116). This infusion rate was estimated to be equivalent to the maximal rate of catecholamine release by the adrenal medulla (116). There is no possibility that the decrease in hepatic blood volume was due to a decrease in flow and therefore a decrease in hepatic venous pressure since adrenaline has been shown to produce an increase in hepatic blood flow (117, 118). A decrease in hepatic blood volume, as measured by changes in liver weight, has also been noted in dogs (238).

Because the hepatic venous smooth muscle constricts to noradrenaline, it can be concluded that the smooth muscle at the capacitance sites is populated with alpha-receptors. Adding the $alpha_1$ and $alpha_2$ agonists, phenylephrine and clonidine to an isolated strip of cat hepatic vein produces marked contractions which are respectively inhibited by the $alpha_1$ and $alpha_2$ antagonists clonidine and yohimbine (K.L. Seaman, unpublished observations). These responses suggest that the cat hepatic capacitance sites possess $alpha_1$ and $alpha_2$ receptors. beta-Receptors appear to be absent from the capacitance sites, as the infusions of isoproterenol directly into the hepatic artery did not increase hepatic blood volume (116). Similarly, adding isoproterenol to isolated strips of cat hepatic vein did not induce a relaxation when these strips were under tonic tension or contracted with

noradrenaline and angiotensin (K.L. Seaman, unpublished observations). However, the hepatic artery appears to have beta-receptors, as the intra-arterial infusion of isoproterenol leads to a hepatic arterial vasodilation in cats (289).

4. Nervous Control of Hepatic Blood Volume

The nervous innervation of the liver has been reviewed (90, 215). The liver receives innervation from parasympathetic and sympathetic nerves mostly through an anterior and posterior plexus. The majority of the nerves are sympathetic, originating from the thoracic splanchnic nerves. The splanchnic nerves leave the paravertebral chain above the diaphragm and terminate in the various splanchnic ganglia. Another group of sympathetic fibers originating in the cephalad region of the lumbar sympathetic trunk has been shown to connect with the splanchnic ganglia. The anterior plexus consists of sympathetic fibers originating from the celiac ganglia and parasympathetic fibers from the anterior vagus. The anterior plexus reaches the liver by travelling primarily with the hepatic artery. The posterior plexus is formed by fibers from the celiac plexus and posterior vagus and travels with the portal vein and bile duct. The anterior and posterior plexuses intercommunicate. Finally, some nerves reach the liver independently of the anterior and posterior plexuses. It has been stated that to obtain total hepatic

denervation, nerves have to be sectioned along the hepatic artery, portal vein, biliary tract and all liver ligaments (215).

It has been shown that all branches of the portal vein and hepatic artery are sympathetically innervated. Nerve endings have been detected in contact with the smooth muscle cuffs of the precapillaries (37) and there is evidence that Kupffer cells lining the sinusoids are adrenergically innervated (88).

Cats which have experimentally sectioned hepatic nerves have larger hepatic blood volumes than intact cats. This suggests the sympathetic nerves which innervate the liver contribute to producing a constant tone within the smooth muscle of the venous system (111).

The effectiveness of any organ as a blood reservoir depends highly on the degree to which the central nervous system controls the blood volume of that organ. In this regard, it is clear from the above discussion that the liver is well innervated. Furthermore, direct stimulation of the nerves supplying the liver leads to marked changes in the hepatic vasculature. Greenway et al. (127) found that before nerve stimulations, hepatic blood volume was 27 ml/100 g of liver weight and that stimulating the hepatic nerves at frequencies ranging from 1-10 Hz resulted in a frequency dependent decrease in hepatic blood volume. At the highest frequency, the hepatic blood content had decreased to 50% of

control. A previous study showed that, except for an initial period of a few minutes, hepatic blood flow does not decrease significantly in response to hepatic nerve stimulation (120). Also, the decrease in blood volume with nerve stimulation was accompanied by an increase in portal pressure, therefore, an active constriction of the venous smooth muscle must have occurred. The volume changes in response to nerve stimulation have been shown to be attenuated by phenoxybenzamine, showing that the volume change is mediated through alpha-receptors on the smooth muscle (141). Thus, the sympathetic nerves innervating the liver, if stimulated, can mobilize large quantities of blood.

It must be added that for the sympathetic nervous system to be considered an effective control mechanism over hepatic blood volume, the response to nerve stimulation should not show an "escape" phenomenon. Such an escape phenomenon is observed with the influence of the hepatic nerves over hepatic arterial resistance. A continuous stimulation of the nerves produces an immediate increase in resistance followed by a gradual, but an eventual nearly complete loss of the resistance response within a few minutes (120). The capacitance response, however, has been shown to be well maintained over a period of at least 20 minutes (127). The physical site within the hepatic venous system that blood is expelled from is not yet clearly known.

Information as to the location of the site of resistance to hepatic venous blood flow which develops upon nerve stimulation comes from experiments where hepatic vein pressure is measured. Stimulating the nerves leads to an increase in hepatic vein pressure. From this, it can be concluded that the resistance site which develops is located post-sinusoidally.

In summary, it can be concluded that the hepatic vasculature is well innervated with sympathetic nerves and that these nerves have the potential to mobilize large quantities of blood. Other studies have shown that not only do the sympathetic nerves have the potential to mobilize blood, but that they are indeed used as a homeostatic mechanism in response to cardiovascular stresses. Many of these studies were carried out using dogs. Brunner et al. showed that after increasing carotid sinus pressure, a significant increase in venous capacitance occurred (35). Most of the increase in capacitance was localized to the splanchnic vasculature. In order to measure the contribution of the spleen to these changes, splenectomies were performed in two dogs. After splenectomy, a 30% attenuation of the volume response occurred. This implies that the liver and intestine contributed to 70% of the total volume response. Similar results as in the above study were found by others whereby changes in carotid sinus pressure led to reflex changes in abdominal vascular capacitance (142). It has also

been shown that changes in pressure distending the aortic baroreceptors alone can lead to significant changes in abdominal capacitance vessels (201).

Another study was carried out which specifically examined changes in liver blood content in response to alterations in carotid baroreceptor activity (43). By changing aortic pressure from 90-250 mm Hg, the liver blood volume changed by 11.1 ml/100 grams of total liver weight. This volume is equivalent to 36% of the amount of blood contained in the normal liver. Calculations made from that study showed that 57-68% of the change in liver blood volume was due to an active constriction of the capacitance sites which was mediated through the sympathetic nerves. The remaining 32-43% of the decrease in blood volume was attributed to a reduction in hepatic blood flow due to an increase in splanchnic arterial resistance.

In cats, the baroreceptor reflex has little effect on hepatic blood volume (219) or on intestinal blood volume (138). To account for this species difference, it has been stated that it may be that in smaller species, such as the cat, postural effects are small and there may not be as much need for an effective venous component to the baroreceptor reflex (111).

Chemoreptors located in the carotid and aortic bodies have also been shown to affect the hepatic circulation. After perfusing the carotid bodies with hypoxic-hypercapnic blood,

it was found that portal vein conductance decreased significantly in dogs (16). Stimulation of carotid and aortic chemoreceptors in dogs resulted in reflex constriction of resistance and capacitance vessels in the abdominal circulation (143, 144).

5. Factors that Optimize the Reservoir Function of the Liver

The effectiveness of an organ as a blood reservoir depends greatly on how well that organ can resist blood volume changes in the face of changes in blood flow to that organ or changes in external pressure around that organ (121). In other words, the reservoir must serve to control circulatory parameters, and not be controlled by circulatory parameters. Several "built-in" mechanisms have been shown to exist which reduce passive effects on hepatic blood volume. These mechanisms have been described previously (111) and three of these mechanisms will be briefly outlined here.

The first mechanism occurs as a result of the fact that the major site of resistance within the hepatic venous system is located at the junction of the hepatic vein and inferior vena cava, i.e., downstream from all capacitance sites. Normally, a sympathetically mediated decrease in blood flow via arteriolar constriction would tend to decrease volume due to a decrease in pressure. However, the simultaneous increase in outflow resistance caused by

sympathetic nerve stimulation tends to raise hepatic venous pressure and therefore counteracts the effect of the decreased flow. Thus liver blood flow can theoretically be decreased without an accompanying large decrease in blood volume although this has not been proven experimentally.

A second mechanism of reducing passive effects on hepatic blood volume is through the hepatic arterial buffer response. The hepatic arterial buffer response was first described in 1911 when Burton-Opitz noted that hepatic arterial flow increased after portal flow was obstructed (39). Also, when portal flow is obstructed to one section of the liver, only that section will receive an increase in arterial flow (123). Thus, the buffer response tends to cause total hepatic blood flow to change in order to cause venous pressure and volume to remain constant when intestinal blood flow is changed. As an added note, the above statement is not meant to imply that the buffer response is regulated by venous pressure or volume.

The mechanism of the arterial buffer response remains unresolved (192, 214, 216). It is clear that these changes in flow do not involve the nervous system (242, 305). Apart from neural mechanisms, several other possibilities have been considered. These possible mechanisms fall into one of three categories whereby changes in arterial blood flow are postulated to occur because of: 1) the anatomical arrangement of the portal and arterial vasculature (39, 135, 306, 338),

2) changes in arteriolar smooth muscle tone due to intrinsic properties of the muscle (86, 126, 152), and, 3) changes in arteriolar smooth muscle tone due to the presence of vasoactive substances (105, 245).

Finally, a third mechanism can be described that optimizes the liver as an effective blood reservoir. This mechanism minimizes passive changes in blood volume that would result from changes in external pressure on the liver. The pressure around the liver does not normally remain constant. It has been shown that normal movements can increase intra-abdominal pressure significantly -- sometimes to as high as 200 mm Hg (128). A pressure as high as this would be expected to force a large quantity of blood out of the hepatic vasculature. Such a mobilization of blood in response to normal body movements would serve to make the cardiovascular system unstable. In fact however, blood is not forced out of the liver after intra-abdominal pressure is raised because outflow vessels also become narrowed under this pressure. The net result is an increase in outflow resistance which in turn prevents blood from leaving the liver. Thus liver volume remains relatively constant (365).

6. Reservoir Function of the Liver in Cardiovascular Homeostasis

Various studies have shown that the body has a reserve of blood which is used during periods of

cardiovascular stress. For example, because of blood reservoirs within the body, up to about 20% of the total body blood volume can be removed before the cardiovascular system begins to become compromised in its function (121, 130).

From the previous discussion, it appears that the liver functions as an effective blood reservoir as it satisfies the following criteria: 1) it can pool or expel a large volume of blood, 2) the volume of blood pooled or expelled is controlled by hormones and the central nervous system and, 3) the hepatic blood volume tends to partially resist changes caused by changes in portal flow and external pressures. There are many instances which illustrate the role of the liver as a blood reservoir in cardiovascular homeostasis during periods of cardiovascular stress. A prime example of this role is during times when abnormal changes in the body blood volume occur. This is clearly seen from the results of a study where dogs were subjected to hemorrhage (33). In the control experiments, dogs were hemorrhaged to the point where a 8 mm Hg drop in blood pressure ensued. The experiments were repeated after the splanchnic organs were denervated and the same degree of hemorrhage led to a 17 mm Hg greater fall in blood pressure. Similar experiments were carried out using cats in which blood was withdrawn to the point where blood pressure had fallen to 45 mm Hg. (218). It was found that 30 ml of blood was required to be removed in control cats, whereas, only 24 ml was removed from cats after

hepatic nerve section. These studies indicate that the hepatic circulation is important in counteracting the deleterious effects of hemorrhage. Although most of the above data have been taken from studies where cats or dogs were used, other data has been produced to indicate that the liver plays an important role as a blood reservoir in humans (279).

Studies have shown that the hepatic circulation plays a large role in homeostasis during volume overload. Studer et al. doubled the plasma volume of rats and noted that the bulk of the increased volume was located in the abdominal area, especially within the liver (332). In a different study, when the blood volume of cats was increased by 10-34%, it was found that the liver pooled 30% of the added blood. The normal liver contains approximately 19% of total body blood volume. Thus, for a given volume overload, the liver pools more blood than would be predicted based on the percentage of total body blood it normally contains.

Another approach in examining the function of the hepatic venous system in cardiovascular homeostasis is by determining its influence on cardiac output. The cardiac output of an animal has been described as being dependent on four variables, namely, 1) preload, 2) afterload, 3) contractility, and 4) heart rate. Preload is defined as cardiac filling pressure and it is this variable that blood reservoirs can affect most directly. The mechanism of increased cardiac output with increased preload involves

Starling's law of the heart. From Starling's law, it can be stated that the greater the preload, the greater the end diastolic pressure which in turn causes a greater length of cardiac muscle fibers and an increase in force of contraction. The fact that cardiac output is highly dependent on preload can be shown from a study where cardiac filling pressure was controlled using a heart lung preparation in dogs (303). In that study, when right atrial pressure was increased by only 2 mm Hg, cardiac output increased by about 50%. However, it is difficult to quantitatively determine what effect changing preload will have by itself on cardiac output. One reason for this is that if any one variable for cardiac output is adjusted, the other remaining variables will tend to be adjusted by reflex mechanisms so as to minimize the effects of the changes produced by the adjusted variable (109). In any event, it is clear that an increase in preload will tend to increase cardiac output.

One study which was carried out in an attempt to quantify the effects of splanchnic nerve stimulation on cardiac output showed a clear relationship between the mobilization of splanchnic blood and cardiac output (113). In that study, arterial pressure was maintained constant by an arterio-venous shunt and it was found that during splanchnic nerve stimulation, cardiac output increased by up to 40%. Thus it can be concluded that if a decrease in hepatic venous compliance occurs during a low cardiac output state, then

that compliance would tend to increase preload, cardiac output and blood pressure.

B. Endotoxin

1. History of Endotoxin

In 1893, Pfeiffer injected lethal amounts of live cholera vibrio suspension into mice. He observed that the mice showed signs of sickness and died after the bacteria were lysed by the host's defense mechanisms. Because lysis preceded the signs of sickness, he speculated that a toxic substance is released after the death of the bacteria. He referred to this toxic substance as "endotoxin" (294).

The discovery of Shiga bacillus as the cause of an epidemic dysentery in Japan in 1898 led to a realization of the clinical importance of toxic substances released by bacteria. It was noted that the bacilli are found in the mesenteric organs but not in the blood of infected animals. This led Lentz (225) to conclude that the local site of infection in the mesentery acted as a reservoir which released a toxin into the circulation and that it was the toxin which gave rise to the clinical picture of dysentery. The conclusion that the cause of dysentery was due to the release of toxins by Shiga bacillus was later tested by Todd (342). Rabbits received an intravenous injection of the filtrate broth from cultures which had been seeded with

bacillus. Todd stated that an intravenous dose of the toxin produced a set of signs remarkably similar to when live bacteria are injected. In an attempt to characterize the toxin, Todd later found that the precipitate produced after adding ammonium sulphate to the filtrate was several times more potent than the filtrate itself (343). As well, injection of the precipitate produced the same effects in rabbits as did the injection of living or dead bacillus.

In agreement with Todd, several other workers produced results to show that bacteria can produce toxins and that these toxins might be causative agents in the mortality of dysentery. However, at that time, it was not known what part of the bacteria was the source of the toxins. By 1919, two lines of thought arose concerning the source of the bacillus toxins. There were those who regarded the toxin as an endotoxin while others regarded it as an exotoxin (268). In 1920, Olitsky was able to separate both an exotoxin and an endotoxin from cultures of Shiga bacillus. They concluded that the two toxins were physically distinct compounds but that the endotoxin produces intestinal lesions in rabbits which were typically found in man in dysentery.

2. Chemistry of Endotoxin

A chemical characterization of endotoxin has been made and it has been found to consist of macromolecules derived from the cell walls of bacteria and is made up of

polysaccharides, lipids and proteins (248, 249, 250, 251). Endotoxin is a heterogenous substance composed of numerous different molecules of different shapes and sizes. Not all components of endotoxin preparations are toxic. Separation of E. coli endotoxin into various components using sucrose density gradient centrifugation showed that 40-50% of the endotoxin material was non-toxic (22). The exact chemical moiety that produces the toxicity of endotoxin is undetermined. In one study, no correlation was found between potency and amount of nitrogen, fatty acids and hexosamine found in various endotoxin preparations (93).

Studies which were concerned with the distribution of endotoxin after its administration to animals showed that it is concentrated in the liver, lung and spleen (30). It has been proposed that endotoxin concentrates in specific organs due to the reticuloendothelial cell activity of those organs (233). These organs are presumably sites where endotoxin is metabolized as enzymes necessary for endotoxin metabolism have been found in liver macrophage homogenates (79). Starzecki et al. (325) studied the clearance of endotoxin by injecting 0.75 mg/kg into dogs and measuring the amount in the blood with respect to time. The dose of endotoxin fitted a two-compartment model in the dogs, with the early distribution phase occurring within approximately 15 minutes. By one hour and six hours, 75% and 92%, respectively, of the endotoxin had been removed from the circulation. Thus,

endotoxin is removed relatively quickly from the blood but the initial effects that it produces lead to long lasting effects and to the eventual demise of the animal some hours later.

3. General Cardiovascular Effects of Endotoxin

Different animal species react differently to endotoxin (209) and different bacterial sources of endotoxin produce different effects (176). However a general picture of Gram-negative bacterial shock in animals (46, 350) and in humans has been well described (46, 99, 313) and will be reviewed here. In humans, initially hypotension and fever occurs. During this initial phase, arterial vasodilation is present and cardiac output increases (26). With time, further changes begin to occur. An arteriolar vasoconstriction with a decrease in cardiac output and tissue perfusion ensues (239). Other signs and symptoms in humans include oliguria, cold extremities, mental confusion, metabolic acidoses, increased blood lactate, organ dysfunction, decreased arteriovenous oxygen difference and increased catecholamine activity. The specific changes in the cardiovascular system which lead to cardiovascular collapse and death are as yet unknown. The collapse has been postulated to occur due to a lack of blood supply to vital organs (327) possibly due to extreme vasoconstriction (178, 227), opening of arteriovenous shunts (316), or increased blood viscosity (327). Another possible

cause of cardiovascular collapse is a decrease in cardiac function (6, 327). There is some evidence that cardiac depression may be a secondary event which is brought about by venous pooling and the consequent low venous return (34, 156, 159, 164). Other studies have indicated that myocardial failure occurs during endotoxin shock as a result of the release of a cardiac depressant factor (29) which may be released from splanchnic organs (224, 349). Nevertheless, the data on the involvement of cardiac depression are controversial (15, 140, 163, 171, 223, 232).

The overall cardiovascular response of the cat (148), rat (358) and dog (351) to endotoxin consists of two phases. In cats, immediately after endotoxin administration, blood pressure falls and pulmonary vascular resistance increases markedly (98). Accompanying the increase in pulmonary resistance is an increase in pulmonary arterial pressure and the development of pulmonary edema (210). The pulmonary vascular responses were noted to precede the fall in systemic blood pressure, suggesting that the blood pressure fall was a result of the pulmonary responses. Dogs show an immediate fall in blood pressure and cardiac output followed by a moderate recovery towards normal. Not all species exhibit an acute phase to endotoxin administration. The primate has been shown to undergo a gradual decline in blood pressure and does not show the precipitous acute phase seen in cats and dogs (97, 169, 209).

If cats survive the acute phase of endotoxin, a secondary delayed phase ensues. The delayed phase is characterized by hypotension (209), a decreased stroke volume and cardiac output, and by severe metabolic acidosis (270). With time an intense mesenteric vasoconstriction ensues in dogs, cats and rats but not in monkeys (97).

In both cats and dogs, preventing or circumventing the acute phase does not necessarily improve survival. In dogs, cardiac output was mechanically maintained to prevent the decrease in cardiac output during the acute phase, however, the late hemodynamic changes still occurred and death still resulted (351). Various procedures have been used to prevent the acute phase in cats. Pretreating cats with acetylsalicylic acid (47, 122) or indomethacin (47, 271) can completely prevent the initial pulmonary vasoconstriction after endotoxin administration, however the delayed phase inevitably ensues.

A study involving patients admitted to hospital with bacterial shock revealed that a close correlation existed between cardiac index at time of admission and survival (350). The decrease in cardiac output which almost always accompanies the later stages of Gram-negative shock has prompted much investigation into the cause of the cardiac output decrease.

4. Effects on the Venous System

As discussed previously, the magnitude of cardiac output is a function of four variables; preload, afterload, heart rate and contractility. Early studies with endotoxin had implicated a decrease in preload as a causative factor for the observed decrease in cardiac output. In dogs, Weil et al. (351) diverted venous blood away from the heart into an extracorporeal reservoir. The volume of blood in the reservoir was monitored after endotoxin was administered to the dogs. It was found that in 7 dogs an average of 54 ml/kg of blood was withdrawn from the reservoir into the animals within 10 minutes. This decrease in reservoir volume occurred in spite of a 71% decrease in cardiac output. Other experiments where the liver and intestinal segments were weighed showed that these organs increased in blood content after endotoxin administration (240). It was concluded that the pooling of blood in the liver and intestines was sufficient to account for the observed hypotension. To confirm that the liver was the major site of pooling in dogs after endotoxin, Hinshaw et al. (172) removed the liver from dogs before administering endotoxin. With the liver removed, endotoxin did not produce a decrease in extracorporeal blood volume.

When Weil et al. (351) administered endotoxin to 7 dogs, an average of 54 ml/kg of blood was pooled from a reservoir during the acute phase and this was accompanied by

an average increase in portal pressure of 19 mm Hg. Since the portal venous pressure increased, it must be concluded that at least part of the hepatic pooling of blood was due to an increase in the distending pressure within the hepatic capacitance sites. The increase in pressure could theoretically be due either to an increase in portal blood flow or an increase in hepatic outflow resistance. It is unlikely that hepatic blood flow increases, as it has been shown that superior mesenteric blood flow decreases (32), and gastric (193) and splenic (91) arterial resistances increase. The increase in portal pressure after endotoxin administration in dogs therefore must be due to an increase in resistance to flow, i.e., due to outflow block of the liver.

Similar to the results using dogs, the administration of endotoxin to cats resulted in a time dependent decrease in extracorporeal blood volume after endotoxin administration (209). At the end of 1 hour and 2 hours after endotoxin, the reservoir blood volume had decreased by 23 ml/kg and 36 ml/kg, respectively. There was no significant rise in portal pressure and a gross examination of the abdominal viscera did not show signs of liver congestion. It was concluded that in the cat, outflow block did not occur and, thus, could not account for any pooling of blood. In a separate group of cats, a short segment of gut was continuously weighed after endotoxin was administered. Gut weight did not change

significantly after endotoxin, indicating that pooling of blood did not occur at this site. Also, using a plethysmographic technique, Falk et al. (76) determined that there was no intestinal pooling of blood after endotoxin.

Cardiopulmonary bypass experiments have been performed in cats to determine the role of the pulmonary system in the response to endotoxin (209). In these experiments, cardiac output was maintained constant and blood volume in an extracorporeal reservoir was recorded. Even though the lungs were excluded from the circulation, the volume of the reservoir decreased. In another study, the weight of a denervated foreleg was found not to increase after endotoxin administration (167). From these studies it can be concluded that pooling of blood tends to occur in the cat during the later stages of endotoxin shock but the sites of this pooling are unknown.

5. Role of Histamine

It has been hypothesized that histamine release plays a mediatory role in the onset of the acute phase in cats (209) and much evidence has accumulated in support of this hypothesis. Histamine is known to be released following a reaction of endotoxin with platelets and blood factors (346). It has been determined that released histamine can produce pulmonary vasoconstriction (270). As well, if cats are pretreated with the histamine liberator 48/80, the endotoxin induced pulmonary vasoconstriction is reduced.

A very plausible explanation for the increase in outflow resistance in dogs after endotoxin is that histamine is being released into the circulation (162). The dog has large quantities of histamine stored in hepatic mast cells which are available for release into the circulation (126). It has been noted that the responses of the dog to histamine are similar to anaphylactic reactions (323, 353) where histamine is known to be released from the liver (5, 267). Also, histamine and the histamine liberator 48/80, when infused into dogs, produced effects similar to those of endotoxin (170, 173). In another study it was found that the administration of antihistamines to dogs prevented the acute blood pressure drop after endotoxin administration (166). Previous studies have indicated that histamine causes a marked increase in resistance to hepatic flow by causing the constriction of the outflow sphincter which is located where the hepatic veins join into the inferior vena cava (200, 348). The observed large increase in hepatic blood volume after histamine administration (12, 20, 238) can be attributed to this outflow block (12, 20, 238). Although other vasoactive substances are released after endotoxin, including serotonin, catecholamines, kinins and other factors, histamine is the only substance known to produce outflow block in the dog (126).

If indeed histamine release is the mechanism of the hepatic pooling of blood, then it would be very difficult to

extrapolate this mechanism to other species. It seems that the canine species is unique in that it has histamine sensitive sphincters at the junction of the hepatic veins with the inferior vena cava. Cats do not have such a sphincter and histamine causes only small changes in hepatic blood volume (13, 20).

6. Role of the Sympathomedullary System

Vasodilators were first proposed for treatment of endotoxin shock in 1955 by Mark Nickerson, former Chairman of the Department of Pharmacology and Therapeutics at the University of Manitoba (262). Previous to 1955 it was generally believed that the major problem in shock was that arterial pressure was low and that the use of adrenergic vasoconstrictors would therefore be considered as rational therapy (252, 345). Five to ten years or so after Nickerson's proposal the sympathomedullary system had become implicated as contributing to the vasoconstriction seen after endotoxin administration (102, 177, 194, 296, 358, 366). Evidence for this sympathetic involvement included the findings that catecholamine levels were elevated during endotoxin shock (64, 147, 153, 194, 265, 295, 296, 324). As well, a sub-lethal dose of endotoxin can become a lethal dose if it is administered simultaneously with a constant infusion of the alpha-agonist, metaraminol (228). Injecting levarterenol bitartrate at 4 hour intervals after endotoxin injection was

also found to increase mortality in rabbits (51). In another study, the sympathetic nervous system of dogs was first blunted by treating the dogs with high doses of epinephrine (64). Blunting the sympathetic nervous system in this manner caused any vasoconstrictor response to noradrenaline and sympathetic nerve stimulation to be attenuated. It was then shown that blunting the sympathetic nervous system caused an improvement in survival after endotoxin administration. Other studies have shown that arterioles can become hyper-responsive to catecholamines after the administration of endotoxin (103, 366). This would tend to exacerbate any deleterious vasconstrictor effects of elevated catecholamine blood levels. Finally, alpha-blockers have been shown to alleviate deterioration and improve survival in shock (2, 9, 80, 101, 103, 263). Thus, many authors came to accept Nickerson's original hypothesis that vasodilators were called for in shock; the rationale being that vasodilators such as alpha-blockers would reverse the vasoconstriction and allow the tissues to be perfused adequately. Unfortunately, human studies using alpha-blockers with positive results are lacking. At the present time it is still accepted that tissue underperfusion is a major problem during endotoxin shock but that there is no definative proof that alpha-blockers produce any overall benefits in humans. As a result,alpha-blockers are not included in standard protocols for the treatment of septic shock (280).

The early conclusion from experiments with alpha-blockers was that they were beneficial because they at least partially alleviated the intense vasoconstriction produced by the sympathetic reflex. It was also assumed that alpha-blockers produced their vasodilatory effects by a competitive blockade of post-synaptic alpha receptors on arteriolar smooth muscle. However, further investigations into the effects produced by alpha-blockers has provided a modified interpretation of these results (2) which suggests that alpha-blockers might be producing a beneficial response by producing a beta-stimulatory effect. It has been shown that stimulation of presynaptic alpha-receptors on noradrenergic nerve terminals produces an inhibitory effect on noradrenaline release by those terminals (74, 104, 213). These presynaptic receptors have been shown to be $alpha_2$-adrenoceptors whereas arterial post-synaptic alpha- receptors are usually of the $alpha_1$-variety (74, 182). The selective blockade of $alpha_1$-receptors using prazosin leads to the expected decrease in blood pressures, through arteriolar vasodilation. However, the blockade of both $alpha_1$- and $alpha_2$-receptors leads to additional effects very similar to those produced by beta-agonists, for example, increased heart rate and cardiac output (302). In the above study, after $alpha_1$ and $alpha_2$ receptors were blocked, plasma concentrations of both noradrenaline and adrenaline rose. Saeed *et al.* concluded that the blockade of presynaptic

alpha$_2$-receptors in effect removed the alpha$_2$- mediated inhibition of noradrenaline release and this resulted in a large excess of noradrenaline release. They postulated that this excess noradrenaline was in sufficient quantity to stimulate beta-receptors within arterioles to produce a vasodilation. In support of this postulate, they found that beta-receptor blockade with nadolol greatly attenuated the phentolamine induced vasodilation. Thus it can be hypothesised that non-selective alpha-blockers may be beneficial during endotoxin shock in animals because of their indirect beta-receptor stimulatory effect on the cardiovascular system. This hypothesis is consistent with the findings that beta-receptor activation produced beneficial effects in endotoxin shock, and that beta-blockade with propranolol has been shown to be deleterious to rats in endotoxin shock (80). Although beta-receptor activation appears to produce a beneficial response in endotoxin shock, the exact mechanism for this response in uncertain. Theoretically, it may be due to cardiac stimulation, arteriolar vasodilation, effects on heart rate or effects on preload. As well, there may be an involvement of indirect mechanisms, for example, a beta-receptor mediated decrease in blood pressure could stimulate the heart via the baroreceptor reflex.

7. Role of Endogenous Opiates

The more recent findings that opiate antagonists produce beneficial cardiovascular effects has led to the conclusion that endogenous opiates may be a contributory factor in endotoxin shock. The i.v. administration of opiate antagonists hasbeen shown to improve cardivascular variables in rats (75), cats (208), dogs (283, 287, 341), mice (362) and humans (66, 129, 277, 339).

Other data have shown that the site of opiate antagonist action in endotoxin shock may be within the CNS. The ventriculo - cisternal administration of naloxone to dogs attenuated the precipitous fall in blood pressure normally seen after endotoxin administration (195). Further support of a centrally mediated action of opiate antagonists comes from the use of iodomethylated naloxone -- a derivative of naloxone to which the blood brain barrier is relatively impermeant. The peripheral administration of this compound did not reverse endotoxin induced hypotension in rats, however, the central administration attenuated the fall in blood pressure (292).

Although naloxone may be working centrally, it appears that it leads ultimately to changes in the periphery. Removal of the adrenal glands prevents the increase in blood pressure normally seen after naloxone administration to rats in endotoxin shock (184). From this study, it has been

concluded that naloxone exerts its beneficial effect by increasing sympathomedullary discharge, possible via a central mechanism (183). Presumably, the sympathomedullary discharge would act by causing an increase in catecholamine release. This conclusion is consistent with other experimental data. Firstly, centrally released opiate peptides have been shown to inhibit sympathomedullary function (59), thus naloxone could act by reversing this inhibition. Secondly, naloxone has been shown to have similar beneficial cardiovascular effects in hemorrhagic shock and these beneficial effects are blocked by alpha-adrenergic antagonists (307). Finally, the administration of naloxone to cats in endotoxin shock caused an increase in preganglionic splanchnic nerve impulses (208). Although the adrenals appear to be the final link in the pathway, it has been pointed out that a generalized increase in sympathetic outflow could improve hemodynamics during shock by acting at sites other than the adrenals (184). For example, activation of sympathetic nerves to the heart could increase cardiac output and blood pressure by acting directly on the heart to increase contractility. Further evidence that endogenous opiates may be an important factor in endotoxin shock comes from experiments where the administration of opiate antagonists led to deleterious cardiovascular effects. Morphine administration to rats after endotoxin caused a significantly greater fall in blood pressure than with

endotoxin alone (186) although this may be due to morphine causing histamine release. The hypotensive effect of morphine was reversed by naloxone.

It should be noted that in most studies concerned with the role of opiates in shock, the parameter chosen to provide indication for a beneficial effect was blood pressure. Unfortunately, there is no established connection between manoeuvres which raise blood pressure per se and increased survival. In fact, as discussed earlier, some agents which raise blood pressure, i.e. metaraminol and levarterenol, cause a decrease in survival times (51, 228). Also, if indeed naloxone acts by increasing endogenous catecholamine release, then any improvement would be expected to be temporary and would yield to the ultimate deleterious effects of catecholamine release.

8. Role of Prostaglandins

Pharmacologic studies have led to the implication that prostaglandins are involved in the pathophysiology of endotoxin shock. Prostaglandins are synthesized from arachidonic acid through three different pathways to form prostacyclin (PGI_2), prostaglandins PGE_2, PGF_2 and PGD_2 and thromboxanes A_2 and B_2 (94). The enzyme, cyclooxygenase, is the first enzyme required in the conversion of arachidonic acid to prostagandins and is inhibited by non-steroidal anti-inflammatory agents (77, 320). Prostaglandins have been

shown to be potent in affecting cardiovascular parameters (94) therefore many of the cardiovascular effects produced by endotoxin could theoretically be accounted for on the basis of prostaglandin production.

In support of the theory that prostaglandins are mediators of endotoxin shock are various studies which have shown that prostaglandin E and $F_{2\text{-alpha}}$, prostacyclin and thromboxane A_2 levels are elevated in plasma during shock (10, 36, 42, 49, 53, 155, 205, 266, 361). Since various prostaglandins can produce vasodilation, vasoconstriction and affect platelet aggregation, their elevated levels in blood could account for the various phases of decreased peripheral resistance, increased peripheral resistance and disseminated intravascular coagulation observed during endotoxin shock.

Prostaglandins have been implicated in the pathophysiology of shock also because of studies which describe the effects of blocking prostaglandin synthesis during shock. The first such study was carried out using dogs (264). In that study it was found that the administration of ASA either before or after endotoxin administration led to the improvement of cardiovascular parameters. Numerous subsequent studies using various species and different drugs have likewise shown that hemodynamics are affected in a beneficial manner when prostaglandin synthesis is inhibited during endotoxin shock (10, 11, 40, 58, 83, 84, 85, 122, 149, 161, 175, 271, 272, 273, 274, 275, 276, 355). Concomitant

with their effect on the cardiovascular system, prostaglandin systhesis inhibitors can also improve survival. Whether survival time was simply prolonged for a short period or prolonged indefinately depended on the lethality of the dose of endotoxin and on when the drug was administered. If an LD_{100} dose was used in animals that had been pretreated with the prostaglandin synthesis inhibitor, then survival time was increased or complete recovery occurred (84, 122, 175, 271, 272, 273, 274, 275, 276), although at least one study showed no improvement in survival (47). If the treatment drug was given after an LD_{100} dose of endotoxin, then no increase in survival occurred (83, 175, 275). However, if a less than an LD_{100} dose of endotoxin was administered before the treatment drug was administered, then survival time was increased (84, 85).

Although the non-steroidal anti-inflammatory agents have been shown to inhibit prostaglandin synthesis, it is possible that these drugs are acting via one or more of their other pharmacological actions. For example, it has been shown that these drugs may affect the sensitivity of vascular smooth muscle to catecholamines (3, 190, 260) and this could account for some of their effects during endotoxin shock. Another secondary effect of these drugs includes the inhibition of serotonin release (61, 72, 146). Serotonin may be a possible mediator in endotoxin shock because of its potent vasoconstrictor activity. Blockade of serotonin

receptors with ketanserin partly prevented the pulmonary hypertension and vasoconstriction normally observed after endotoxin administration in dogs (241). Also, non-steroidal anti-inflammatory (NSAI) drugs have been reported to stabilize lysosomal enzyme release (246) -- a property shared by steroid drugs which, as discussed later, also happen to improve survival in endotoxin shock. Finally, ASA decreases vascular permeability during endotoxin shock in dogs (10) and this action might be important in maintaining an adequate circulatory volume.

Recent studies with more specific prostaglandin inhibitors, i.e., thromboxane inhibitors, have indicated that the effects of NSAI drugs are indeed due to prostaglandin synthesis inhibition. Many of these inhibitors have chemical structures unrelated to NSAI drugs. They therefore posess pharmacologic profiles that are different than those of NSAI drugs. One of the few properties that all of these drugs have in common is that they inhibit prostaglandin synthesis. Therefore, if these newer thromboxane inhibitors modify the usual cardiovascular effects of endotoxin administration, then it can be more readily concluded that the beneficial effects produced are due to prostaglandin inhibition. The administration of a variety of these thromboxane inhibitors to different species had beneficial effects. Imidazole (53, 319), 7-(1-imidazolyl) heptanoic acid (361), dazoxiben (18) and two pyridine-based inhibitors (8) have all been found to

improve survival or to provide beneficial cardiovascular effects. Thromboxane production during shock therefore appears to be a contributory factor to the shock-state presumably through the vasoconstrictor and pro-aggregatory effects of thromboxane B_2 and A_2.

9. Role of the Immune System and Corticosteroids

Normally, during periods of stress, corticosteroid release by the adrenal cortex is increased. During septic shock however, it has been noted that corticosteroid levels are not increased (41) or can in fact be lower than normal (315). The mechanism of adrenocorticoid suppression has not been determined, however, it is known that blood plasma from rabbits in shock interferes with corticosteroid production by isolated adrenocortical cells (44, 204).

Corticosteroid therapy has been found to produce beneficial effects and to significantly improve survival times of animals which are in the state of endotoxin shock (52, 165, 168, 256, 261, 278, 310, 357). Beneficial effects of corticosteroids have also been reported in studies using humans (212, 226, 256, 311, 352). At this time however, there exists a debate as to whether corticosteroids should be used for septic shock in light of the fact that these drugs impair the immune system so as to make the host more susceptable to microorganism attack (27, 231, 312).

The use of corticosteroids has led to a possible mechanism of how endotoxin produces its destructive effects. This mechanism involves the immune system and has been summarized recently (157). It has been found that methylprednisolone inhibits free-radical production by polynorphonuclear leukocytes and monocytes (92, 151). When endotoxin is introduced into the blood of animals, complement is activated which in turn leads to the production of the complement component C5A (191). Polymorphonuclear leukocytes are in turn activated by C5A. When polymorphonuclear leukocytes become activated, they adhere to endothelial cells and release oxygen free radicals, lysosomal enzymes and arachidonic acid metabolites (356). Leukocytes require the production of these oxygen free radicals in order to kill and phagocytose (206, 356). Normally, oxygen free radicals are beneficial to the host in that they are used to help rid the body of invading microorganisms. However, it has been proposed that after endotoxin administration, a large amount of free radicals are produced, and that this leads to extensive tissue damage. The tissue damage is thought to be produced by the build-up of hydroxyl radicals which are normally maintained at low levels. Some reported damaging effects of free radicals include endothelial cell damage (62), phospholipid membrane lysis (237), mitochondrial damage (230), disruption of lysosomes (87), vascular permeability increases (63) and interference with calcium dispostion by

cardiac sarcoplasmic reticulum (158). It has been pointed out that changes very similar to those noted above have been observed during endotoxin shock which lends support to the theory that free radicals are the source of tissue damage by endotoxin (157). Since corticosteroids inhibit free radical production, it has been proposed that this is their mechanism of producing beneficial effects.

There are other theories on the mechanism of beneficial effects on the cardiovascular system produced by corticosteroids. As stated earlier, endogenous beta-endorphin release may be detrimental to animals during endotoxin shock. Corticosteroids have been found to inhibit beta-endorphin secretion (133). It has also been found that plasma vasopressin levels are elevated during endotoxin shock (359, 360). Vasopressin is a potent vasoconstrictor and could possibly account for part of the vasoconstriction observed in the late stages of shock. Corticosteroids have been found to decrease the release of vasopressin in rats (329) and in humans (65). Finally, corticosteroids inhibit the release of arachidonic acid (67). As described above, prostaglandins have been implicated as one of the factors responsible for cardiovascular changes which occur during shock, therefore corticosteroids might be acting by virtue of their prostaglandin inhibitory property.

10. Other Mediators

The antidiuretic role of vasopressin has long been established. However, relatively recently, evidence has accumulated which suggests that vasopressin has important physiological constrictor effects on the vasculature. If nervous reflexes are suppressed in dogs, the normal circulating levels of vasopressin act to maintain arterial blood pressure by 5-10 mm Hg. Also, vasopressin secretion is able to attenuate an induced fall in blood pressure by 75% (56, 57). Other studies have provided data for a physiological role of vasopressin in causing vasoconstriction (4, 243, 247, 335). Vasopressin infusions into dogs have been shown to produce an intense, sustained decrease in mesenteric arterial flow (160, 203, 244).

Intense vasoconstriction has been found after endotoxin in various species (81, 226) which suggests that vasopressin is playing a mediatory role (360). Blood vasopressin concentrations have been measured in dogs and baboons after endotoxin administration (360) and were found to be elevated for the duration of the 4-6 hour experiments. The vasopressin concentrations were often above 300 pg/ml whereas normal concentrations are approximately 4 pg/ml. Further evidence that vasopressin has a role in regulating cardiovascular function during endotoxin shock comes from a study where Brattleboro rats were used (28). The Brattleboro strain of rats have a hereditary hypothalamic deficiency

which results in lack of vasopressin release. This strain of rats is normal in other respects in that it shows a normal pressor response to various stimuli such as noradrenaline, angiotensin II and vasopressin. The administration of endotoxin to these rats resulted in a much larger drop in blood pressure as compared to Sprague-Dawley rats. Thus, without the release of vasopressin, rats are apparently much more susceptible to the hypotensive effects of endotoxin.

Apart from causing a direct vasoconstriction, vasopressin could theoretically act in other ways (28). Firstly, vasopressin has been shown to potentiate vasopressor responses to catecholamines in rats and cats (19). Secondly, vasopressin can potentiate post-ganglionic sympathetic nerve responses as demonstrated using isolated cat spleen (25). Therefore, vasopressin might be improving blood pressure in endotoxin shock by potentiating the effects of sympathetic nerve discharge and adrenal medulla release of catecholamines.

Serotonin is a potent vasoconstrictor which has been investigated as a potential mediator of the endotoxin induced vasoconstriction. However, serotonin levels were found to decrease to approximately 20% of control levels in dogs (295, 296). As well, pretreatment of dogs with the serotonin receptor antagonist, ketanserin, was found not to affect long term cardiovascular variables during endotoxin shock (241). Thus, it appears that serotonin plays no direct, significant role in the deleterious effects produced by endotoxin.

A good deal of diverse information and observations has accumulated regarding the pathology of endotoxin shock. However, no definative conclusion regarding the specific cause of endotoxin shock can be drawn from this information. It seems that the apparently beneficial effects of many of the above described pharmacological agents are due to their ability to counteract not the primary defect but rather the secondary effects of endotoxin, eg., hypotension, tissue underperfusion, etc. Although discovering the primary defect caused by endotoxin would be the ideal aim of further research, it must be noted that directing research efforts toward the secondary effects of endotoxin may be profitable as well. This is because it is the secondary effects which are the immediate cause of death in animals during endotoxin shock. By finding ways to counteract the onset of secondary effects, the animal in endotoxin shock might remain alive long enough to repair any primary defect caused by endotoxin and ultimate survival may ensue.

II. GENERAL METHODS

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A. HEPATIC BLOOD VOLUME MEASUREMENTS

Hepatic blood volume was measured by the method of plethysmogaphy as described previously (108, 364). Plethysmography was used because this technique can provide a continuous record of hepatic blood volume in intact animals throughout experimental procedures. An added advantage is that small, slowly developing changes in blood volume can be measured. The following is a description of the technique used in these experiments.

A longitudinal cut was made through the skin, underlying subcutaneous tissue, linea alba and parietal peritoneum from approximately just below the umbilicus to the xyphoid process. A lateral cut was then made along the subcostal margin on both sides for about 3 cm. Care was taken to prevent blood loss, especially from the superior epigastric artery which courses along near the midline. After the surface of the liver was exposed by these cuts the ligaments which anchor the liver to the abdominal walls were cut. Specifically, the falciform ligament and the left triangular ligaments were cut. The entire liver, excluding the right lateral and caudate lobes were then lifted while the bottom half of the plethysmograph was slid under the liver. The liver was then lowered into the cup-shaped bottom

half of the plethysmograph such that flow through the hepatic inflow and outflow vessels was not significantly obstructed and so that the liver was twisted as minimally as possible. The top half of the plethysmograph was then put in place and sealed with a hydrocarbon base gel (Plastibase, Squibb) so as to cause the liver to be in a fluid-tight container. The plethysmograph was filled with Ringer-Locke solution at 37° C. The only outlet from the plethysmograph was connected to a rubber tube which emptied into a reservoir. An increase or decrease in liver volume would therefore force Ringer-Locke solution into or out of the reservoir respectively. The change in reservoir volume was detected by a potentiometer and recorded on a Beckman type RM Dynograph. The system was calibrated such that a 2 ml change in reservoir volume produced a 1 cm deflection of the pen. Changes in liver volume of less than 0.25 ml could be detected (Fig. 3). A servo-control system lowered or raised the entire reservoir when Ringer-Locke was forced into or out of it so that there remained a constant hydrostatic pressure at the fluid surface of the reservoir. Thus, the hydrostatic pressure was maintained within the plethysmograph so that it was always constant regardless of any hepatic volume changes.

At the end of the above procedures, a visual inspection of the liver was made to ensure that it was not damaged. Also, portal pressure was compared before and after the procedure to obtain any indication of an obstruction to

blood flow through the liver. Experimental procedures were not performed for at least 20 minutes after the preparation was set-up and then only if the hepatic blood volume produced a stable baseline on the tracing. In other studies using this identical technique, hepatic blood volume remained stable for at least 6 hours (107).

The amount of blood that was in the liver at the end of the experiment was determined by the following procedure. All hepatic inflow and outflow vessels were occluded simultaneously, thus trapping hepatic blood in the liver. This blood was then washed out of the liver by perfusing it with approximately 175 ml of Ringer-Locke solution. One ml of the washout fluid was diluted with 24 ml of an EDTA/Ringer-Locke solution and the light absorption of this final product was determined by using a Bausch and Lomb, type Spectronic 20 spectrophotometer, at wavelength 540 nm. Arterial blood (0.2 ml) was also diluted in 25 ml of EDTA/Ringer-Locke solution and the light absorption of this product was likewise determined. The volume of blood in the liver was calculated by comparing the absorption of the washout fluid to that of whole blood after taking into account the various dilutions. By obtaining a value of hepatic blood volume at the end of the experiment and by assigning this value to the position of the pen on the chart at the end of the experiment, the value of hepatic blood volume at any time of the chart could then be calculated.

B. CARDIAC OUTPUT MEASUREMENTS

In experiments where cardiac putput was measured the method of thermodilution was used. A polyethylene cannula (PE 160, inside diameter 0.045", outside diameter 0.062") was inserted through the right jugular vein with its tip in the superior vena cava just above the heart. This cannula was used to infuse Ringer-Locke solution into the vena cava. The chest was opened through the fifth right intercostal space and a 2F thermistor probe was placed in the pulmonary artery. At the end of each experiment the location of the thermistor was checked to determine if it was in the pulmonary artery. This probe was connected to a cardiac output measurement system (IL 701, Instrumentation Laboratory Inc.). The chest was then closed and a negative intrathoracic pressure of 2 mm Hg was maintained by suction. Cardiac output was determined by injection of 1 ml Ringer-Locke solution over 4 seconds at room temperature using an infusion pump (Harvard Apparatus Co.).

The accuracy and reproducibility of this specific method of cardiac output measurement have been determined previously (113). Those determinations involved pumping blood at 37° through the inferior and superior vena cava with a constant perfusion pump. It was found that the least variability in measurements occurred when one millilitre of Ringer-Locke solution was injected over 4 seconds. The coefficient of variation for multiple measurements was 5-10%.

C. ARTERIAL PRESSURE MEASUREMENTS

Mean arterial pressure in all experiments was recorded from the right femoral artery using a polyethylene cannula (P.E. 90, inside diameter 0.066", outside diameter 0.095") which was narrowed slightly at the tip to facilitate insertion into the artery. The cannula was filled with Ringer-Locke solution containing heparin (200 units/ml) to minimize obstruction of the bore of the cannula with clotted blood. Pressures were measured by using a Beckman, type 4-327-C transducer and recorded on a Beckman, type RM Dynograph. The recorder was calibrated such that a 25 mm Hg change in pressure caused a 0.5 cm deflection of the pen. The recording apparatus was checked periodically and recalibrated when necessary. A typical calibration tracing is shown in Fig. 2.

D. PORTAL PRESSURE MEASUREMENTS

Portal pressure was recorded from a polyethylene cannula (PE 90, inside diameter 0.03", outside diameter 0.048") which was inserted through a small branch of the mesenteric vein located in the mesentery close to the appendix. The cannula was passed down this branch, through the mesenteric vein and then into the portal vein. The tip of the cannula was located close to the base of the liver. The cannula was filled with a heparin solution (200 units/ml) to prevent blood from clotting at its tip. After each experiment

the cannula was examined to ensure that it was in the portal vein. Pressure was measured by using a Beckman, type 4-327-C transducer and recorded on a Beckman, type RM Dynograph. The recorder was calibrated such that a 10 mm Hg change in pressure caused the pen to deflect by 2 cm (Fig. 2). The recording apparatus was checked periodically.

Portal pressure was used to provide a measure of pressure within the hepatic venous system. The validity of obtaining hepatic venous pressure in this manner is discussed in the Introduction section.

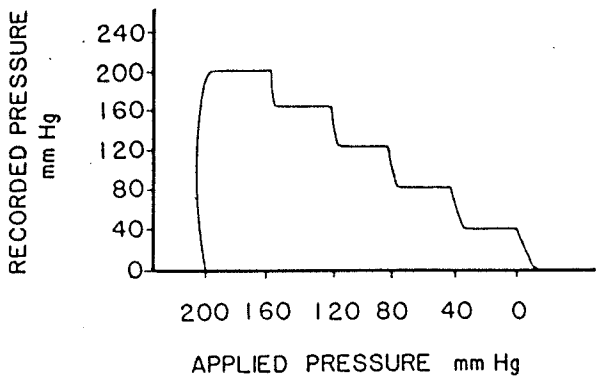

FIG. 1. Calibration tracing for arterial blood pressure measuring apparatus showing pen deflection versus pressure applied to the transducer. A mercury manometer was used to set the applied pressure at the given values.

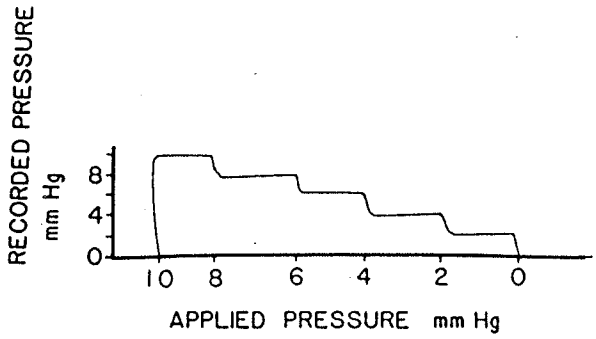

FIG. 2. Calibration tracing for portal vein blood pressure measuring apparatus showing pen deflection versus pressure applied to the transducer. A mercury manometer was used to set the applied pressure at the given values.

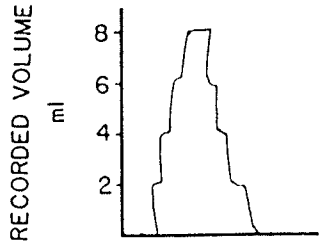

FIG. 3. Calibration tracing for hepatic blood volume measuring apparatus. Shown is the recorder pen stepwise deflection when 2 ml aliquots of fluid were infused into the plethysmograph. Chart speed was 5 mm/min.

III. RESULTS

The results of work presented here are divided into four sections. Each section contains a brief introduction which emphasizes material already discussed plus provides new material where required along with an outline of the rationale for experiments performed. Each section also contains a description of specific methods used for the experiments and a discussion of the results.

A. EFFECT OF ENDOTOXIN ON HEPATIC VENOUS RESPONSIVENESS

INTRODUCTION

Circulatory shock is a complex syndrome involving measurable disturbances of many or all body systems. The mortality rate remains high in spite of intensive studies of many treatment regimens. Estimates of the incidence of septic shock range from 70,000 to 300,000 cases each year in the United States (291). Comparable figures, adjusted for population, probably exist for Canada. Syndromes resembling circulatory shock may occur due to many different causes and a variety of animal models have been studied. Models produced by hemorrhage, infusions of live bacteria, bacterial extracts such as endotoxin, mesenteric artery occlusion and acute renal failure differ in many details and responses can be very different in different species. One feature common to at least the terminal stages of all types of circulatory shock is a progressive and marked reduction in cardiac output in association with peripheral arteriolar vasoconstriction (55, 96, 282, 313, 321).

In the 1950-60's, studies with endotoxin administration to dogs attributed the reduction in cardiac output to venous pooling, especially in the splanchnic bed (96, 353). Outflow block in the dog liver involves intense constriction of sphincter-like smooth muscle in the hepatic veins resulting in passive engorgement of the splanchnic venous bed (124, 126, 173). While this response can be

produced by histamine (124, 126), the mechanism causing outflow block in conditions causing circulatory shock in the dog remains to be elucidated.

It was gradually appreciated that this dramatic phenomenon occurred only in dogs (209) and attention shifted from venous pooling to the role of cardiac depression as the mechanism causing the reduced cardiac output. Although cardiac depression may play some role, circulatory shock does not resemble heart failure in that central venous pressure tends to be low, normal or at most modestly elevated. This suggests that peripheral venous pooling may play some role in circulatory shock in all species although the mechanism in other species may be quite different from that in the dog. In this study the effects of endotoxin on the hepatic venous bed in anesthetized cats were studied.

Other studies have examined some hemodynamic responses to endotoxin administration in cats. Acute administration of an intravenous bolus of endotoxin caused an immediate, frequently lethal, response resembling anaphylactic shock. Hypotension and a marked rise in central venous pressure occurred (122). This acute anaphylactic response varied with the type of endotoxin preparation used. It was severe after Salmonella enteriditis endotoxin (122), and after E. coli endotoxin (78, 270), but less marked after administration of a non-steroidal anti-inflammmatory agent such as acetylsalicylic acid or indomethacin (122, 271) and

could be ameliorated by giving the endotoxin as a slow intravenous infusion (47). In the presence of acetylsalicylic acid or indomethacin, endotoxin administered to anesthetized cats caused an intense lethal mesenteric vasoconstriction (122) and if this vasoconstriction was ameliorated by aminophylline, pulmonary damage resembling the adult respiratory distress syndrome occurred (47). However, after infusion of live E. coli, the mesenteric vasoconstriction was not seen (76).

The important role of the splanchnic venous system in overall cardiovascular homeostasis and control of cardiac output has been discussed on previous pages. Briefly, hepatic nerve stimulation, noradrenaline and angiotensin can cause a large redistribution of blood volume from the hepatic venous bed to the central circulation. It therefore seemed of interest to examine the effects of endotoxin on hepatic venous responsiveness to nerve stimulation, noradrenaline and angiotensin. In these experiments the cats were protected against the acute anaphylactic response to endotoxin by pretreatment with indomethacin. In a few experiments, the responsiveness of the hepatic venous system was examined in the absence of indomethacin to ensure that indomethacin did not itself alter the effects of endotoxin on venous responsiveness.

METHODS

Cats (2.4-3.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg, by intraperitoneal injection) and additional doses were administered (3 mg/kg, i.v.) when reflex limb movements returned. The trachea was cannulated so as to facilitate breathing. Mean arterial pressure and portal pressure were recorded as described. In some experiments lobar hepatic venous pressure was recorded from a polyethylene cannula (PE 60, inside diameter 0.03", outside diameter 0.048") inserted through a jugular vein and passed down through the heart to lie within a lobar hepatic vein 3-6 cm from the junction of that vein with the inferior vena cava. This cannula was not wedged (108, 111).

In experiments where cardiac output was measured, the method of thermodilution as described in the General Methods section was used.

Hepatic blood volume was recorded using the plethysmographic method described in the General Methods section.

For experiments in which nerve stimulation was performed, the hepatic nerves were separated from the common trunk of the hepatic and gastroduodenal arteries, and ligated; the peripheral end was placed in a bipolar ring electrode for stimulation from a Grass SD5 stimulator. Stimulus parameters were square wave pulses, 15V, 1 msec, and 1-8 Hz.

For experiments in which drugs were infused into the hepatic artery, the hepatic nerves were left intact but a cannula was placed in the ligated gastroduodenal artery with its tip close to the junction of this artery with the hepatic artery.

After all surgery was completed, a 30 minute stabilization period was allowed to elapse before any experimental procedures were started.

A control series of responses to nerve stimulation or drug infusions was then obtained. These responses were measured after steady state was reached and the measured variables were stable. Indomethacin (1 mg/kg body weight in phosphate buffer, pH 8.0) was given intravenously to prevent the acute lethal effects of endotoxin. Endotoxin (3 mg/kg) was suspended in 5 ml of 0.9% w/v sodium chloride solution. This suspension or an equivalent volume of sodium chloride solution (for controls) was administered as a bolus intravenous injection over 30 seconds. Changes in blood volume and portal pressure were measured at 30 min and 150 min after endotoxin and the responses to nerve stimulation or drug infusions were then re-examined. To confirm that the effects of endotoxin observed in these experiments were not dependent on the presence of indomethacin, some experiments were carried out in which indomethacin was not given but the endotoxin was infused slowly over 60 minutes. In another series of cats, saline was given instead of endotoxin to

obtain time-control data. These cats received indomethacin pretreatment.

At the end of each experiment, and as described in the General Methods section, the inflow and outflow vessels of the liver were simultaneously occluded. The liver was weighed and washed out with 150-175 ml of Ringer-Locke solution. The hemoglobin contents in the washout fluid and in diluted venous blood were compared photometrically after dilution with EDTA solution. The calculated value of hepatic blood content was used to calibrate the hepatic volume record at the point at which the hepatic vessels were ligated (108).

Noradrenaline bitartrate (1 mg base per ml water) was diluted immediately before use in Ringer-Locke solution containing ascorbic acid (200 mg/l) and infused intravenously in doses of 0.2, 0.5, and 1.0 ug/kg/min or intra-arterially into the hepatic artery in doses of 0.1, 0.2 and 0.5 ug/kg/min for 3 min at each dose. Angiotensin II stock solution (0.5 mg/ml water) was diluted immediately before use in Ringer Locke solution and infused intra-arterially in doses of 0.1, 0.2 and 0.5 ug/kg/min for 3 min at each dose. All comparisons before and after endotoxin were made by blocked analysis of variance and Duncan's multiple range test (328).

RESULTS

Endotoxin on arterial pressures and cardiac output

Arterial pressures and cardiac outputs were recorded in 6 cats pretreated with indomethacin and given endotoxin, in 5 control cats given saline instead of endotoxin, and in 7 cats given a slow intravenous infusion of endotoxin without pretreatment with indomethacin. There was a tendency for arterial pressure to decrease with time in both the control cats and in the cats which received endotoxin with indomethacin pretreatment (Fig. 4). However, the changes were not statistically significant. Similarly cardiac outputs were not significantly different and did not change with time in these two groups (Fig. 5). In the group which received the slow infusion of endotoxin over 60 min (Fig. 2), arterial pressure and cardiac output fell significantly during the infusion (Fig. 4 and Fig. 5). Arterial pressure then recovered somewhat while cardiac output remained depressed.

Endotoxin on hepatic blood volume

The effect of endotoxin on baseline liver blood volumes and portal pressures was examined in the groups of cats which received indomethacin pretreatment and in which the hepatic nerves were not sectioned. In 10 cats, after all surgery was completed and the 30 minute stabilization period had elapsed, the mean hepatic blood volume was 18.2 $\pm$ 0.6 ml/100 g of liver and the mean portal pressure was 9.1 $\pm$ 0.4

mm Hg. Within 30 min after the administration of endotoxin to the cats, hepatic blood volume had increased significantly to 130% of control and portal pressure had decreased significantly to 80% of control (Fig. 6). Hepatic blood volume and portal pressure then remained stable for at least 2 hours. In two of these cats, hepatic lobar venous pressure was also recorded. This pressure was slightly but not significantly lower than portal pressure during the control period and throughout the period after endotoxin. This is in agreement with data from other studies (111). Since pressure within the hepatic venous system did not increase after endotoxin, it can be concluded that pooling of blood occurred as a result of loss of smooth muscle tone in the hepatic venous system. In a control group of 10 cats with intact hepatic nerves, administration of saline instead of endotoxin caused no significant changes in hepatic blood volume or portal pressure over 150 min (Fig. 7).

The effects of endotoxin on baseline hepatic blood volumes and portal pressures were examined in eight cats with denervated livers (Fig. 8). Before endotoxin, hepatic blood volume was 25.0 $\pm$ 0.95 ml/100g liver and portal pressure was 8.5 $\pm$ 0.2 mm Hg. After endotoxin, hepatic blood volume and portal pressure did not change significantly -- at 150 min after endotoxin, hepatic blood volume was 26.1 $\pm$ 0.95 ml/100 g liver and portal pressure was 8.7 $\pm$ 0.2 mm Hg. Thus endotoxin had no detectable effect on baseline hepatic blood volume and portal pressure when the liver was denervated.

Hepatic nerve stimulation

Stimulation of the hepatic nerves caused a frequency dependent decrease in blood volume (Fig. 9) and increase in portal pressure (Fig. 10) in all cats before endotoxin was administered. At 150 min after the administration of endotoxin, the hepatic blood volume and portal pressure changes in reponse to hepatic nerve stimulation were markedly reduced. Attenuation of the hepatic blood volume (Fig. 11) and portal pressure (Fig. 12) responses after endotoxin was also observed in cats which did not receive indomethacin pretreatment. Data from previous studies have shown that there is no significant reduction in the hepatic blood volume or portal pressure responses to hepatic nerve stimulation over 150 min in the absence of endotoxin (107).

From these data, it can be concluded that by 150 minutes after endotoxin administration, the sympathetic nervous system can no longer redistribute significant amounts of blood from the liver to the central circulation.

Intravenous noradrenaline infusions

To examine whether this impairment of nerve function was a failure of noradrenaline release by the nerves or a failure of the smooth muscle to respond to agonist, the reponses to intravenous noradrenaline infusions were studied. Infusions of noradrenaline produced a dose dependent decrease in blood volume and increase in portal pressure in all cats

before any endotoxin was administered. At 150 minutes after endotoxin administration, the hepatic blood volume (Fig. 13) and portal pressure (Fig. 14) responses to noradrenaline infusion were markedly reduced. These attenuated blood volume and portal pressure responses to noradrenaline also occurred in cats which did not received indomethacin pretreatment (Fig. 15 and Fig. 16). In a control group of 5 cats which received indomethacin but not endotoxin, the hepatic blood volume (Fig. 17) and portal pressure (Fig. 18) responses to noradrenaline were not significantly different ($P>0.05$) after 150 minutes compared with responses at zero time.

Noradrenaline infusions into the hepatic artery

This group of experiments was carried out in order to circumvent the possibility that endotoxin might interfere with the delivery of intravenously infused noradrenaline to the hepatic venous system due to intestinal vasoconstriction (47, 232).

Infusions of noradrenaline into the hepatic artery produced dose-dependent decreases in hepatic blood volume and increases in portal pressure. At 150 minutes after endotoxin administration, the hepatic blood volume and portal pressure responses to noradrenaline infusion into the hepatic artery were markedly attenuated (Fig. 19 and Fig. 20). In a control group of 4 cats which received indomethacin but no endotoxin, the hepatic blood volume and portal pressure responses to

noradrenaline infusions into the hepatic artery were not significantly different after 150 minutes compared to the responses at zero time ($P>0.05$) (Fig. 21 and Fig. 22).

Angiotensin II infusions into the hepatic artery

These experiments were performed in order to assess whether the decrease in responsiveness of the hepatic venous system after endotoxin was specific to noradrenaline or more generalized to involve other agonists. Infusions of angiotensin II produced dose-dependent decreases in hepatic blood volume and increases in portal pressure before endotoxin administration. The blood volume and portal pressure responses to infused angiotensin II were markedly attenuated at 150 minutes after endotoxin administration (Fig. 23 and Fig. 24). In the control group of 5 cats which received no endotoxin, the hepatic blood volume and portal pressure responses to intra-arterially infused angiotensin II were not significantly different after 150 minutes compared to the responses at zero time ($P>0.05$) (Fig. 25 and Fig. 26).

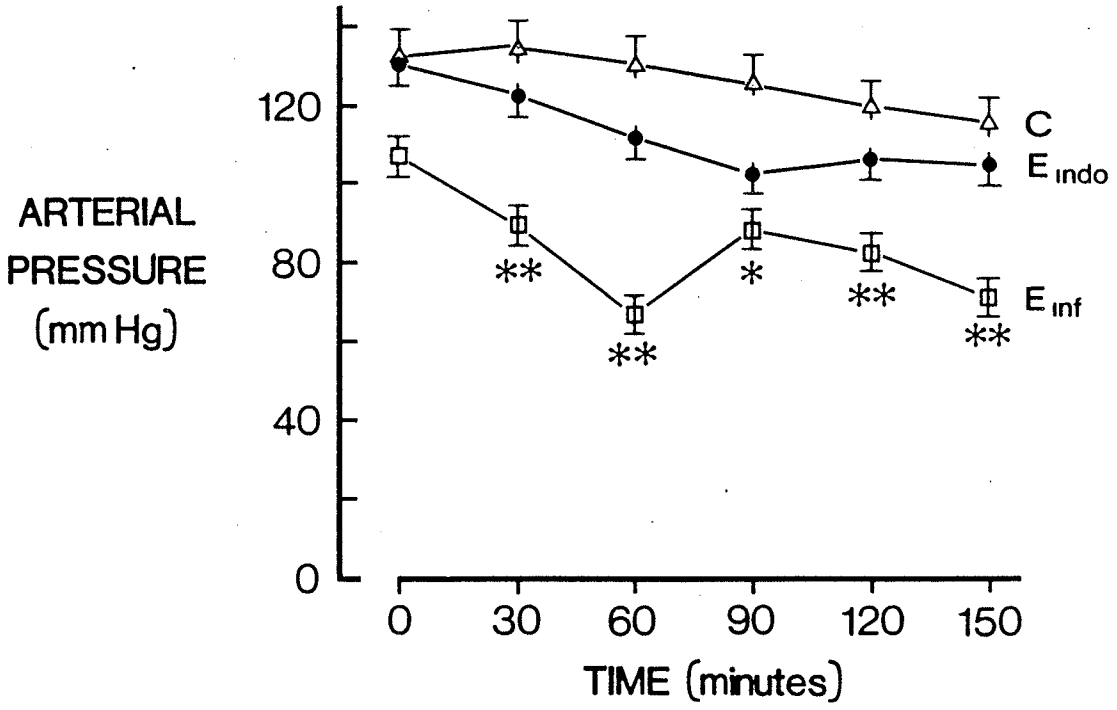

FIG. 4. Arterial pressures in three groups of cats: those which received indomethacin pretreatment but no endotoxin (controls C, n=5), those which received indomethacin pretreatment and a bolus injection of endotoxin (E_{indo}, n=6) and those that received a slow intravenous infusion of endotoxin without indomethacin pretreatment (E_{inf}, n=7). * = $P<0.05$, ** = $P<0.01$ compared with control values averaged over the 10 min prior to treatment and shown in the figure as zero time.

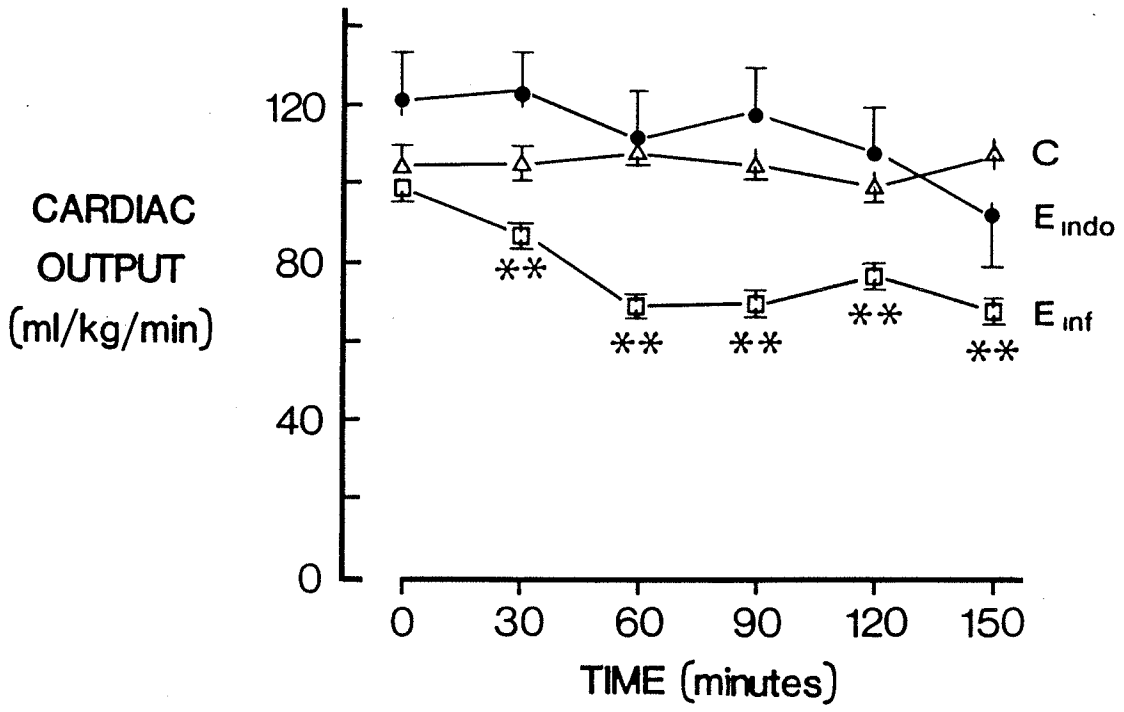

FIG. 5. Cardiac outputs in three groups of cats: those which received indomethacin pretreatment but no endotoxin (controls C, n=5), those that received indomethacin pretreatment and a bolus injection of endotoxin (E_{indo}, n=6) and those that received a slow intravenous infusion of endotoxin without indomethacin pretreatment (E_{inf}, n=7). ** = $P<0.01$ compared with control values averaged over the 10 min prior to treatment and shown in the figure as zero time.

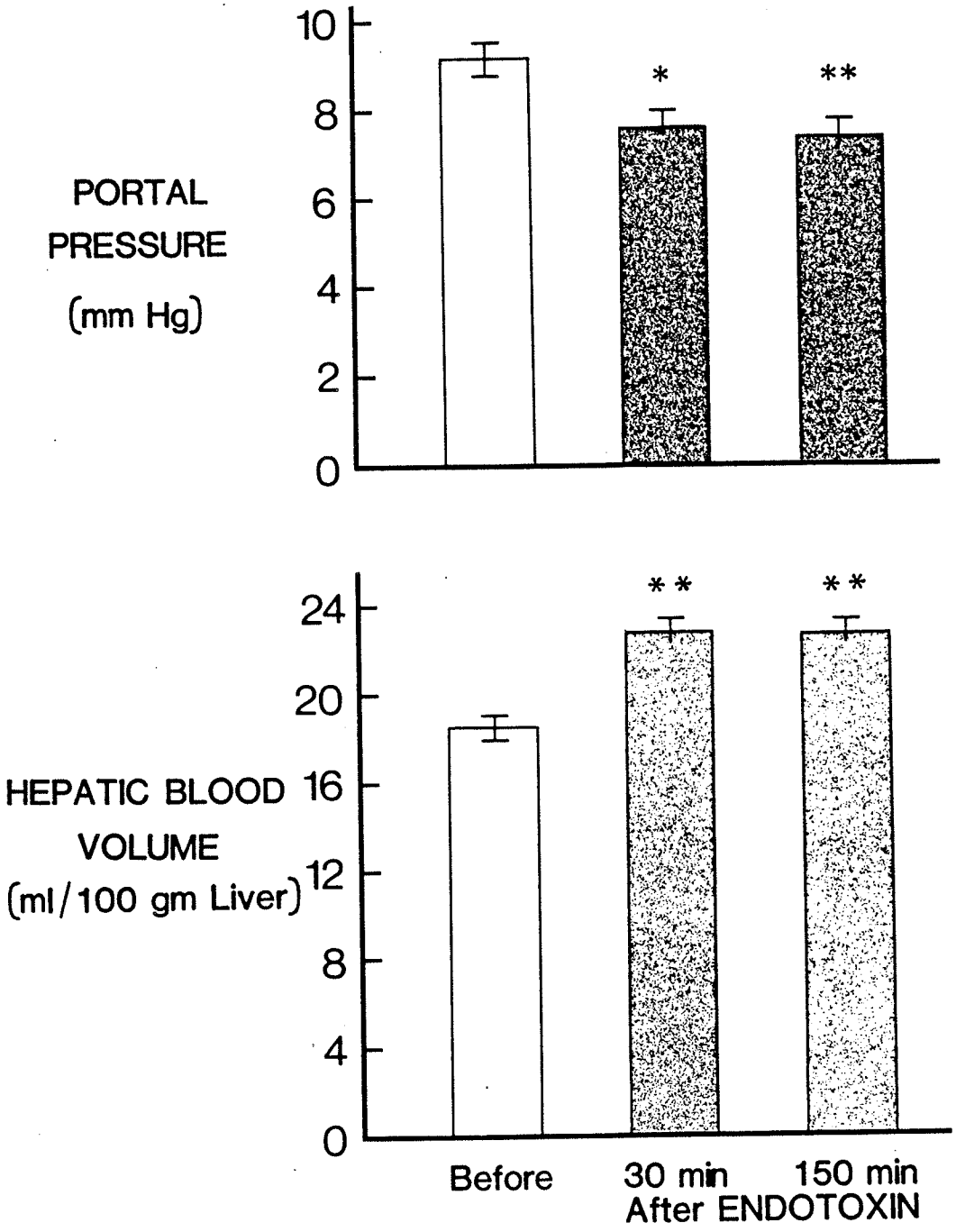

FIG. 6. Portal pressure and hepatic blood volume responses before, 30 min and 150 min after administration of endotoxin (n=10) in cats with innervated livers which received indomethacin pretreatment. * = $P<0.05$, ** = $P<0.01$ compared with control values over 10 min before endotoxin.

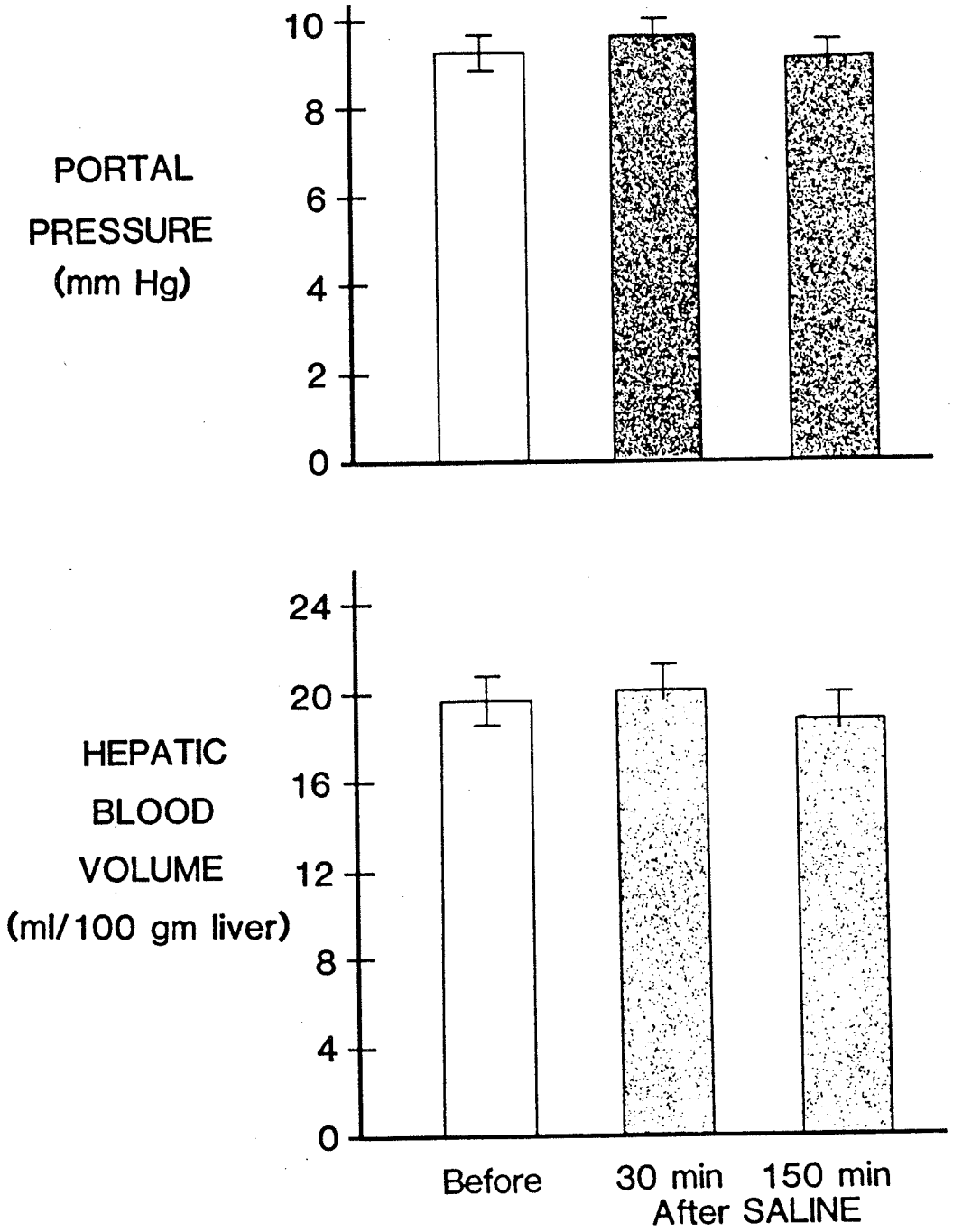

FIG. 7. Portal pressure and hepatic blood volume responses before, 30 min and 150 min after administration of endotoxin saline vehicle (n=10) in cats with innervated livers which received indomethacin pretreatment. * = $P<0.05$, ** = $P<0.01$ compared with control values.

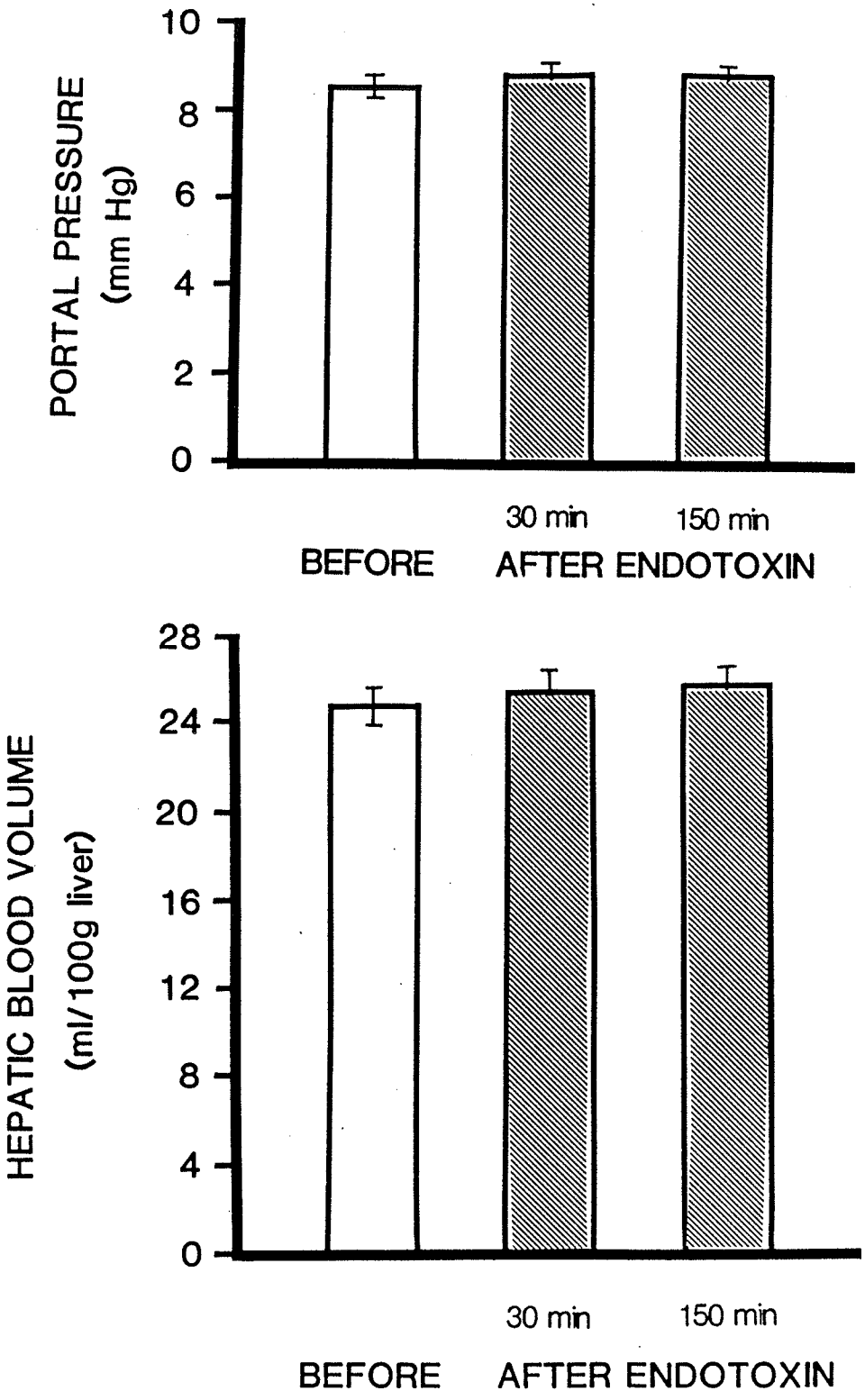

FIG. 8. Portal pressure and hepatic blood volume responses before, 30 min and 150 min after administration of endotoxin (n=10) in cats with denervated livers which received indomethacin pretreatment. * = $P<0.05$, ** = $P<0.01$ compared with control values over 10 min before endotoxin.

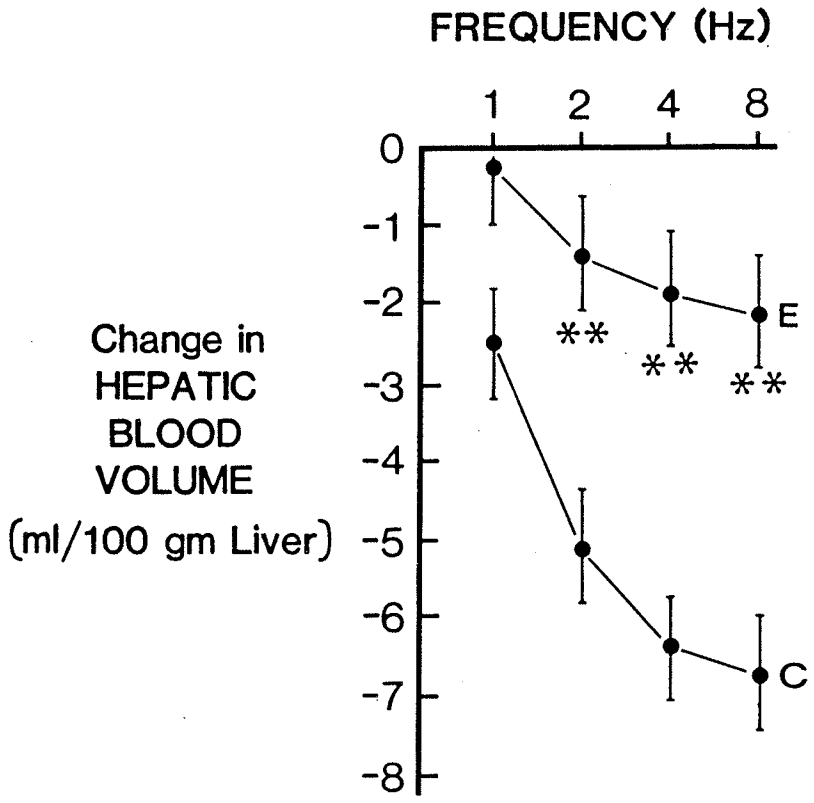

FIG. 9. Changes in hepatic blood volume during hepatic nerve stimulation before (C) and 150 min after infusion of endotoxin (E) with indomethacin pretreatment (n= 5). ** = P<0.01 comparison of before and after endotoxin.

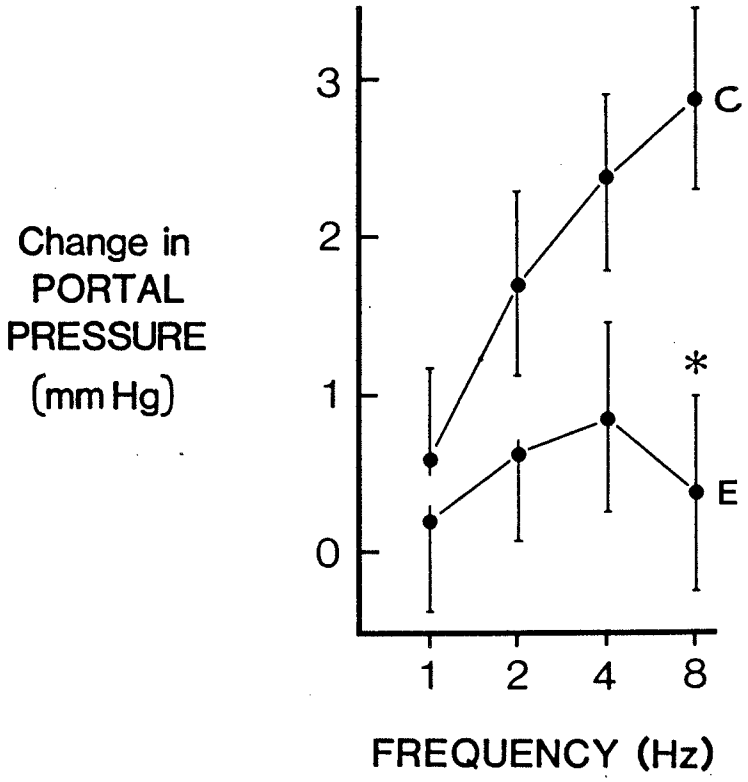

FIG. 10. Changes in portal pressure during hepatic nerve stimulation before (C) and 150 min after infusion of endotoxin (E) with indomethacin pretreatment (n=5). * = $P<0.05$, comparison of before and after endotoxin.

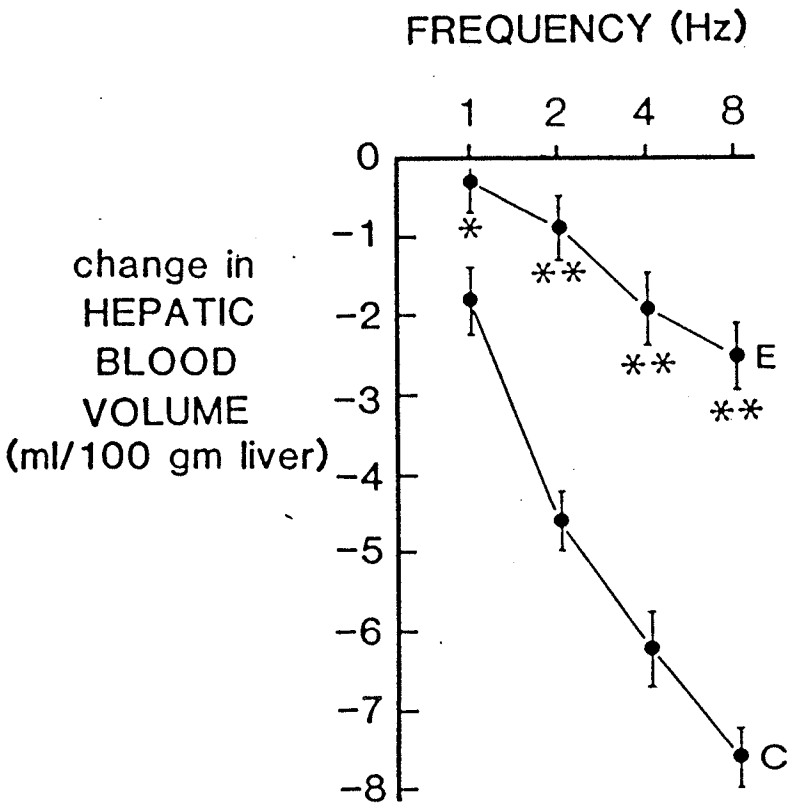

FIG. 11. Changes in hepatic blood volume during hepatic nerve stimulation before (C) and 150 min after infusion of endotoxin (E) in cats which did not receive indomethacin pretreatment (n=4). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

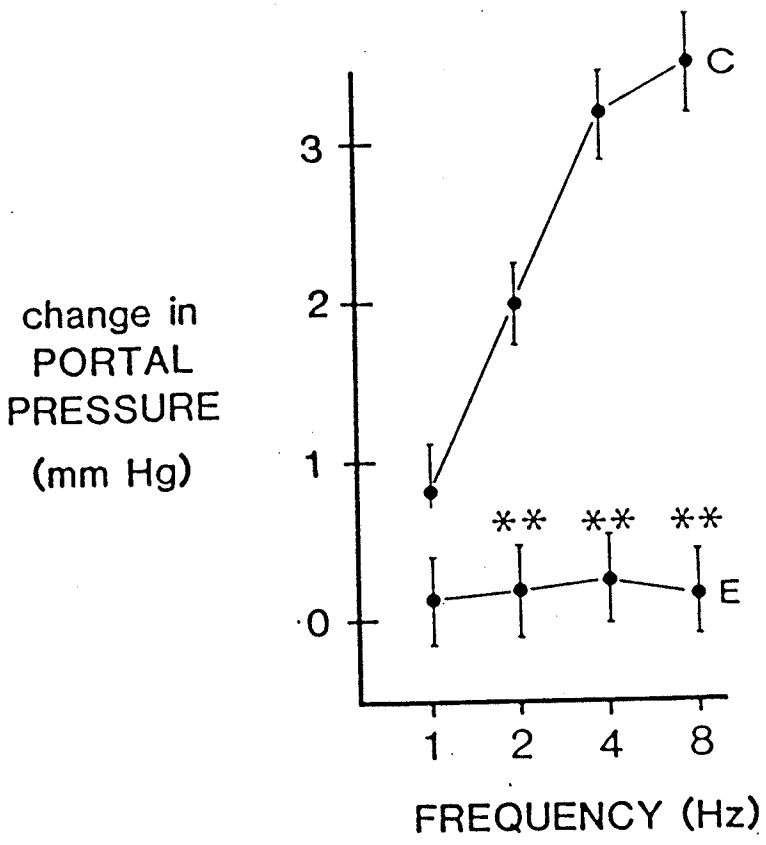

FIG. 12. Changes in portal pressure during hepatic nerve stimulation before (C) and 150 min after infusion of endotoxin (E) in cats which did not receive indomethacin pretreatment (n=4). ** = $P<0.01$ comparison of before and after endotoxin.

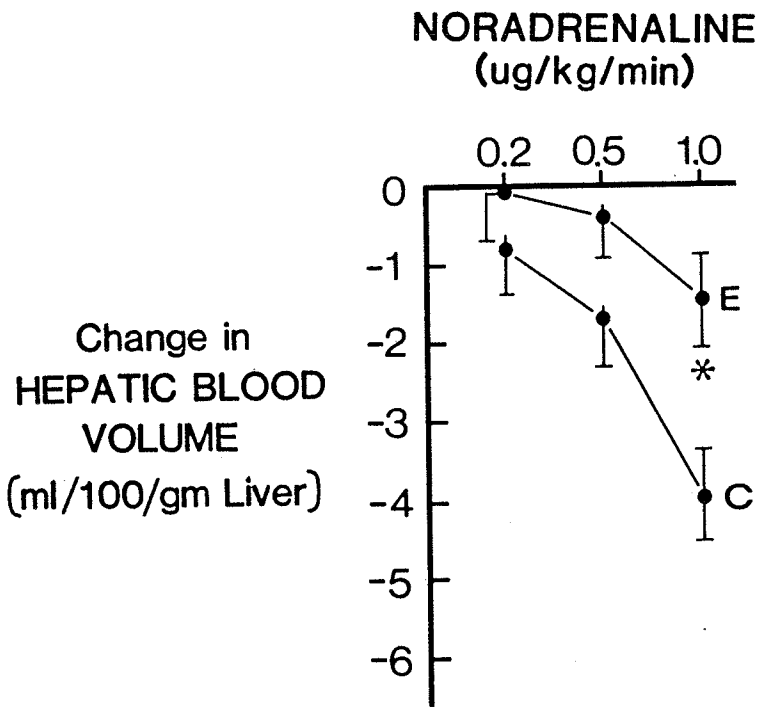

FIG. 13. Changes in hepatic blood volume during intravenous noradrenaline infusions before (C) and 150 min after injection of endotoxin (E) in cats pretreated with indomethacin (n=4). * = $P<0.05$ comparison of before and after endotoxin.

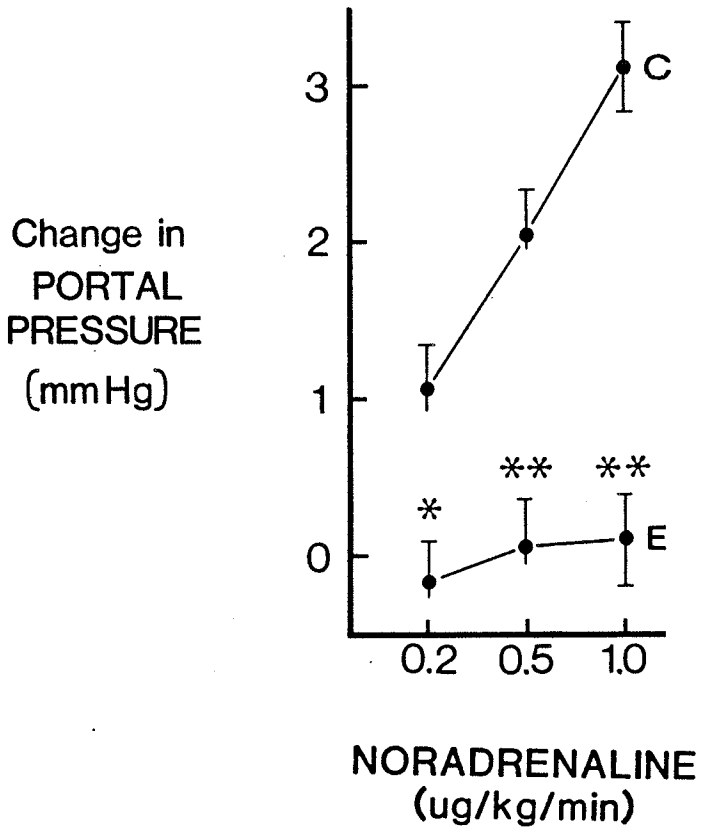

FIG. 14. Changes in portal pressure during intravenous noradrenaline infusions before (C) and 150 min after injection of endotoxin (E) in cats pretreated with indomethacin (n=4). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

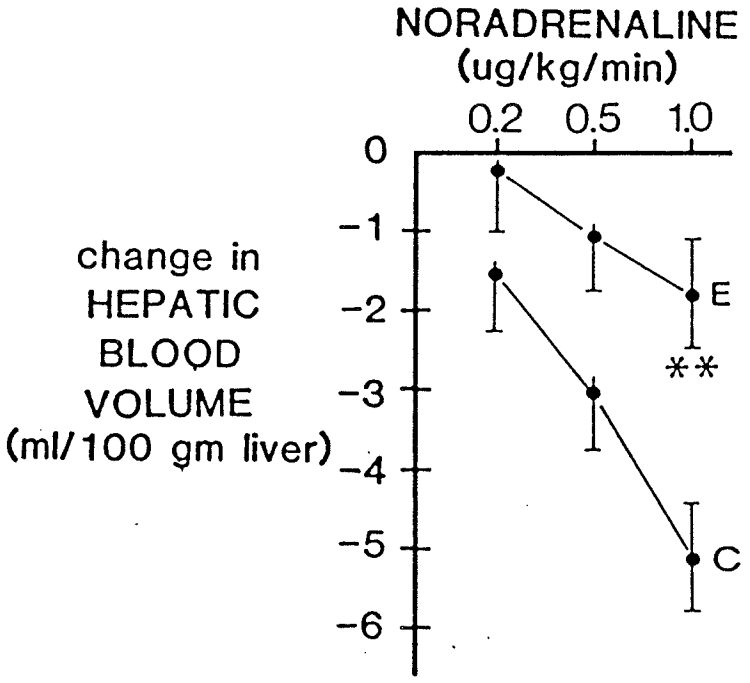

FIG. 15. Changes in hepatic blood volume during intravenous noradrenaline infusions before (C) and 150 min after injection of endotoxin (E) in cats not pretreated with indomethacin (n=4). ** = $P<0.01$ comparison of before and after endotoxin.

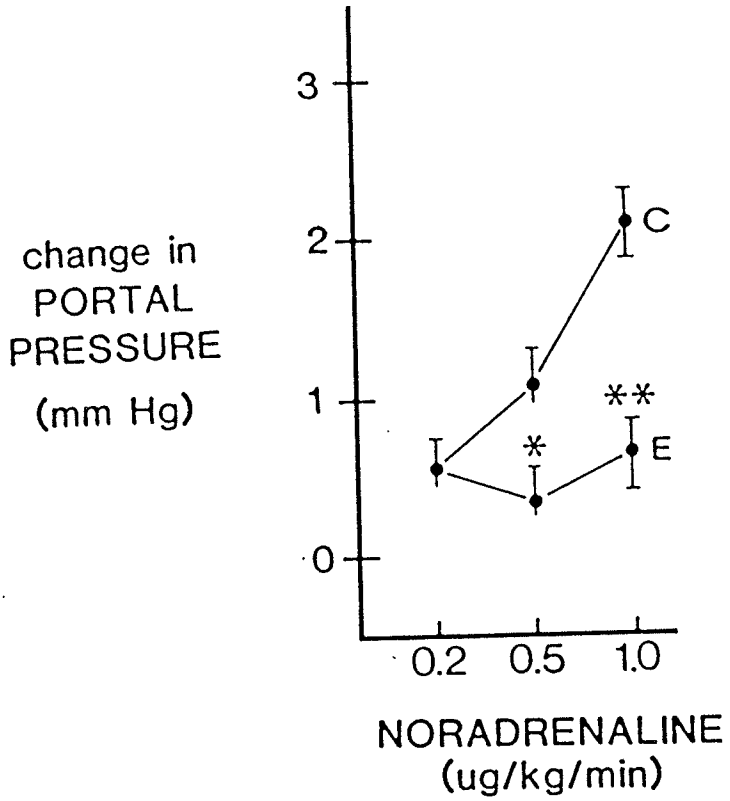

FIG. 16. Changes in portal pressure during intravenous noradrenaline infusions before (C) and 150 min after injection of endotoxin (E) in cats not pretreated with indomethacin (n=4). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

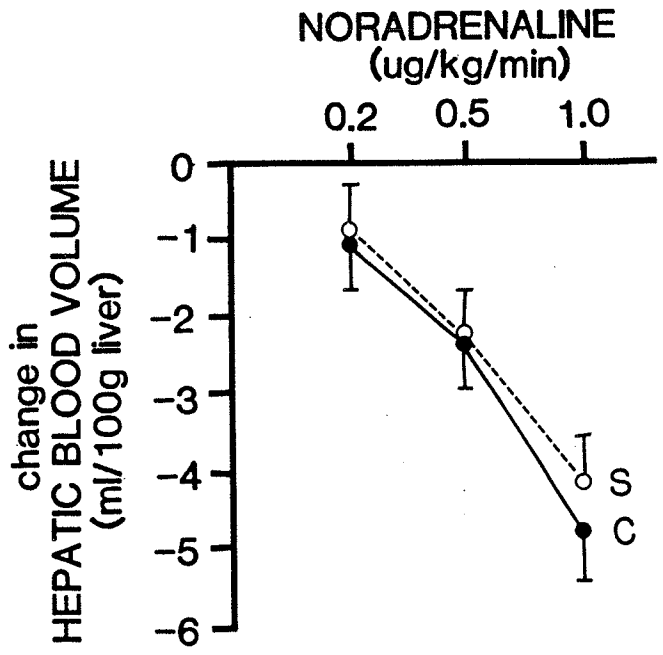

FIG. 17. Changes in hepatic blood volume during intravenous noradrenaline infusions before (C) and 150 min after injection of saline vehicle in cats pretreated with indomethacin (n=4). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

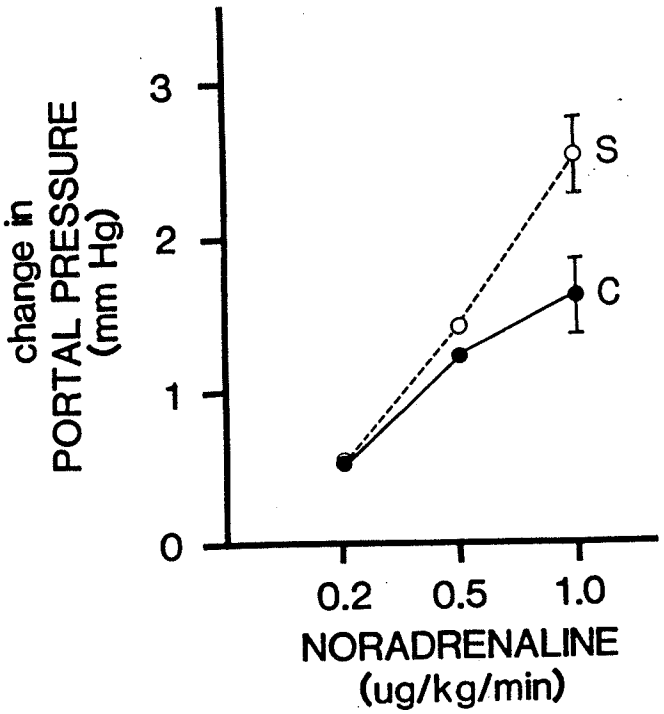

FIG. 18. Changes in portal pressure during intravenous noradrenaline infusions before (C) and 150 min after injection of saline vehicle in cats pretreated with indomethacin (n=4). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

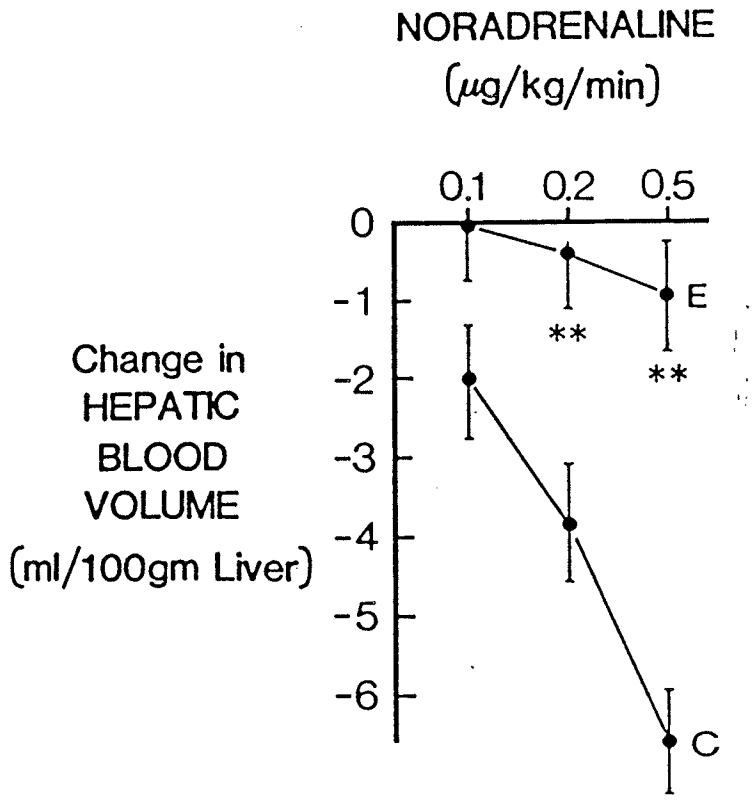

FIG. 19. Changes in hepatic blood volume during infusions of noradrenaline into the hepatic artery before (C) and 150 min after injection of endotoxin (E) into cats pretreated with indomethacin (n=5). ** = $P<0.01$ comparison of before and after endotoxin.

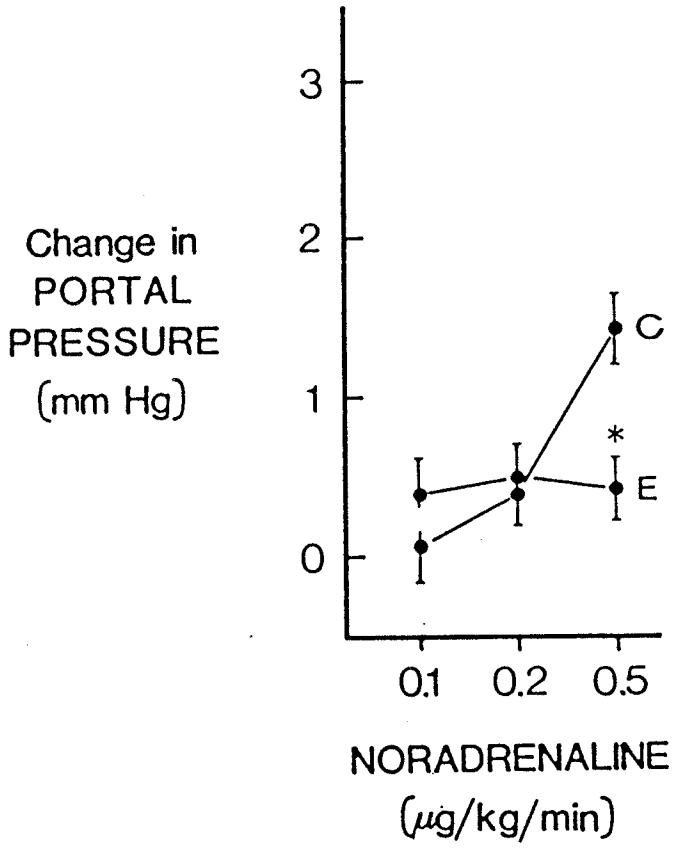

FIG. 20. Changes in portal pressure during infusions of noradrenaline into the hepatic artery before (C) and 150 min after injection of endotoxin (E) into cats pretreated with indomethacin (n=5). * = $P<0.05$ comparison of before and after endotoxin.

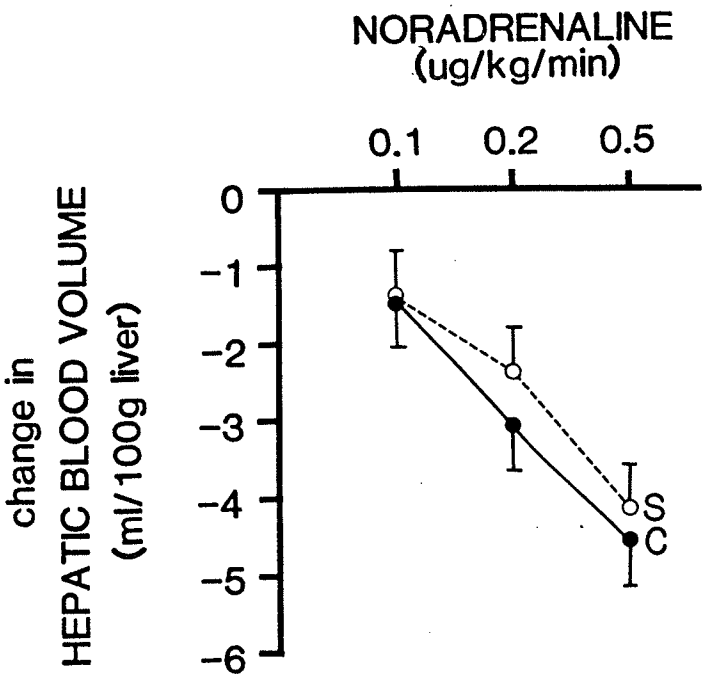

FIG. 21. Changes in hepatic blood volume during infusions of noradrenaline into the hepatic artery before (C) and 150 min after injection of saline vehicle into cats pretreated with indomethacin (n=5). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

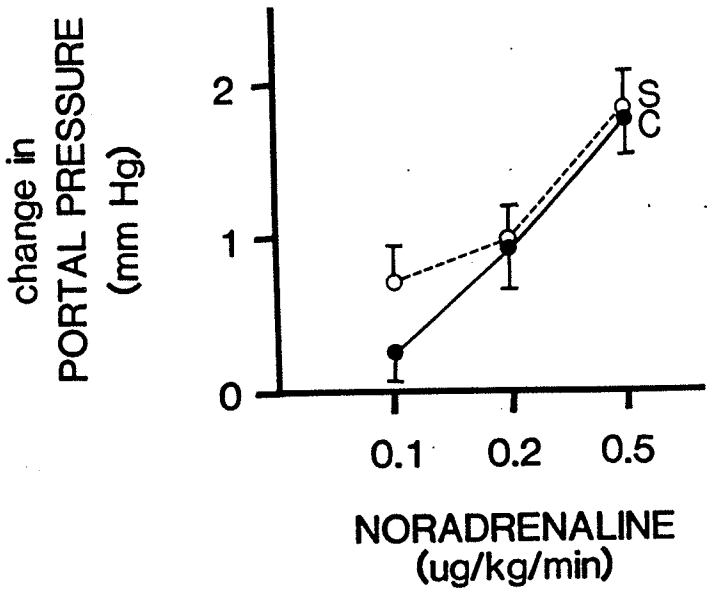

FIG. 22. Changes in portal pressure during infusions of noradrenaline into the hepatic artery before (C) and 150 min after injection of saline vehicle into cats pretreated with indomethacin (n=5). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

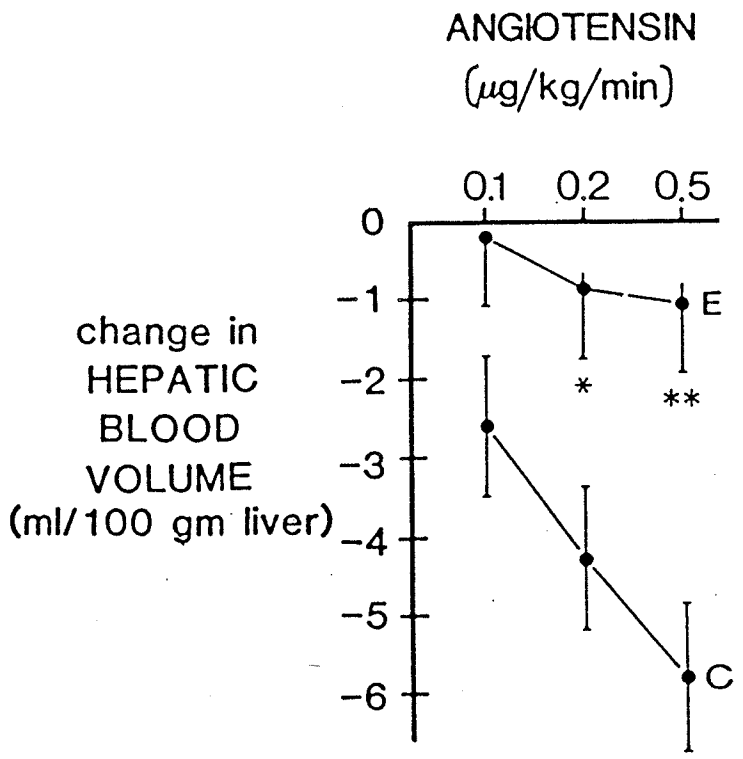

FIG. 23. Changes in hepatic blood volume during infusions of angiotensin II into the hepatic artery before (C) and 150 min after injection of endotoxin (E) into cats pretreated with indomethacin (n=5). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

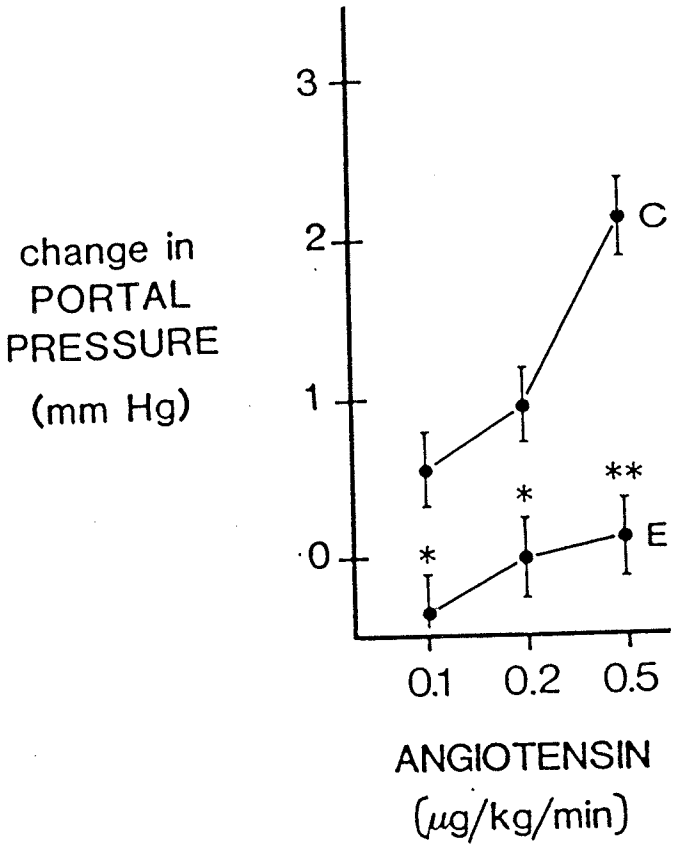

FIG. 24. Changes in portal during infusions of angiotensin II into the hepatic artery before (C) and 150 min after injection of endotoxin (E) into cats pretreated with indomethacin (n=5). * = P<0.05, ** = P<0.01 comparison of before and after endotoxin.

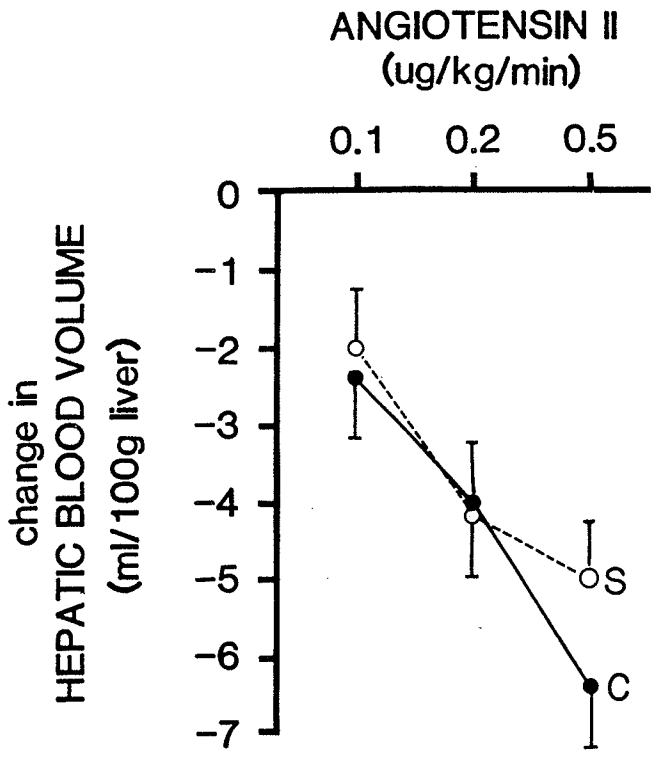

FIG. 25. Changes in hepatic blood volume during infusions of angiotensin II into the hepatic artery before (C) and 150 min after injection of saline vehicle into cats pretreated with indomethacin (n=5). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

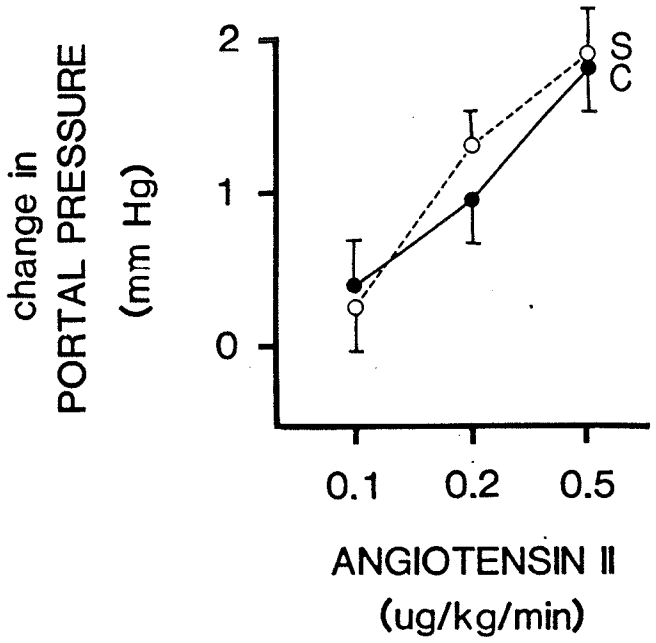

FIG. 26. Changes in portal pressure during infusions of angiotensin II into the hepatic artery before (C) and 150 min after injection of saline vehicle into cats pretreated with indomethacin (n=5). * = $P<0.05$, ** = $P<0.01$ comparison of before and after endotoxin.

DISCUSSION

The volume of blood within the hepatic venous system is under control of the sympathetic nervous system (111). Nerve stimulation (1-8 Hz) has been shown to lead to a constriction of hepatic venous smooth muscle resulting in the expulsion of up to 50% of the total hepatic blood content. Mobilization of hepatic blood in turn leads to an increase in cardiac filling pressure (113) and the effects of this on cardiac output have been analysed (109, 113). This study shows that the constriction of venous smooth muscle in response to hepatic nerve stimulation is markedly attenuated after endotoxin. A decreased response to hepatic nerve stimulation was observed in cats which received indomethacin and in cats which received no indomethacin. Thus, indomethacin did not alter the effects of endotoxin.

The inability of hepatic nerve stimulation to evoke a normal venoconstrictor response after endotoxin could be explained if the hepatic nerves failed to release normal amounts of noradrenaline, if venous smooth muscle failed to respond normally to noradrenaline, or if a combination of these occurred. Since the constrictor response to parenterally administered noradrenaline was attenuated after endotoxin, it appears that the responsiveness of the smooth muscle of the hepatic venous system was impaired by endotoxin. Whether or not noradrenaline release was also impaired by endotoxin cannot be determined from these experiments.

However, the data which show an attenuated response to intravenously infused noradrenaline could also be explained by postulating that the noradrenaline did not reach the liver due to a marked reduction in portal blood flow. Previous studies have shown that mesenteric arterial flow is decreased by 70% within 90 minutes of endotoxin administration (47, 122). This possibility was examined by infusing noradrenaline directly into the hepatic artery. After endotoxin, any reduction in portal blood flow would result in a significntly higher concentration of noradrenaline within the liver when the same dose is infused into the hepatic artery. Thus if the smooth muscle responsiveness was unimpaired, then responses should have been larger than before endotoxin. In fact they were markedly attenuated which is consistent with the conclusion that venous smooth muscle responsiveness is impaired.

After endotoxin the venous reponsiveness to angiotensin II infusions into the hepatic artery was also attenuated. There are various possibilities for the decreased responsiveness of the hepatic venous system. The decreased responsiveness to noradrenaline and angiotensin II could be due to reduced numbers of membrane receptors to noradrenaline and angiotensin or to a defect in the excitation-contraction coupling mechanisms and contractile elements in the smooth muscle. It has recently been shown that after endotoxin, the number of alpha and beta adrenergic receptor binding sites

were decreased in dog liver cell membranes (95) and it was suggested that these changes may play an important role in the development of hepatic glucose dyshomeostasis during shock. Thus alterations in the smooth muscle receptors is an attractive hypothesis but further studies are required to elucidate the mechanism of the impaired responsiveness.

Before administration of endotoxin, the mean hepatic blood volume was 18 ml/100 g liver in the innervated livers and 25 ml/100 g in the denervated livers. These values are similar to those reported previously and they suggest a considerable sympathetic tone on the hepatic venous bed in anesthetized, surgically-operated cats (111). After administration of endotoxin, hepatic blood volume increased and portal pressure decreased in the innervated livers but not in denervated livers. The pooling of blood in the innervated livers after endotoxin could therefore be due to impairment of pre-existing sympathetic tone mediated through the hepatic innervation. Thus the increase in resting hepatic blood volume and the attenuated responses to sympathetic nerve stimulation may be due to the same action of endotoxin.

This study suggests that after endotoxin, hepatic venous sympathetic tone and the ability of the sympathetic nervous system to redistribute blood volume from the hepatic venous reservoir is severely impaired. It is clear that this impairment, by itself, does not result in a reduced cardiac output (Fig. 5). In these experiments, arterial pressure and

cardiac output were well maintained in cats given endotoxin after pretreatment with indomethacin. In the cats given a slow infusion of endotoxin, early hypotension and reduced cardiac output were seen but since these effects could be blocked by indomethacin, it seems more likely they were due to release of autacoids, especially prostaglandins, than to impaired adrenergic responsiveness. Other compensatory mechanisms are therefore sufficient to maintain cardiac output in the face of modest hepatic venous pooling and the impaired ability to mobilize the hepatic venous reservoir. As the general condition of the animal deteriorates due to the multiple other actions of endotoxin, the impaired ability to mobilize the hepatic venous reservoir may play an increasingly important role in the progressive decline in cardiac output. However, this cannot be a major factor in the lethality of endotoxin since blood volume expansion by dextran does not overcome the lethal effects (47).

An interesting question which was not examined in this study is whether the resistance vessel reponses to sympathetic nerve stimulation and noradrenaline are impaired in parallel with the venous responses. This is difficult to determine. In cats, mesenteric resistance vessels contract markedly during the first two hours after endotoxin but this mesenteric vasoconstriction is not mediated through the sympathetic nerves nor noradrenaline (47, 122). Hepatic arterial resistance decreases very slightly (47). The renal

bed is dilated (47) but it is not known whether this dilation is due to impaired arteriolar responsiveness to the sympathetic nerves, noradrenaline or angiotensin.

B. EFFECTS OF ISOPROTERENOL ON THE HEPATIC VENOUS SYSTEM

INTRODUCTION

Isoproterenol is a non-selective beta-receptor agonist which relaxes most vascular smooth muscle and stimulates the heart. These actions result in increased cardiac output and hypotension (109). In previous studies it has been shown that infusions of isoproterenol into the hepatic artery caused a vasodilation of the hepatic arterial bed (119) but no significant changes in hepatic blood volume (116). This lack of effect on hepatic blood volume was not simply due to the absence of a venous basal tone since the responses to sympathetic nerve stimulation were not inhibited during isoproterenol infusions (107). It was concluded that beta-receptors mediating smooth muscle relaxation were present in the hepatic arterioles but not in the hepatic capacitance vessels.

These observations are consistent with observations in other organs and species (reviewed by Greenway, (109)). Isoproterenol reduces arteriolar resistance in all vascular beds although the vasodilation appears to be greatest in the splanchnic region and least in the kidney (7, 17, 45, 179, 189, 207, 288, 308, 334). Isoproterenol in small to moderate doses has little effect or causes weak relaxation of venous smooth muscle, in isolated strips (333, 337) and vein segments (60, 125, 197). In the human hand, isoproterenol relaxes venous smooth muscle preconstricted by norepinephrine

suggesting some beta-receptors are present in these veins (50).

However, more recently, it has been reported that when isoproterenol is infused into a peripheral vein, a slowly developing decrease in hepatic blood volume occurs (107, 114). This apparent venoconstrictor effect would appear to be important. Although cardiac stimulation and arteriolar vasodilation increase cardiac output, this increase is limited by the resulting decrease in cardiac preload. Redistribution of blood volume from the splanchnic reservoir by venoconstriction would minimise this reduction in preload and allow a larger increase in cardiac output (109, 111). Redistribution of splanchnic blood volume by isoproterenol has been reported previously in dogs and attributed to either hepatic venoconstriction (198) or relaxation of hepatic outflow sphincters (106, 188, 301) which are present in dogs but not in cats (125).

In this study the hepatic venoconstrictor response to isoproterenol given intravenously is confirmed and possible mechanisms are studied in more detail.

It was anticipated that the results from these experiments would be useful in the elucidation of cardiovascular homeostatic mechanisms and for determining the effects of endotoxin on these mechanisms. This section of the results deals with the effects of isoproterenol in normal cats, while the next section is concerned with these effects

during endotoxin shock. Hepatic blood volume, intrahepatic pressures and blood flow are examined in anesthetized cats. The blood flow experiments were performed by co-workers in this laboratory. The results of these blood flow experiments have been previously published (314) and are included here because of their importance to the conclusions of this study. Possible indirect mediators of the isoproterenol induced response are eliminated by hepatic denervation, adrenalectomy, nephrectomy and indomethacin infusion.

METHODS

Cats (2.2-3.3 kg body weight, mean 2.8 kg) were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and supplementary doses (3 mg/kg) were given intravenously as required throughout the experiments. Arterial pressure and portal pressure were recorded as described in the Methods section. Right atrial pressure was obtained through a polyethylene cannula (PE 60, inside diameter 0.03", outside diameter 0.048") inserted through a jugular vein. Artificial respiration was maintained using a Harvard respirator and expired carbon dioxide levels were monitored by a Beckman Gas Analyser LB-2 and maintained between 4 and 5%. Rectal temperature was monitored and maintained at the temperature measured immediately after the animal was anesthetized. Further surgical preparation of the animals varied in the different series of experiments as described below. In all series, a control period of 20 minutes was allowed to elapse after completion of the surgery. Isoproterenol hydrochloride was prepared in Ringer-Locke solution containing ascorbic acid (20 mg/100 ml) and given by intravenous infusion into all cats in each of the following 5 series of experiments. Cats received the infusions into a peripheral cutaneous vein in doses of 0.2, 0.5 and 1.0 ug/kg/min. Each dose level was maintained for 5-10 min until the responses were stable. After the

isoproterenol infusions were completed a 20 minute recovery period was allowed before any further experimental procedures were carried out.

Series 1: Hepatic blood volume responses.

The plethysmographic method for recording changes in hepatic blood volume as described in the General Methods section was used for this series of cats. Isoproterenol was administered as described above into 6 cats.

Series 2: Hepatic blood flow responses.

Total hepatic blood flow was recorded using the long-circuit technique previously described (108, 115). The inferior vena cava was ligated below the liver and flow from regions below this point was drained to an extracorporeal reservoir through cannulae in both femoral veins. The inferior vena cava was ligated above the liver through an incision in the fourth intercostal space, and cannulated to drain total hepatic blood flow through an extracorporeal probe of an electromagnetic flowmeter (Nycotron, Oslo) to the extracorporeal reservoir. Outflow pressure was set at zero relative to the right atrium. Blood was pumped from the reservoir back to the animal through cannulae in both jugular veins. The reservoir and tubes were primed with donor blood from another cat and the volume of blood in the extracorporeal reservoir was maintained constant by

adjustment of the pump. The flow probe was calibrated periodically by timing the collection of 10 ml of hepatic flow and the zero was checked by diverting flow through a bypass round the flowmeter without interference with hepatic flow or pressure.

Series 3: Intrahepatic lobar venous pressures.

Pressure in a lobar hepatic vein 3-6 cm within the liver was recorded as previously described (108). A catheter (PE90, I.D. 0.86 mm, O.D. 1.27 mm) was inserted through the right jugular vein and passed through the right atrium into the thoracic inferior vena cava. It was then directed by gentle pressure into a lobar hepatic vein and inserted 3-6 cm from the junction of the hepatic veins with the inferior vena cava. This catheter had a sealed tip and pressure was recorded through side-holes 2-3 mm back from the tip. This ensured that a wedged pressure was not recorded. Characteristic cardiac venous pressure oscillations were present in undamped recordings from this catheter. The position of the catheter tip in the lobar vein was verified by dissection at the end of each experiment.

Series 4: Hepatic denervation.

The responses to isoproterenol infusions in cats after section of the hepatic nerves have been determined and published by others (107). The protocol of those experiments

was identical to the protocol of these experiments and the data from that study will be included here for purposes of discussion.

Series 5: Hepatic denervation, adrenalectomy and nephrectomy.

These cats were prepared as in Series 1 but the hepatic nerves were separated from the hepatic artery, ligated and cut, the adrenal glands were excluded from the circulation by tight ligatures and both kidneys were removed after ligating the renal pedicels. Isoproterenol was then infused and responses were measured. Later, after the cats recovered from the isoproterenol infusions, indomethacin (1 mg/kg) was administered intravenously and isoproterenol infusions were repeated again.

RESULTS

A record from an individual experiment is shown in Fig. 27 to illustrate the protocol and general type of response that was seen in Series 1. When the intravenous infusion of isoproterenol was begun, arterial pressure declined and portal pressure increased. Hepatic blood volume began to increase but within 1-2 min this increase was reversed to a decrease and blood volume stabilized at a level below the pre-infusion control level. The decrease in hepatic blood volume thus began 1-2 min after starting the infusion and required about 3 min to stabilize. Infusions at the higher doses caused further small decreases in arterial pressure, no further rise in portal pressure, and a further decrease in hepatic blood volume. On cessation of the isoproterenol infusion, arterial pressure and portal pressure returned rapidly to control levels. However hepatic blood volume initially decreased further. It began to recover towards the control level after 1-2 min and reached the pre-infusion control level after 5-10 min.

Responses to infusion of isoproterenol into a peripheral cutaneous vein (Series 1) were determined in 6 cats. All data were analysed by blocked analysis of variance with multiple comparisons by Duncan's multiple range test (328). Control values for arterial pressures, portal pressures and hepatic blood volumes were within the ranges previously reported (111). Infusions of isoproterenol caused

a dose-related fall in arterial pressure (Fig. 29) , a rise in portal pressure (Fig. 30) which occurred with the smallest doses and did not increase further with larger doses, and a dose-related fall in hepatic blood volume (Fig. 28) measured at equilibrium 4-5 min after starting each dose level. Right atrial pressures (control 2.4 $\pm$ 0.2 mm Hg) showed no significant change during isoproterenol infusions. Control infusions of vehicle caused no significant changes in any measured parameter. In 3 cats, after administration of propranolol (1 mg/kg) intravenously, isoproterenol infusions in the same doses caused no significant effects on the variables measured.

The effects of isoproterenol infusions on total hepatic flow were determined in the long-circuit experiments (Series 2) in 8 cats. Arterial pressures were similar to those in Series 1 (Fig. 31). Control hepatic flow was 27 ml/min/kg body weight and mean liver weight (after passive drainage of blood) was 22 g/kg body weight in these cats. During isoproterenol infusions, portal pressure and total hepatic blood flow increased with the lowest dose and further increases with larger doses were small (Fig. 32). Hepatic venous resistance, calculated as hepatic lobar venous pressure (outflow pressure was zero) divided by total hepatic blood flow showed no statistically significant change.

Although portal pressure can be used as an index of intrahepatic pressure (108, 111), measurements of lobar

hepatic venous pressure were carried out in 6 cats to determine the relationships between portal pressure and intrahepatic lobar venous pressure during isoproterenol infusions in Series 3. The results are shown in Fig. 33. The control portal pressure was slightly but not significantly higher than the control hepatic venous pressure confirming previous obervations. This should not be taken to indicate that no gradient for flow was present (see discussion). During isoproterenol infusions both pressures increased, but the increases in portal pressures were significantly larger than the increases in hepatic venous pressures. However no decrease in hepatic venous pressure during isoproterenol infusions in any animal was observed; this pressure always increased. The decreases in hepatic blood volume during isoproterenol infusions cannot therefore be explained as passive emptying of the liver secondary to decreases in intrahepatic pressure.

The data in Series 4 were taken from experiments where it was determined whether or not the hepatic blood volume responses to isoproterenol were blocked by hepatic denervation. These data for 7 cats were published previously (107) but are shown here in greater detail in Fig. 34. The responses to isoproterenol were not blocked after denervation and were in fact very similar to those seen in cats with innervated livers (Fig. 28 and Fig. 30).

In Series 5 an examination was made as to whether or not the hepatic blood volume responses to isoproterenol were blocked by hepatic denervation, adrenalectomy and nephrectomy combined. The responses in a representative cat are shown in Fig. 35. Compared to the animals in Series 1, these cats showed lower arterial pressures, lower portal pressures and greater hepatic blood volumes. The hypotensive response to isoproterenol was enhanced in this series of cats (Fig. 36). The increased portal pressures (Fig. 37)and decreased hepatic blood volumes (Fig. 38) were still present in response to isoproterenol after these surgical procedures. These cats were then given indomethacin (1 mg/kg) and the responses to isoproterenol were again assessed. No significant alterations in the portal pressure and hepatic blood volume responses could be seen (Fig. 39 and Fig. 40).

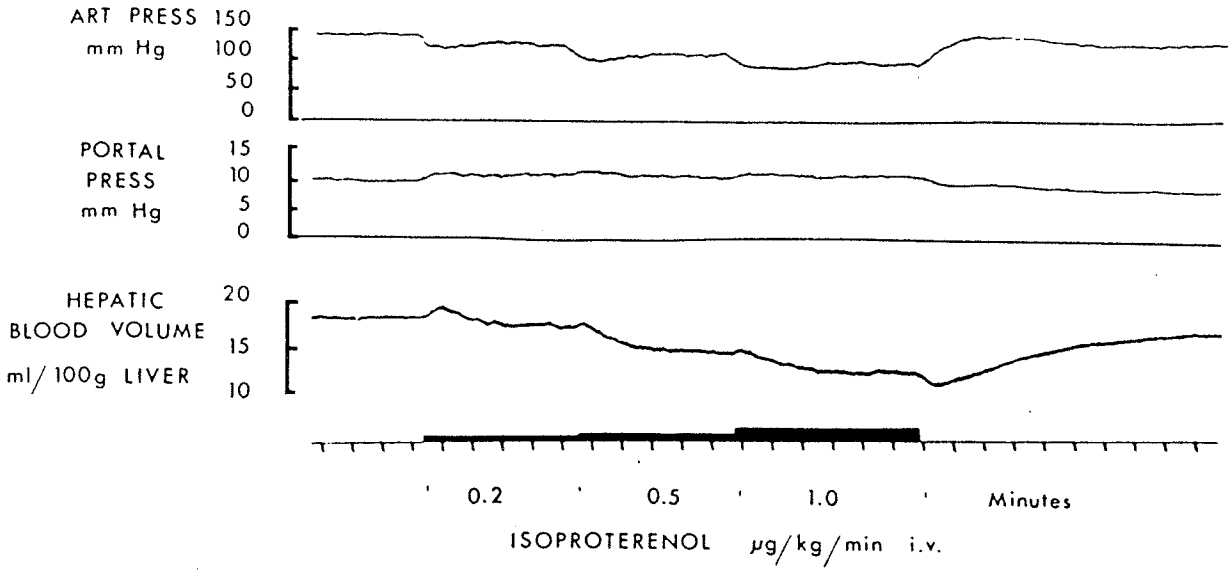

FIG. 27. Responses to isoproterenol infusion in a representative cat (2.4 kg) from Series 1 to illustrate the protocol and recordings obtained.

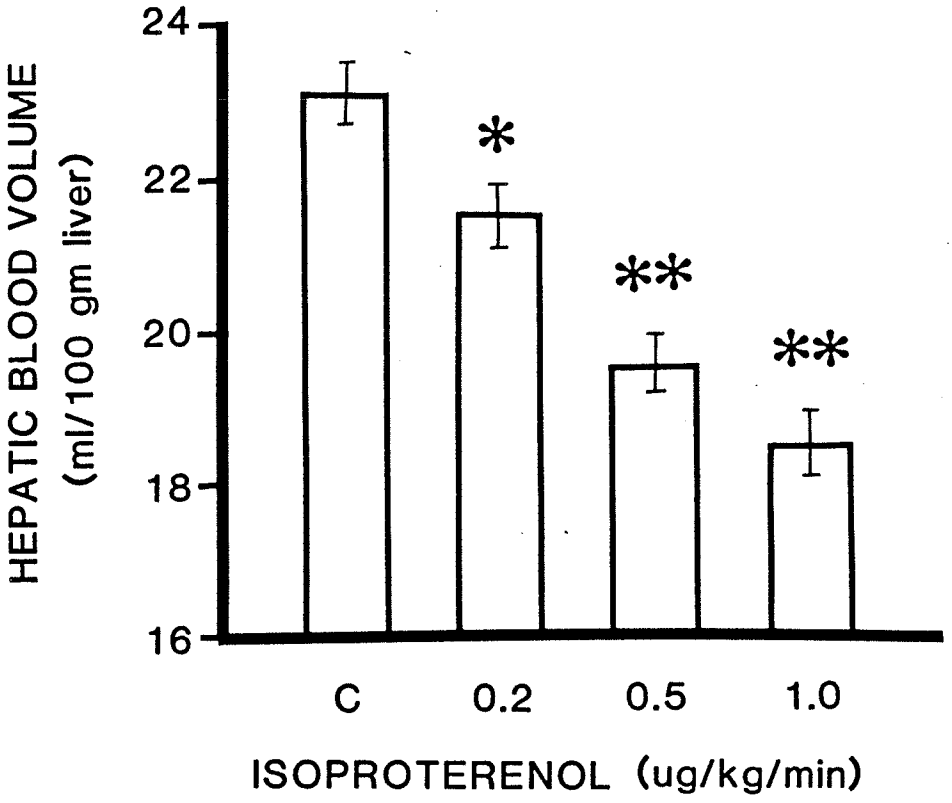

FIG. 28. Values of hepatic blood volume during control period (C) and during cumulative intravenous infusions of isoproterenol. Means ± S.E.; n=6; * = P<0.05, ** = P<0.01 compared with control.

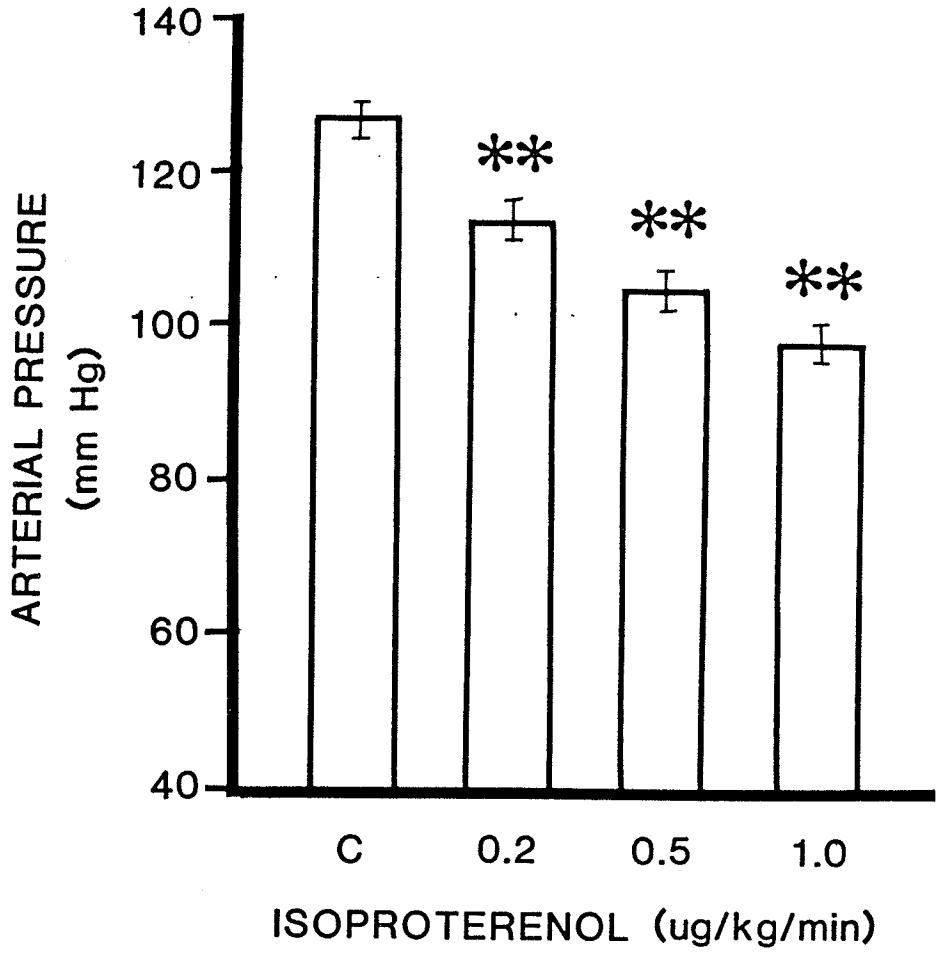

FIG. 29. Values of arterial pressure during control period (C) and during cumulative intravenous infusions of isoproterenol. Means ± S.E.; n=6; ** = P<0.01 compared with control.

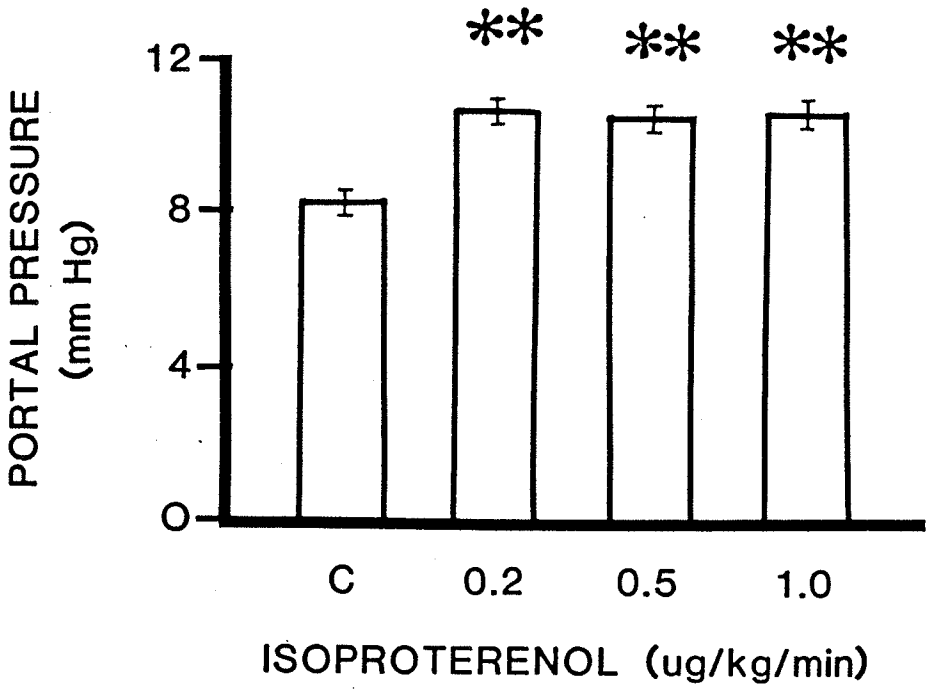

FIG. 30. Values of portal pressure during control period (C) and during cumulative intravenous infusions of isoproterenol. Means ± S.E.; n=6; ** = P<0.01 compared with control.

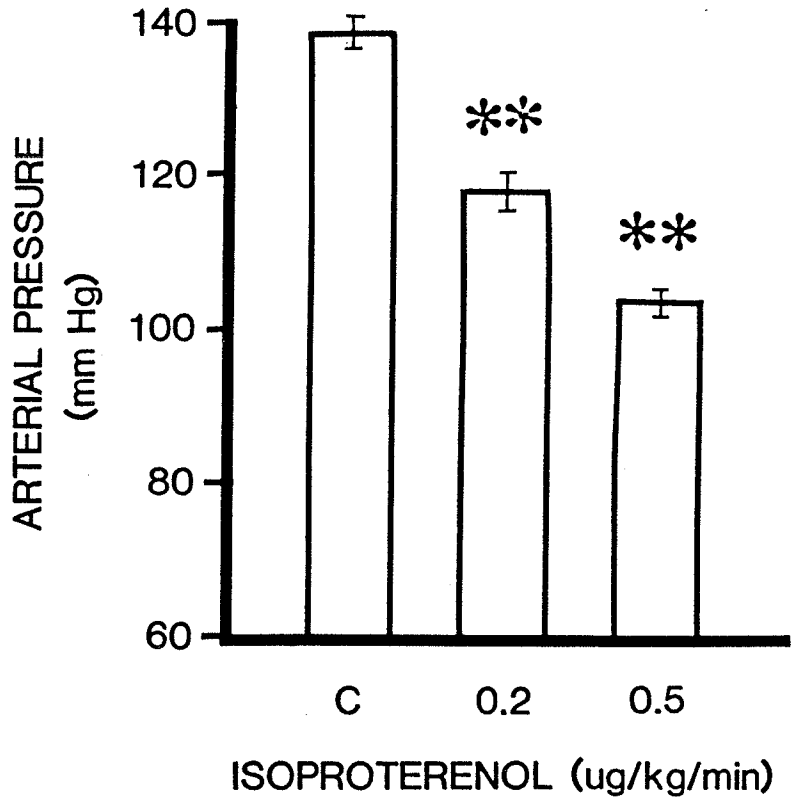

FIG. 31. Hepatic venous long-circuit experiments. Values of arterial pressure during the control period (C) and during cumulative infusions of isoproterenol. Means ± S.E.; n=7; ** = P<0.01 compared with control.

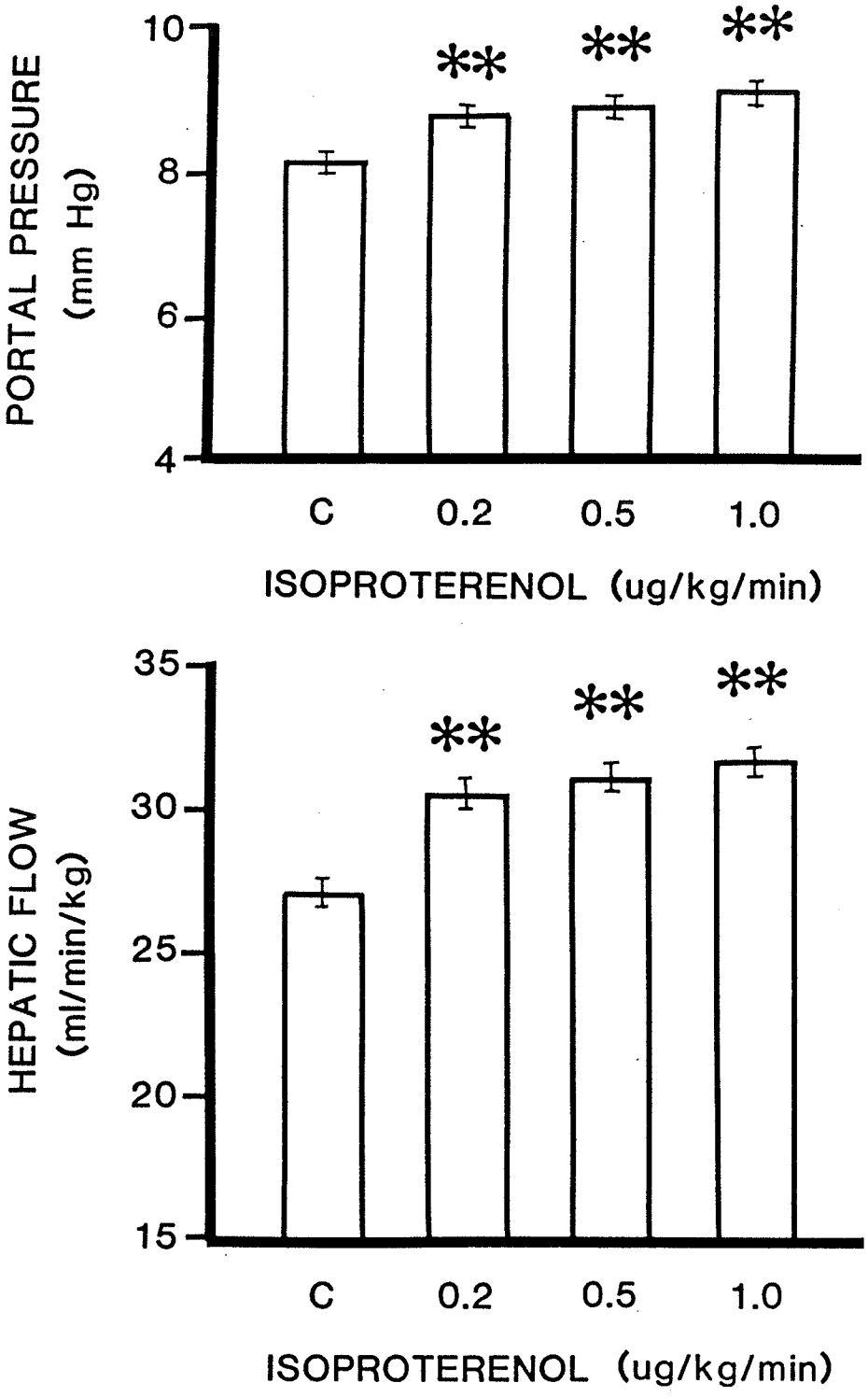

FIG. 32. Hepatic venous long circuit experiments. Values of portal pressure and hepatic flow during the control period (C) and during cumulative infusions of isoproterenol. Means ± S.E.; n=8; ** = $P<0.01$ compared with control.

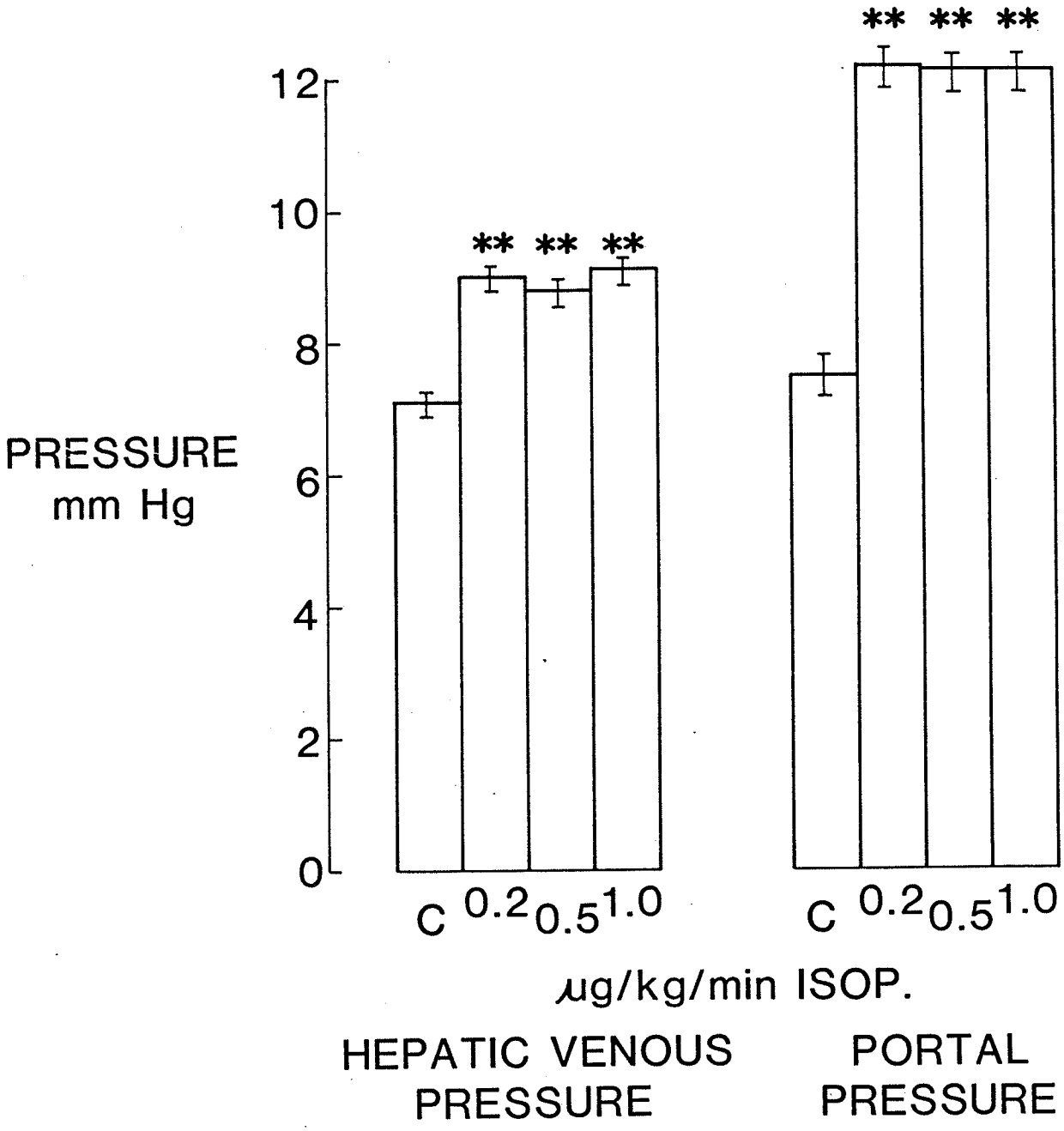

FIG. 33. Hepatic venous pressures recorded 3-6 cm within the liver, and portal pressures recorded simultaneously during the control period (C) and during cumlative intravenous infusions of isoproterenol. Means $\pm$ S.E.; n=6; ** = $P<0.01$ compared with control.

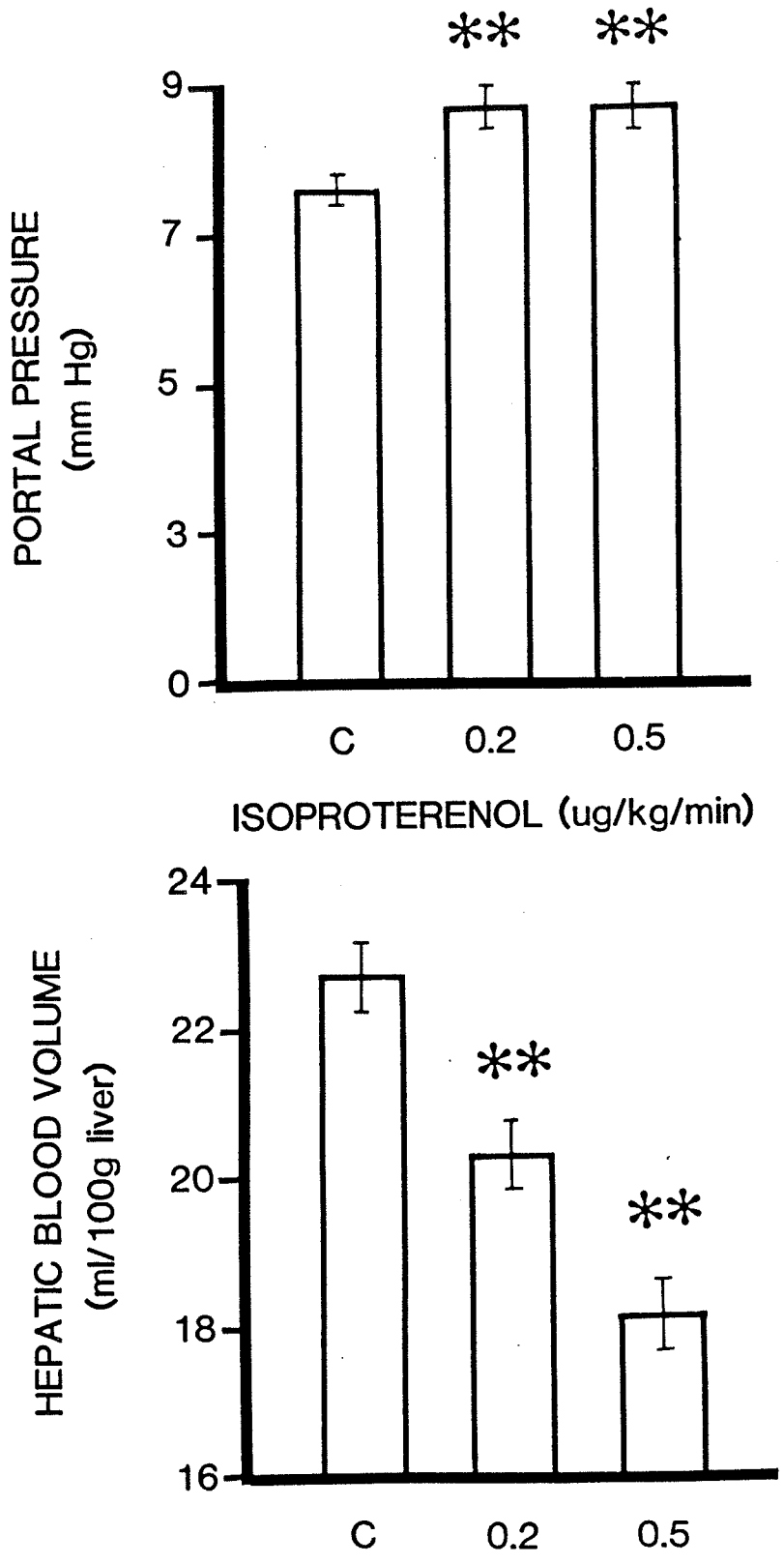

FIG. 34. Cats with hepatic denervation. Values of portal pressure and blood volume during the control period (C) and during cumulative infusions of isoproterenol. Means $\pm$ S.E.; n=7; ** = $P<0.01$ compared with control.

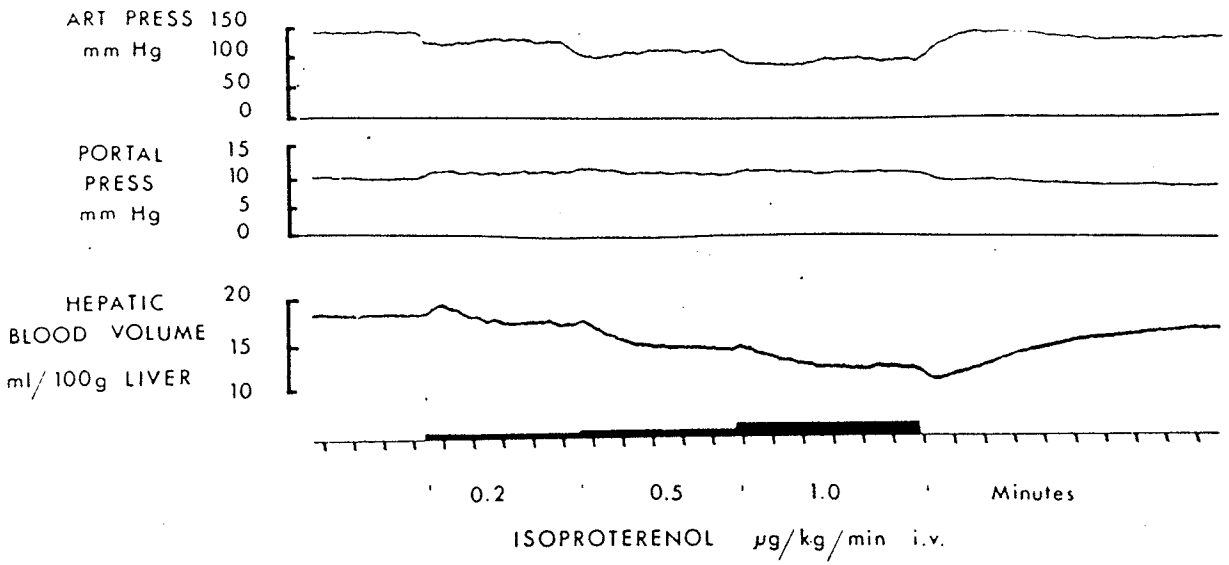

FIG. 35. Responses to isoproterenol infusions in a representative cat (2.8 kg) from Series 5 (denervated, adrenalectomized and nephrectomized).

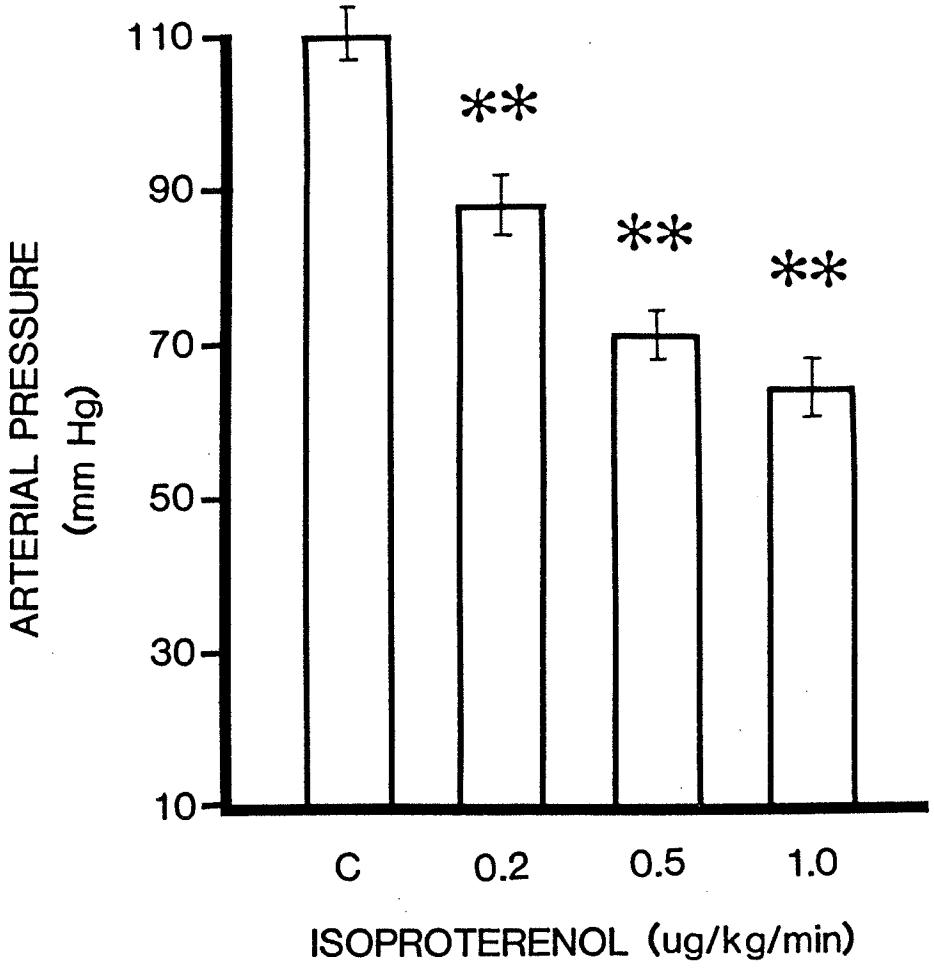

FIG. 36. Cats with hepatic denervation, adrenalectomy and nephrectomy. Values of arterial pressure during control period (C) and during cumulative infusions of isoproterenol. Means $\pm$ S.E.; n=7; ** = $P<0.01$ compared to control.

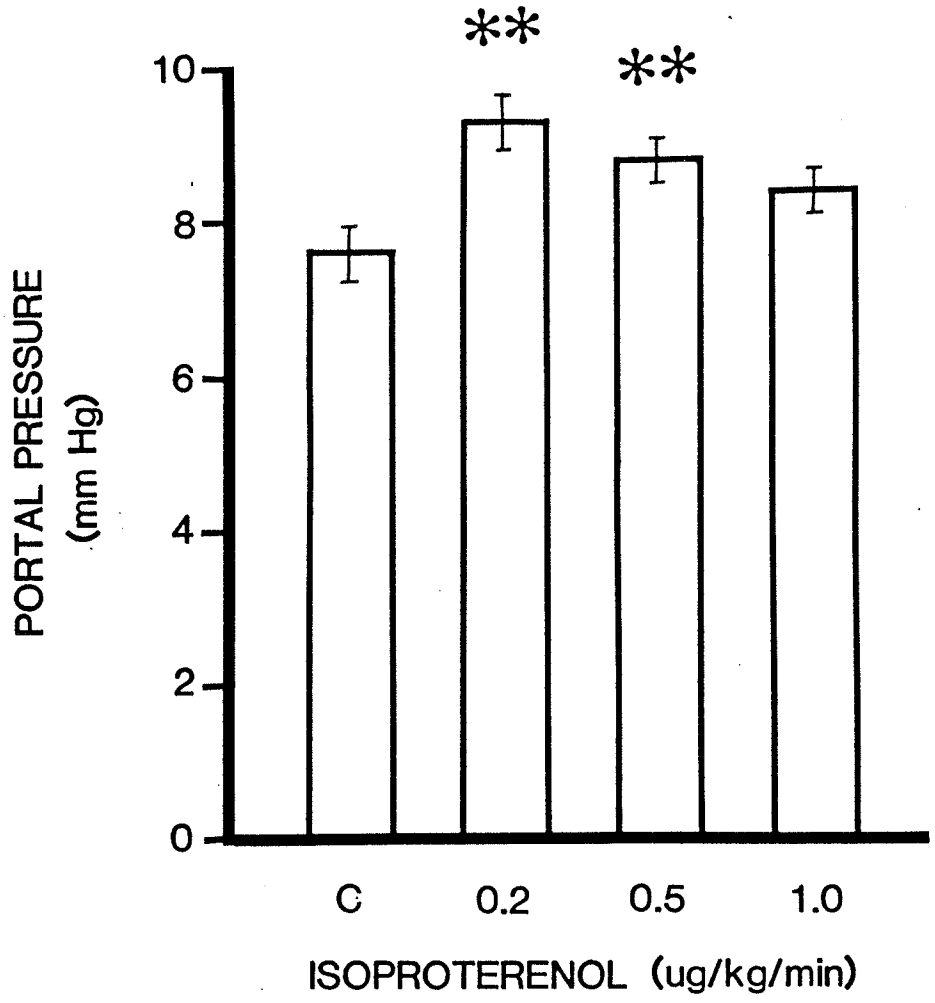

FIG. 37. Cats with hepatic denervation, adrenalectomy and nephrectomy. Values of portal pressure during control period (C) and during cumulative infusions of isoproterenol. Means $\pm$ S.E.; n=7; ** = $P<0.01$ compared to control.

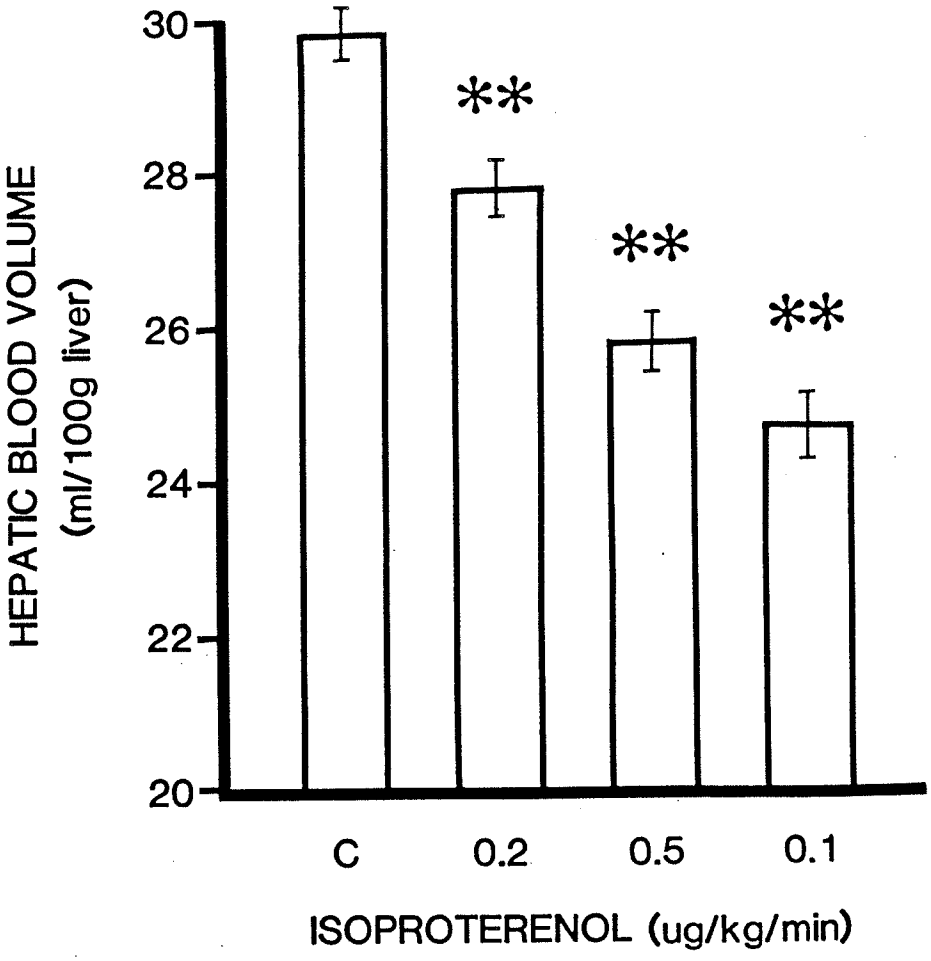

FIG. 38. Cats with hepatic denervation, adrenalectomy and nephrectomy. Values of hepatic blood volume during control period (C) and during cumulative infusions of isoproterenol. Means $\pm$ S.E.; n=7; ** = $P<0.01$ compared to control.

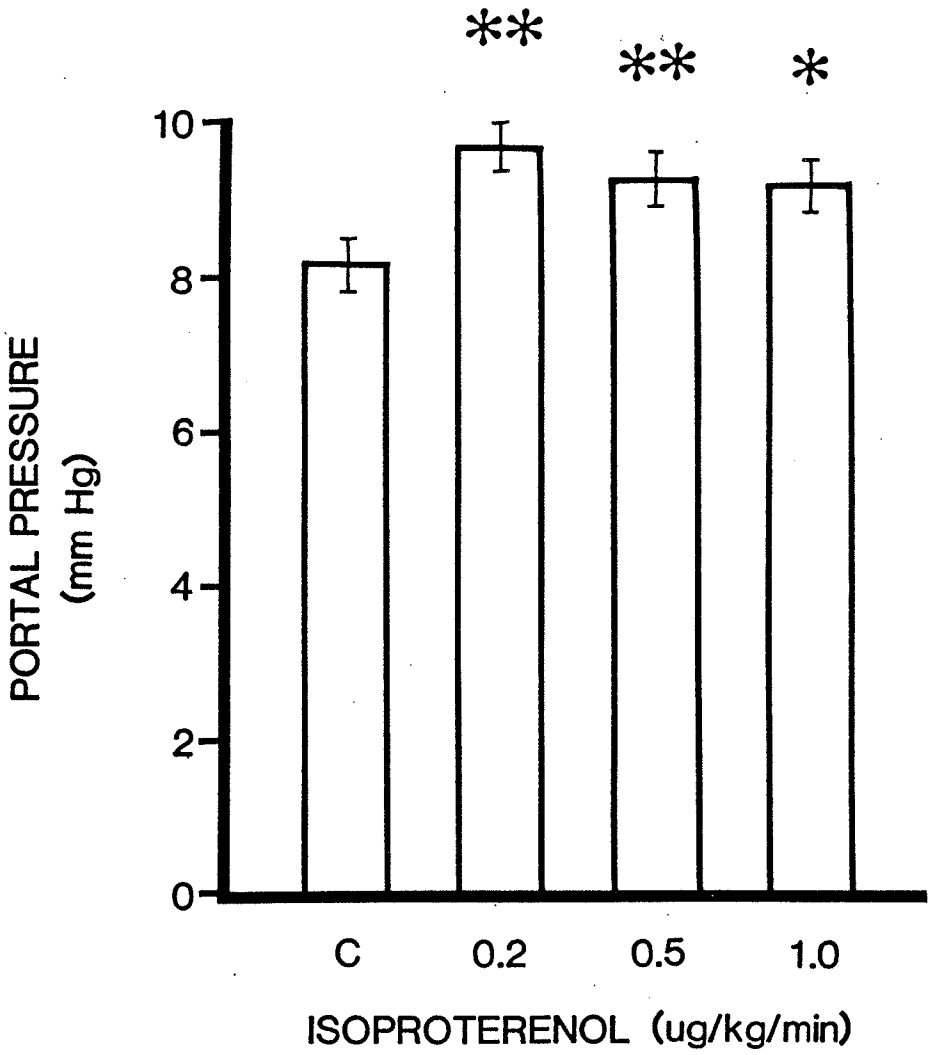

FIG. 39. Cats with hepatic denervation, adrenalectomy and nephrectomy and pretreated with indomethacin. Values of portal pressure during control period (C) and during cumulative infusions of isoproterenol. Means $\pm$ S.E.; n=7; * = $P<0.05$, ** = $P<0.01$ compared to control.

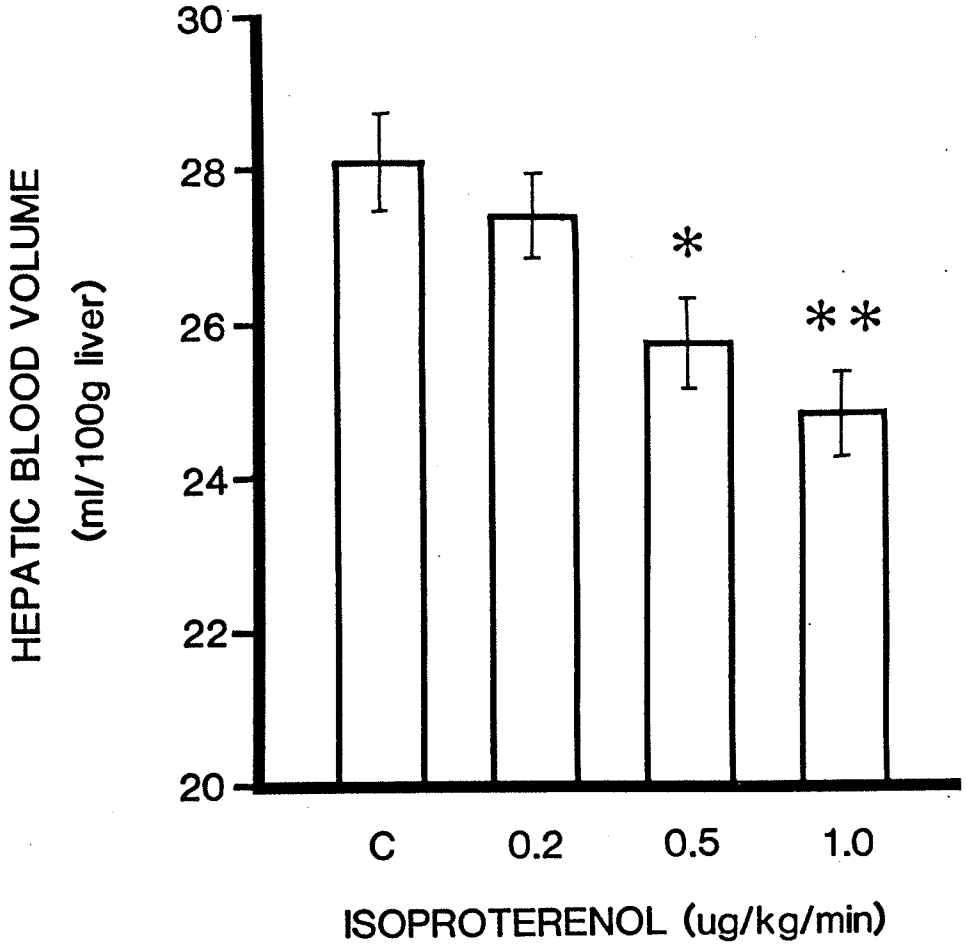

FIG. 40. Cats with hepatic denervation, adrenalectomy and nephrectomy and pretreated with indomethacin. Values of hepatic blood volume during control period (C) and during cumulative infusions of isoproterenol. Means ± S.E.; n=7; * = $P<0.05$, ** = $P<0.01$ compared to control.

DISCUSSION

The first series of experiments confirm previous reports (114, 107) that isoproterenol given by infusion into a peripheral cutaneous vein reduced hepatic blood volume in anesthetized cats. Other workers have studied venous responses to isoproterenol in dogs using different techniques. In one study the effects of isoproterenol were examined in a canine cardiopulmonary bypass preparation where total systemic blood flow was held constant and the volume of the extracorporeal reservoir was used as an index of the reciprocal changes in intravascular volume. Isoproterenol caused an increase in reservoir volume and this was interpreted to indicate a venoconstrictor effect of isoproterenol. Similar results obtained in similar preparations were interpreted rather differently by others (106, 188, 301). They suggested isoproterenol relaxed hepatic venous smooth muscle causing a decreased splanchnic venous resistance. This resulted in decreased pressure with passive emptying of blood from the splanchnic circulation to the reservoir. These responses in dogs differ significantly from our experiments in cats.

The extensive surgery required to prepare the cardiopulmonary bypass preparation can, in the dog, cause hepatic outflow block. This was seen in a dramatic form in early attempts to perfuse the dog liver (reviewed by 126).

This phenomenon, perhaps mediated by intrahepatic histamine release, occurs more readily in dogs than in other species and involves contraction of the lobar hepatic veins (hepatic sphincters) resulting in raised intrahepatic pressure and resultant congestion. In some studies where isoproterenol was shown to reduce hepatic venous resistance (301), control values for portal pressures were abnormally high (21-27 cm water) and arterial pressures were low (55-60 mm Hg). Both isoproterenol and norepinephrine infusions reduced portal pressures to 13-20 cm water. Isoproterenol antagonises anaphylactic-type responses both by inhibiting release of autacoids such as histamine and by preventing the smooth muscle contraction resulting from this release (354). Thus the reduction of hepatic venous resistance by isoproterenol could be due to antagonism of outflow block in the canine liver. In the studies by Green (106), portal pressures are not reported but calculation from the data given suggests they were not abnormally elevated. Splanchnic venous resistance decreased substantially during isoproterenol and by calculation from the flows and resistance data, pressure in the splanchnic venous bed decreased from 10.1 to 8.5 mm Hg. This would result in passive emptying of the venous bed in accordance with the passive pressure-volume curves previously reported for the liver (220, 24). In addition, the increase in hepatic outflow pressure required to produce an increase in portal pressure was decreased by isoproterenol

and venous compliance was unchanged (106). The responses in these dogs can therefore be interpreted as passive emptying of the splanchnic venous bed secondary to reduced hepatic venous resistance. However quantitative comparisons between the changes in pressures and volumes observed during isoproterenol infusions and the pressure-volume curves measured by Bennett and Rothe (24) cannot be made and active contraction of the splanchnic capacitance vessels cannot at present be ruled out. Since venoconstriction can occur as a result of a change in unstressed volume without significant change in venous compliance (300), only measurements of unstressed volume before and during isoproterenol would exclude this possibility.

Hepatic venous responses in cats differ significantly from those in dogs. The canine hepatic venous sphincters, which are extremely responsive to histamine (23, 126), are not seen in cats (116). In the dog, this sphincter appears to account for the absence of a change in portal pressure until hepatic outflow (or inferior vena cava) pressure is elevated by 3-4 mm Hg (106). In cats, any increase in outflow pressure (above zero) increases portal pressure although the increase in portal pressure is quantitatively smaller (115). In this study, infusions of isoproterenol resulted in increased hepatic blood flow and increases in portal and hepatic lobar venous pressures. The intrahepatic lobar venous pressure during the control period was slightly but not significantly

lower than portal pressure confirming previous observations in cats and dogs that resistance to flow in the portal circuit through the liver is predominantly located in the lobar hepatic veins close to their entrance into the inferior vena cava (108, 111).

Calculated hepatic venous resistance did not change significantly during isoproterenol. However, the pressures throughout the intrahepatic venous bed increased. It would appear to be impossible to conceive of any realistic mechanism by which changes in any splanchnic resistances could reduce hepatic blood volume at the same time that portal, hepatic venous, and by implication intrahepatic, and transmural pressures increased. As in the dog (24), elevations in intrahepatic pressure in cats produce marked increases in hepatic blood volume (220). It is therefore concluded that the decrease in hepatic blood volume involved an active venoconstriction.

The hepatic volume response during isoproterenol infusions appears to consist of two components. On beginning and ending the infusion there is evidence of a rapid passive response to the change in intrahepatic pressure (Fig. 27 and Fig. 35) but this appears to be overshadowed by a slowly developing and slowly disappearing hepatic venoconstrictor response. The slow development of this venoconstrictor response and its absence when small doses of isoproterenol

are infused directly into the liver (116) suggest an indirect mechanism secondary to some action of isoproterenol elsewhere in the body.

Since the major mechanism controlling hepatic venous compliance appears to be the sympathetic nerves to the liver (111), and since the hypotensive effects of isoproterenol might produce reflex sympathetic activation an examination was made of previously published data on the effects of intravenous isoproterenol infusions in denervated livers (107). Results from those experiments are shown in Fig. 34. The responses were not abolished by denervation. Thus the hepatic venoconstriction during isoproterenol infusions does not appear to be a reflex consequence of the hypotensive effect. This is consistent with previous observations that in cats the carotid baroreceptor reflex does not influence hepatic blood volume (217). Here again the hepatic venous bed in cats appears to differ from that in dogs (300).

However isoproterenol is well known to cause renin release from the kidneys (202) and angiotensin II is known to cause hepatic venoconstriction (119, 330). Therefore the renal renin-angiotensin system was eliminated by nephrectomy in a series of experiments. In these experiments the adrenal glands were also excluded from the circulation and the livers were denervated. Although there was no reason to suspect the involvement of the prostaglandin system in the response, since PGE_1 does not alter hepatic blood volume in cats (126),

indomethacin was infused later in these experiments. The hepatic venoconstrictor effect of isoproterenol was not obviously modified by any of these procedures.

From these observations it can be concluded that intravenous infusions of isoproterenol cause hepatic venoconstriction which results in a decrease in hepatic blood volume in spite of an increase in intrahepatic pressure. This response is mediated indirectly by an unknown mechanism which does not involve the anterior plexus portion of the sympathetic innervation of the liver, the adrenal glands, the renal renin-angiotensin system or endogenous prostaglandins.

C. EFFECTS OF ISOPROTERENOL ON THE HEPATIC VENOUS SYSTEM DURING ENDOTOXIN SHOCK

INTRODUCTION

The effects of isoproterenol on the cardiovascular system have been extensively studied and it would seem that many of its observed effects would be beneficial in the treatment of shock. Some of the more desirable effects include: 1)increasing cardiac contractility which would counteract any myocardial depressant effects of endotoxin, 2) causing peripheral vasodilation which would tend to reverse the endotoxin induced vasoconstriction, and, 3) increasing cardiac output through a venoconstrictor effect which might restore tissue perfusion.

Previous studies with isoproterenol have shown that this drug can cause cardiovascular improvements during endotoxin shock in sheep (148) and dogs (69, 347). Administering isoproterenol to dogs after endotoxin has produced an increase in survival (326). One recent study has shown that endotoxin caused a decrease in the binding affinity and the number of beta-adrenergic receptors in dog liver plasma membranes *in vivo* (95). Thus isoproterenol may be acting by restoring an understimulated beta-adrenergic system back to normal.

Further indication that beta-receptor activation may be useful during shock comes from the interpretation of experiments using alpha-blockers which was described in the Introduction section. In that interpretation it was proposed that the beneficial effects of alpha-blockers during shock

were in fact due to a secondary beta-adrenergic effect of the alpha-blockers.

The results of experiments in the previous section have provided indications that isoproterenol may directly counteract a specific deleterious action of endotoxin. It was shown that the hepatic venous system pools blood after endotoxin and that the hepatic venous system cannot mobilize blood in response to normally effective stimuli. It was also shown that isoproterenol infusions lead to hepatic venoconstriction. The purpose of the following experiments was to determine if isoproterenol can produce its hepatic venoconstrictor effects during endotoxin shock. Such an action would help to explain any beneficial cardiovascular effects of isoproterenol during endotoxin shock.

METHODS

Cats (2.5 - 3.4 kg body weight, mean 2.9 kg) were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and supplementary doses (3 mg/kg) were given intravenously as required throughout the experiments. Arterial pressure, hepatic blood volume, portal pressure and cardiac output were recorded as described in the General Methods. Pulmonary arterial temperature was monitored and maintained at 37.5^{o} C. After all surgery was completed, 20 minutes were allowed to elapse before any experimental procedures were started.

For all cats, endotoxin (3 mg/kg) was suspended in 5 ml of 0.9% w/v sodium chloride solution and administered as a bolus intravenous injection over 30 seconds. All cats were pretreated with indomethacin (1 mg/kg body weight in phosphate buffer, pH 8.0) 20 minutes before endotoxin was administered. Isoproterenol hydrochloride was prepared in Ringer-Locke solution containing ascorbic acid (20 mg/100 ml) and given by intravenous infusion into a peripheral cutaneous vein in doses of 0.2, 0.5 and 1.0 ug/kg/min 150 min after the endotoxin was administered. Each dose was maintained for 6 minutes. Cardiac output values were determined before the isoproterenol infusion was started and during each dose level.

RESULTS

Similar to the results in normal cats, isoproterenol infusions produced a dose dependent decrease in arterial pressure (Fig. 41) and peripheral resistance (Fig. 42) and increase in cardiac output (Fig. 43) and heart rate (Fig. 44) in endotoxin treated cats. However, during endotoxin shock, compared to healthy cats, the portal pressure (Fig. 45) and blood volume (Fig. 46) responses after endotoxin were attenuated. Isoproterenol no longer could produce a significant decrease in hepatic blood volume at any dose and portal pressure did not rise except at the highest dose.

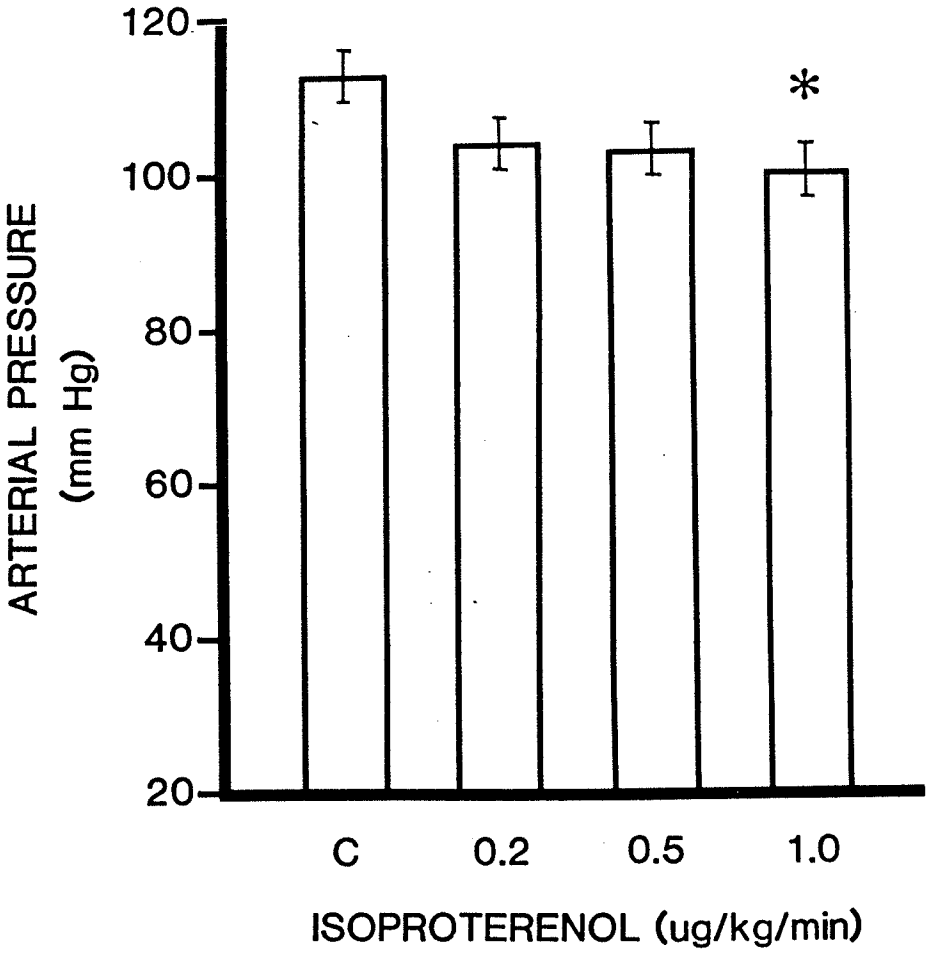

FIG. 41. Values of arterial pressure during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration. Means ± S.E.; n=4; * = P<0.05, ** = P<0.01 compared with control.

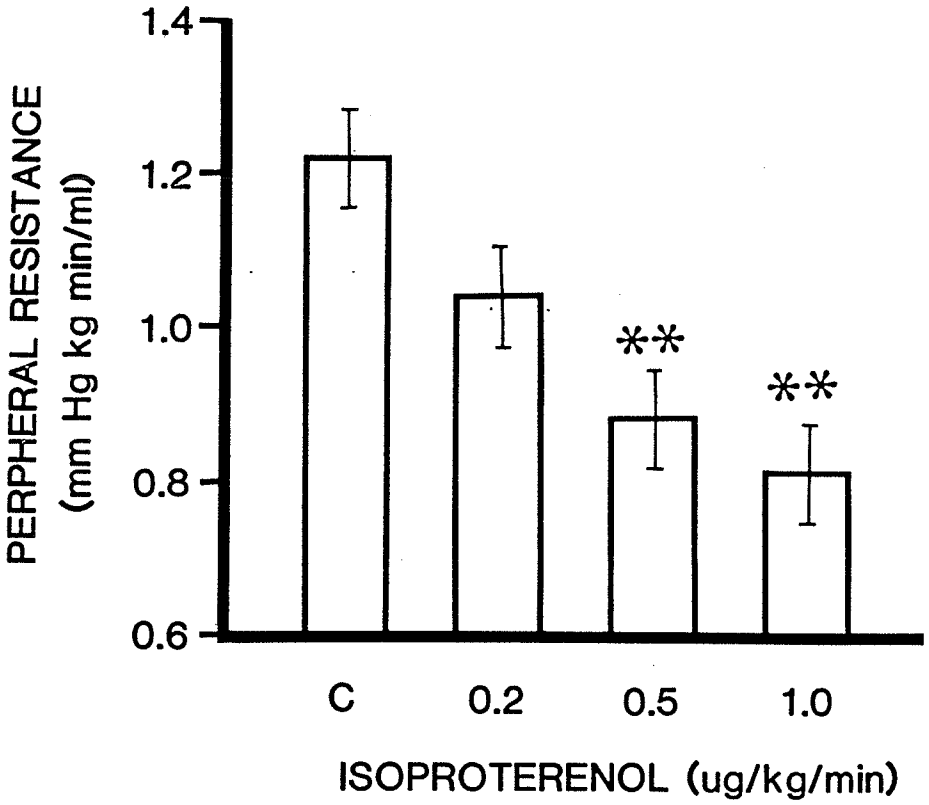

FIG. 42. Values of total peripheral resistance during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration. Means $\pm$ S.E.; n=4; * = $P<0.05$, ** = $P<0.01$ compared with control.

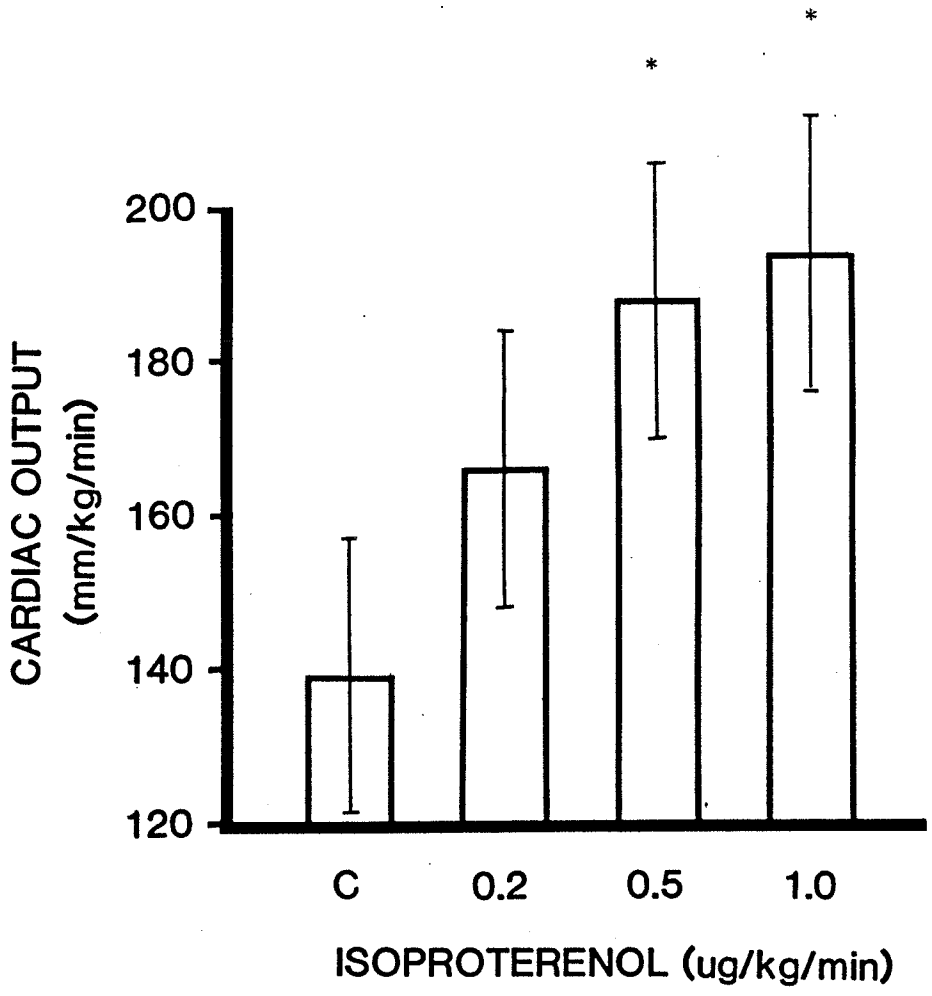

FIG. 43. Values of cardiac output during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration. Means ± S.E.; n=4; * = P<0.05, ** = P<0.01 compared with control.

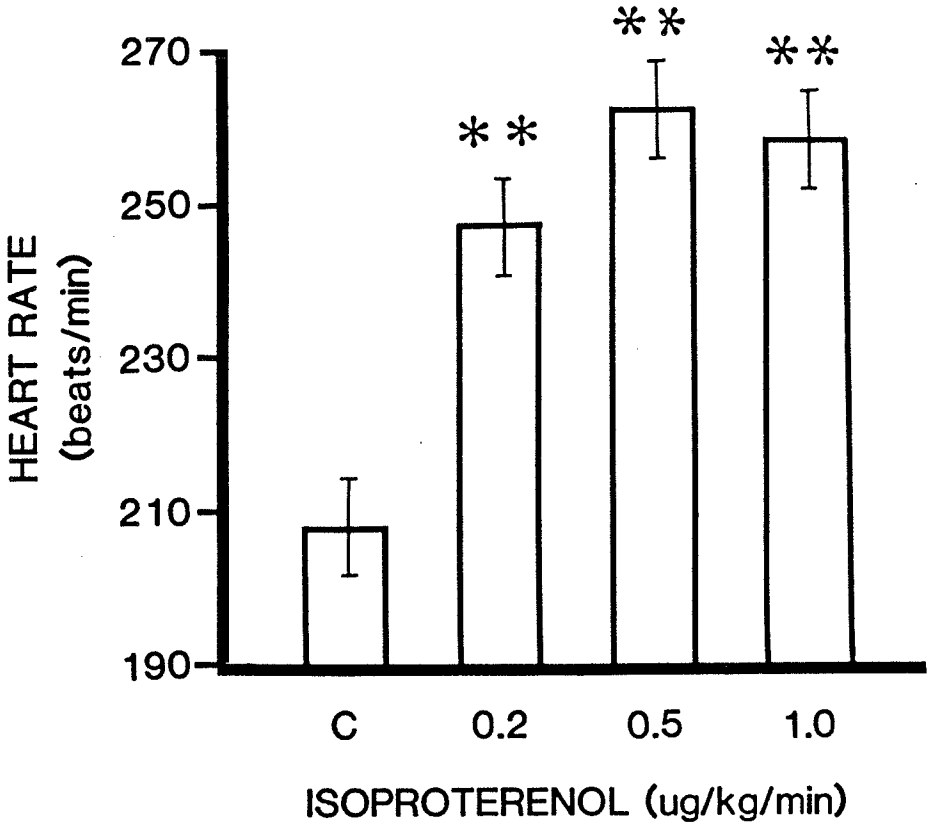

FIG. 44. Values of heart rate during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration.

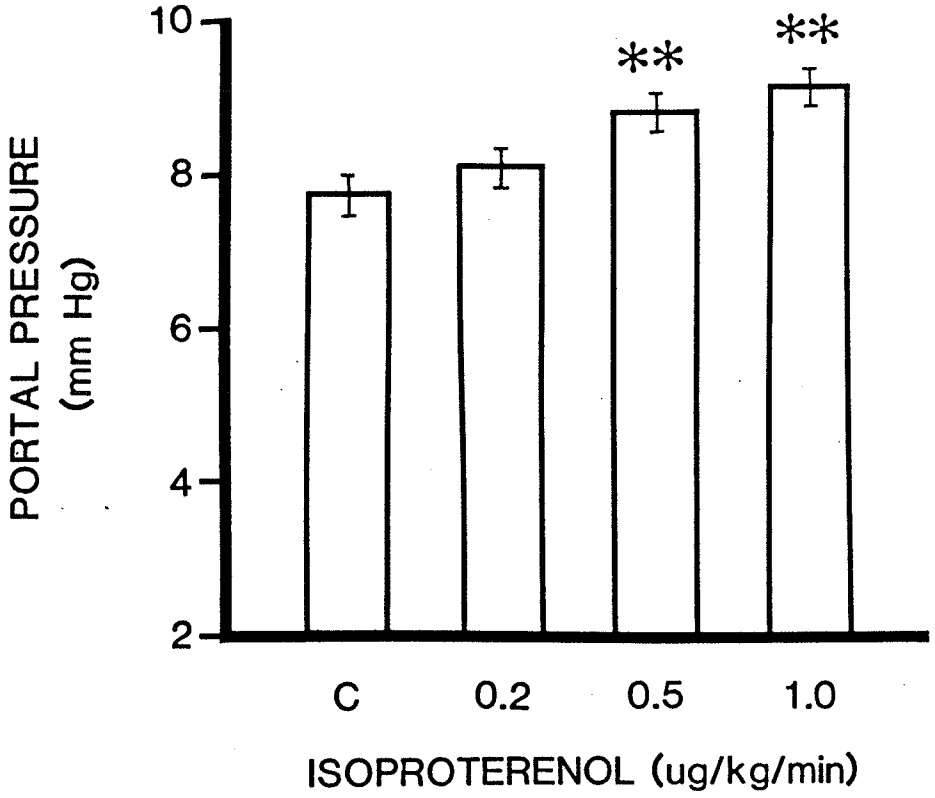

FIG. 45. Values of portal pressure during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration. Means ± S.E.; n=4; * = P<0.05, ** = P<0.01 compared with control.

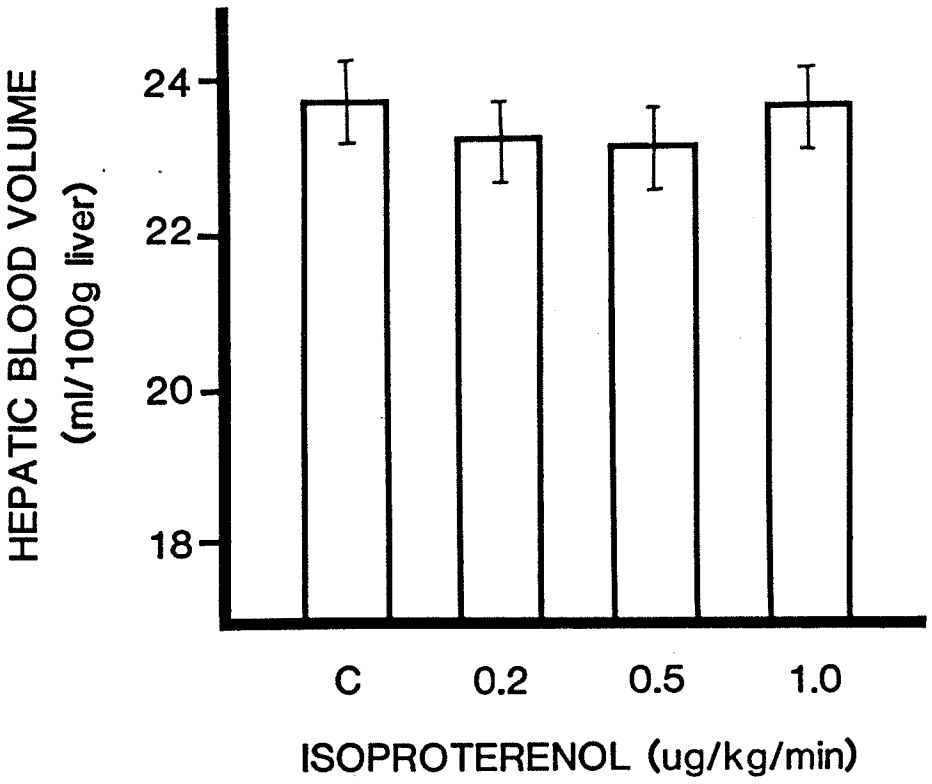

Fig. 46. Values of portal pressure during the control period (C) and during cumulative intravenous infusions of isoproterenol at 150 min after endotoxin administration. Means ± S.E.; n=4; *=P<0.05 compared with control.

DISCUSSION

Unlike the response in healthy cats, isoproterenol infusion did not produce hepatic venoconstriction during endotoxin shock. A venoconstrictor action would have counteracted two problems during endotoxin shock, namely, pooling of venous blood and low cardiac output. This lack of venoconstrictor effect after endotoxin is consistent with the finding that the hepatic venous system loses its responsiveness to agonists after endotoxin. On the other hand, the arterial system remained responsive to isoproterenol after endotoxin as the calculated total peripherial resistance fell significantly during infusions of isoproterenol. As well, the heart appeared to remain responsive to beta-receptor stimulation as judged by the increase in heart rate after isoproterenol. It is concluded that cardiac output increased due to a combination of cardiac stimulation and decreased peripheral resistance and that the hepatic venous system was not involved in this increase in cardiac output.

Isoproterenol infusions during cardiogenic shock have also been shown to improve cardiac performance and increase cardiac output, however, this occurs at the expense of myocardial oxygenation (257). In view of the fact that heart failure may occur in the later stages of endotoxin shock (see Introduction), this effect of isoproterenol may in the long term be counterproductive.

Part of the increase in cardiac output after isoproterenol appears to be due to peripheral vasodilation and this may be an undesirable event to an animal in endotoxin shock. Since blood pressure may already be unduely low in the later stages of shock, a further reduction in peripheral resistance may worsen the situation.

At present, beta-agonists are not part of any standard protocol for the treatment of endotoxin shock. The results from this study provide no further indication that beta-agonists would be useful in the treatment of shock.

D. EFFECTS OF NIFEDIPINE ON THE HEPATIC VENOUS SYSTEM IN HEALTHY CATS AND IN CATS DURING ENDOTOXIN SHOCK

INTRODUCTION

In 1964 a new class of drugs, the "calcium antagonists" were first reported in the literature (82). Specifically, the effects of verapamil and prenylamine on cardiac tissue were published. Since that time a large number of related compounds have been developed. Calcium antagonists can be classified as such on the basis of the following characteristics 1) their effects are reversed by calcium, 2) they inhibit tension development in potassium depolarized cardiac muscle, 3) they prevent calcium overload in myocardium which is treated with isoproterenol, and, 4) they decrease the calcium dependent component of the myocardial action potential (259). The molecular mechanism of action of these compounds is generally regarded as being unknown (31), although ultimately they act to interfere with the normal function of the membrane calcium channel, or affect intracellular calcium disposition (304). Evidence for other possible mechanisms of action exist. A summary of these possible mechanisms would include: 1) competative blockade of alpha-adrenoceptors, 2)inhibition of phosphodiesterase, 3) directly affecting calmodulin, and 4) stimulating Na, K-ATPase (318). Investigations into these compounds have shown that they may vary in their ability to block calcium channels in different tissues (344). Especially notable is a selectivity between cardiac and smooth muscle shown by some calcium antagonists (344).

During the years 1970-72 the calcium antagonistic properties of a new drug, nifedipine, were published (82). The pharmacokinetic characteristics of nifedipine in humans are such that 90% is protein bound in blood, 75% is excreted via the kidneys in a metabolized form, 15% is excreted in bile in a metabolized form and the plasma half-life is 4 to 5 hours after an oral dose (89, 331) and approximately 2 hours after an i.v. dose (89).

Nifedipine appears to exhibit a calcium antagonistic effect which is selective for vascular smooth muscle as opposed to cardiac muscle (154). Administration of nifedipine to humans thus leads to a decrease in total peripheral resistance (331) without producing a depressant action on the heart. Furthermore, it has been proposed that this decrease in resistance causes a reflex generalized adrenergic response which nullifies any negative inotropic, chronotropic and dromotropic effects that nifedipine would tend to produce (331). This sympathetic reflex is exemplified by the finding that plasma noradrenaline levels are acutely elevated after nifedipine administration to normotensive humans (54, 222).

The effect of nifedipine on left ventricular end-diastolic pressure has been shown to be variable; ranging from an increase, no change or a decrease (331).

Therapeutic doses of nifedipine have been reported to have either little effect on plasma renin activity (269, 322, 340) or to cause an increase in plasma renin activity (14,

54). Vasopressin is released after nifedipine administration, presumably because of the decrease in peripheral resistance (322).

Although peripheral resistance decreases acutely in normotensive humans after a 10-20 mg dose of nifedipine, blood pressure has been reported not to decrease (54, 221). Conversely, other studies have indicated that blood pressure in normotensive people does decrease acutely after nifedipine (221, 285). In any event, it is clear that blood pressure does decrease in hypertensive patients (221). It has also been found that there is an inverse correlation between pretreatment plasma renin activity and blood pressure fall upon nifedipine administration (71). Thus it appears that the net effect of nifedipine on blood pressure varies with the state of the animal prior to treatment. Nifedipine has been shown to increase cardiac output in dogs (131) and humans (132, 293) which would account for any lack of blood pressure drop in the face of decreased peripheral resistance. It is believed that the increase in cardiac output is due to the direct vasodilatory property of nifedipine combined with reflex cardiovascular compensatory mechanisms which are evoked by the nifedipine induced drop in blood pressure (131, 293).

Nifedipine was found to not inhibit noradrenaline induced contracture of hand veins (293). Isotope studies have indicated no increase in blood volume within arms and legs of

patients after oral nifedipine (255). In one study, nifedipine was shown to produce a decrease in leg but not arm venous distensibility (254). It has been concluded by several authors that nifedipine has little effect on capacitance vessels (73, 131, 132, 199, 235, 236).

In view of some of the described effects of nifedipine on the cardiovascular system, it would seem reasonable to speculate on the possible beneficial effects of nifedipine during endotoxin shock. Firstly, its vasodilatory action would tend to counteract the vasoconstriction which occurs during shock. Secondly, its action to increase cardiac output would tend to reverse the tissue underperfusion which is postulated to occur during shock. Thirdly, its apparent lack of negative inotropic and venodilator effects would be beneficial in that it would tend not to exacerbate the myocardial failure and venous pooling of blood which have been postulated to occur during endotoxin shock. Finally, its ability to decrease peripheral resistance without decreasing blood pressure is a beneficial feature of this drug in view of the fact that blood pressure is already low during endotoxin shock.

Nifedipine may also theoretically afford protection against endotoxin for other than hemodynamic reasons. As described in the Introduction, it has been postulated that endotoxin may be producing destructive effects via leukocyte

activity. Recently, it has been shown that enzyme release from polymorphonuclear leukocytes is inhibited by nifedipine (70).

The purpose of these experiments was to determine the effect of nifedipine on the cardiovascular system during endotoxin shock.

Nifedipine has been used in clinical trials for the treatment of hypertension and angina pectoris. It is generally believed that the hypotensive action is due to an effect on resistance vessels and that cardiac performance and the venous system is not directly involved in this action. At present however there is little information on the effects of nifedipine on the venous system in vivo. Theoretically, the venous system could play an important role in determining blood pressure change by affecting preload, which in turn would affect cardiac output and therefore blood pressure. A measure of the effects of nifedipine on the hepatic venous system in healthy cats was therefore made in order to further elucidate its hypotensive action. Such a measure would also be useful in determining the mechanism of the antianginal effect of nifedipine (100, 253).

METHODS

Cats (2.1 - 3.8 kg, mean 3.0 kg body weight) were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and supplementary doses (3 mg/kg) were given intravenously as required throughout the experiments. Arterial pressure, hepatic blood volume, portal pressure and cardiac output were recorded as described in the General Methods section. Pulmonary arterial temperature was monitored and maintained at 37.5° C. Further surgical preparation of the animals varied in the different series of experiments as described below. In all series, a control period of 20 minutes was allowed to elapse after completion of surgery. Nifedipine was dissolved in ethyl alcohol (10 mg/3 ml) and diluted with 7 ml distilled water. All doses of nifedipine were injected slowly over 1 min into a peripheral cutaneous vein in cumulative doses. "Cumulative doses" refers to the fact that the sose specified is the sum total of drug administered from the start of the experiment in each cat. The maximum volume of injectate was 0.2 ml/kg.

Series 1:

This series consisted of a group of 5 cats. Nifedipine was injected into a peripheral vein as described above in cumulative doses of 30, 50 100 and 200 ug/kg with 20 minutes being allowed to elapse between each dose. Blood pressure, portal vein pressure, hepatic blood volume and

cardiac output were noted at 20 minutes after the injection of each dose of nifedipine. In 3 separate cats injectate vehicle (0.05 - 0.3 ml/kg) was injected into a peripheral vein in order to ensure that the vehicle alone produced no effects.

Series 2:

This series consisted of a group of 5 cats. These cats were treated as in Series 1 except that the hepatic nerves were ligated and cut, the adrenal glands were excluded from the circulation by tight ligatures and both kidneys were removed after ligating the renal pedicles. Nifedipine was injected into a peripheral vein in cumulative doses of 15, 30, 50, 100 and 200 ug/kg.

Series 3:

A group of 4 cats were used in this series. These cats were treated identically to those in Series 1 except that the nifedipine injections were made 150 minutes after endotoxin (3 mg/kg, i.v.) was administered. The cats were also pretreated with indomethacin (1 mg/kg) 20 minutes before endotoxin administration. Cardiac output was measured after each dose of nifedipine.

RESULTS

Series 1:

A record from an individual experiment is shown in Fig. 47 to illustrate the type of response that was seen after nifedipine administration to healthy cats. Immediately after administration of a dose of nifedipine, arterial pressure declined, reaching its lowest value at approximately 2 minutes. Pressure then gradually rose to reach a steady state value after approximately 10 minutes. Portal pressure and hepatic blood volume likewise showed an immediate increase and decrease, respectively, followed by a small movement toward control values and finally reaching a steady state after about 10 minutes.

Hepatic blood volume and portal pressure responses to nifedipine were determined and the mean results are shown in Fig. 48 and Fig. 49. Injections of nifedipine caused a dose-related fall in hepatic blood volume and rise in portal pressures. At the highest doses, hepatic blood volume and portal pressure were 69% and 121% of control, respectively. Thus nifidepine administration led to a constriction of capacitance vessels.

Arterial pressure was decreased significantly after all doses of nifedipine (Fig. 50). Cardiac output tended to rise with all doses and was significantly higher at the 2 highest doses (Fig. 51). At the highest dose, cardiac output was 27% higher than the control value. Total peripheral

resistance, as calculated by dividing the value of arterial pressure by the value of cardiac output, was found to be decreased significantly at all doses of nifedipine (Fig. 52).

The injectate vehicle alone had no effect on any measured variable (Fig. 53), except heart rate at one dose, but this effect was minimal.

Series 2:

In this Series, an examination was made as to whether or not the effects of nifedipine on the measured cardiovascular variable were altered by hepatic denervation, adrenalectomy and nephrectomy. The responses of a representative cat are shown in Fig 54. Hepatic blood volume did not decrease as in Series 1 but rather tended to increase (Fig. 55). At the highest dose of nifedipine, hepatic blood volume was 17% higher than the control volume. Portal pressure rose after nifedipine but much less than in Series 1 (Fig. 56).

Similar to Series 1, arterial pressure decreased from control values at all doses of nifedipine (Fig. 57). However, unlike Series 1, peripheral resistance decreased significantly only at the highest dose. As well, the two lowest doses tended to cause peripheral resistance to increase (Fig. 58) although the increase is statistically not significant. A comparison of the effect of nifedipine on peripheral resistance showed that resistance decreased

significantly less after nephrectomy, adrenalectomy and hepatic nerve section at the lowest dose (Fig 59).

Unlike in Series 1, cardiac output did not increase after nifedipine and in fact decreased significantly with the lowest doses (Fig. 60).

Series 3:

As in healthy cats, nifedipine produced a dose dependent decrease in arterial pressure (Fig. 61) and peripheral resistance (Fig. 62) and a small increase in portal pressure (Fig. 63) after endotoxin administration. However, unlike the results in healthy cats, nifedipine did not produce a significant decrease in hepatic blood volume (Fig. 64), or a significant increase in cardiac output (Fig. 65), except at the highest dose administered. Heart rate was decreased at the highest dose of nifedipine (Fig. 66). Thus, the effects of nifedipine on hepatic blood volume and cardiac output were attenuated after endotoxin.

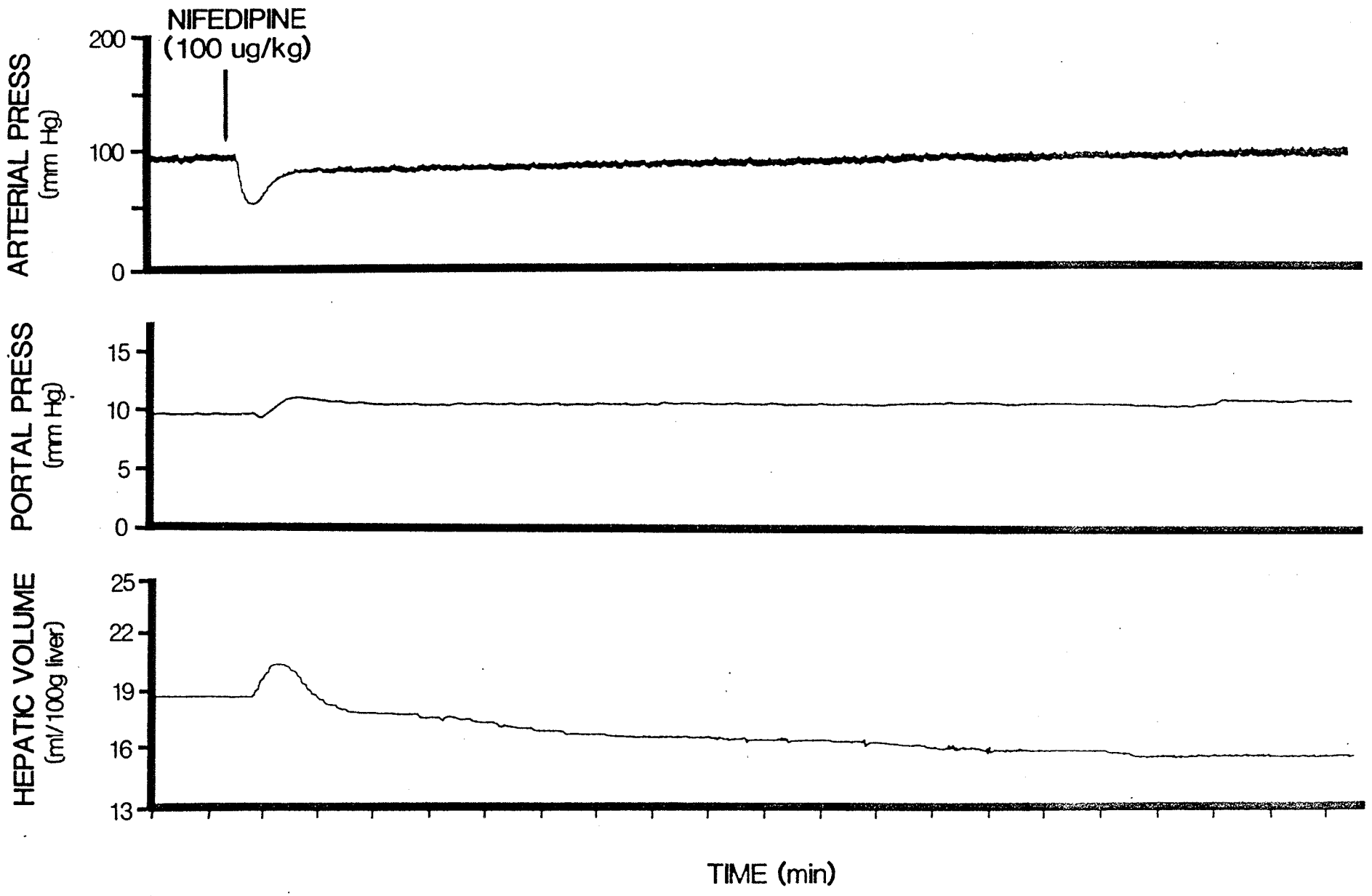

FIG. 47. Responses to intravenous nifedipine injection (100 ug/kg) in a representative cat to illustrate the protocol and recordings obtained.

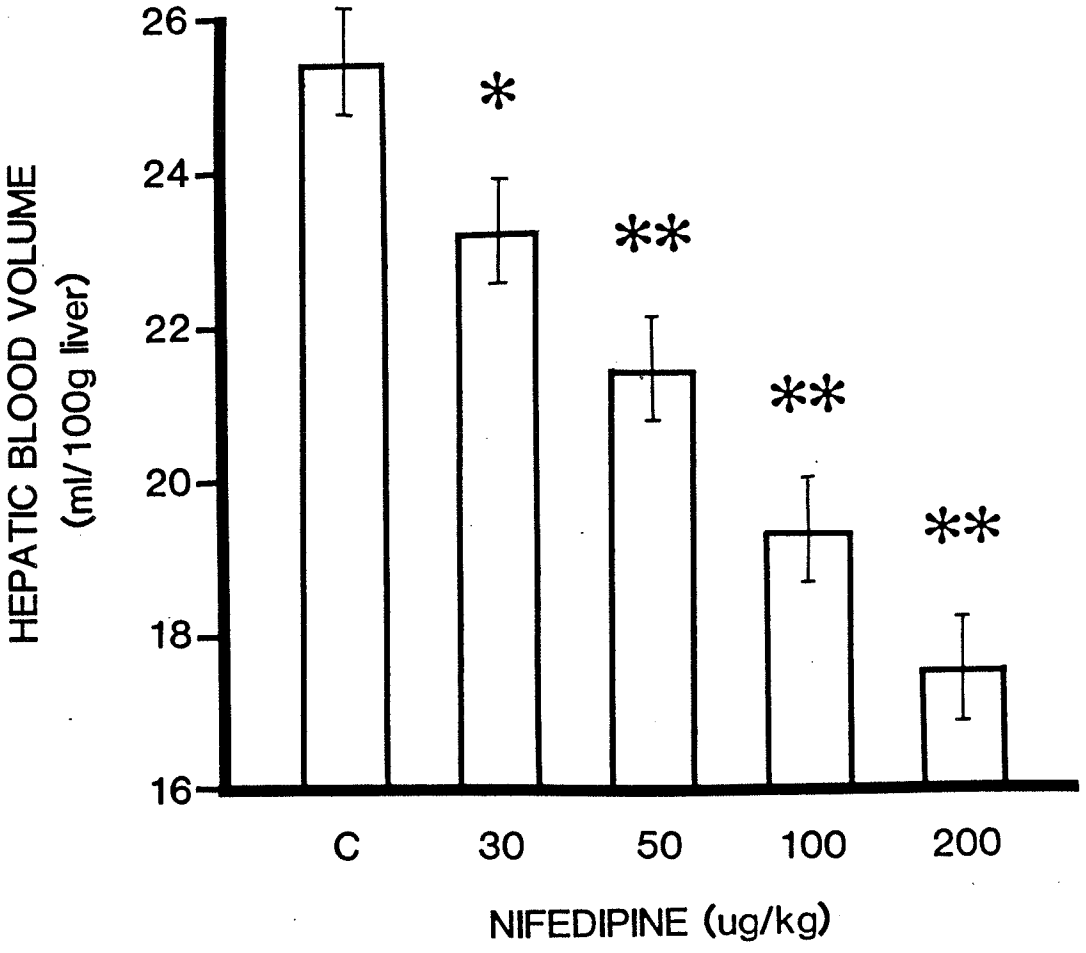

FIG. 48. Values of hepatic blood volume during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

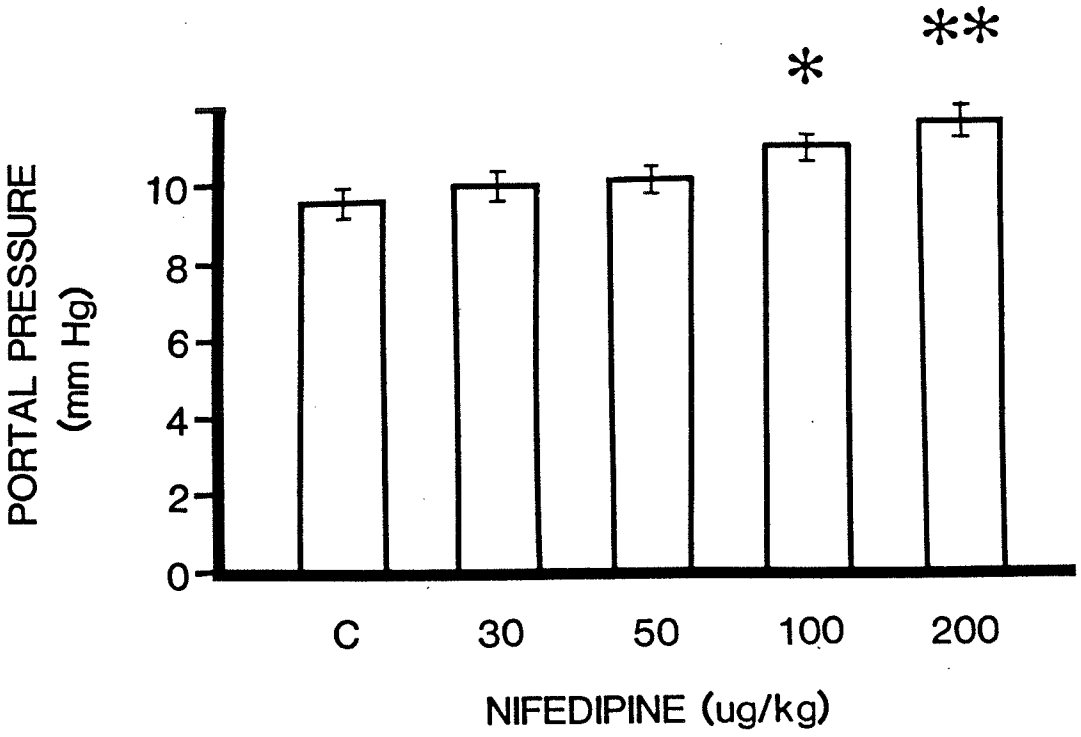

FIG. 49. Values of portal pressure during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = P<0.05, ** = P<0.01 compared with control.

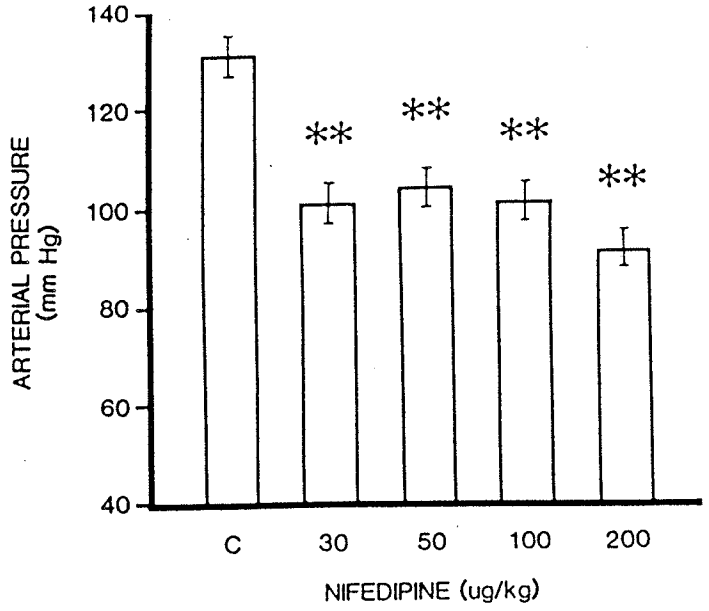

FIG. 50. Values of arterial pressure during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = P<0.05, ** = P<0.01 compared with control.

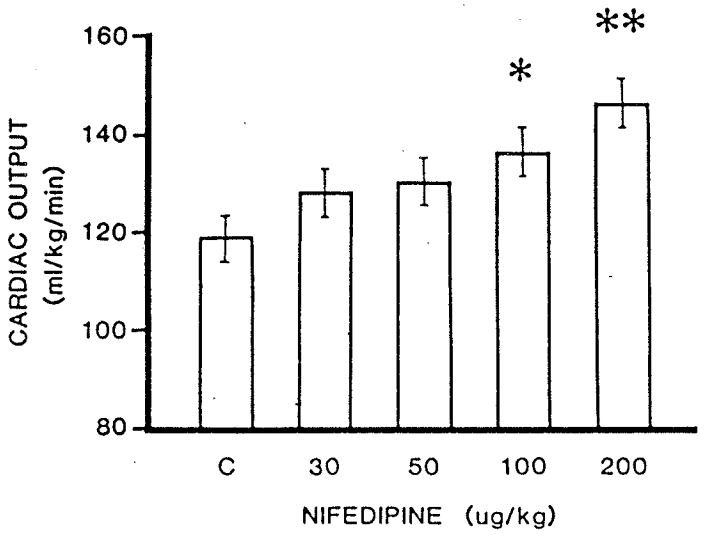

FIG. 51. Values of cardiac output during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = P<0.05, ** = P<0.01 compared with control.

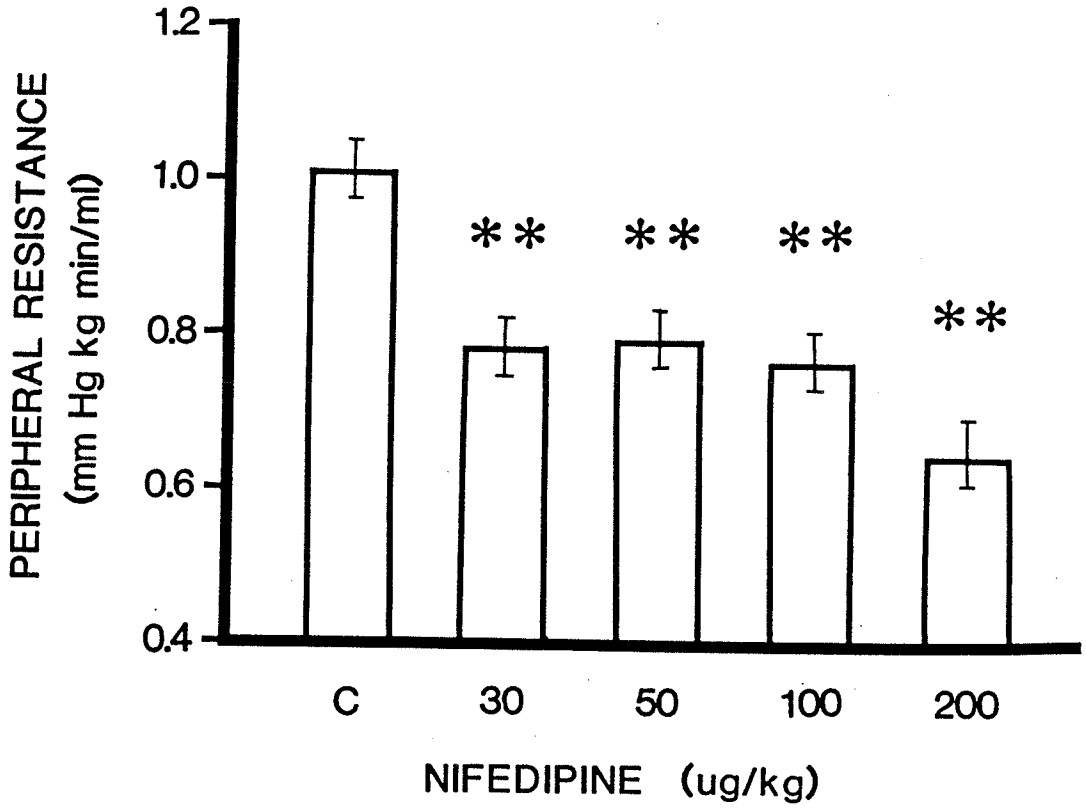

FIG. 52. Values of total peripheral resistance during the control period (C) and during cumulative doses of nifedipine. Means $\pm$ S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

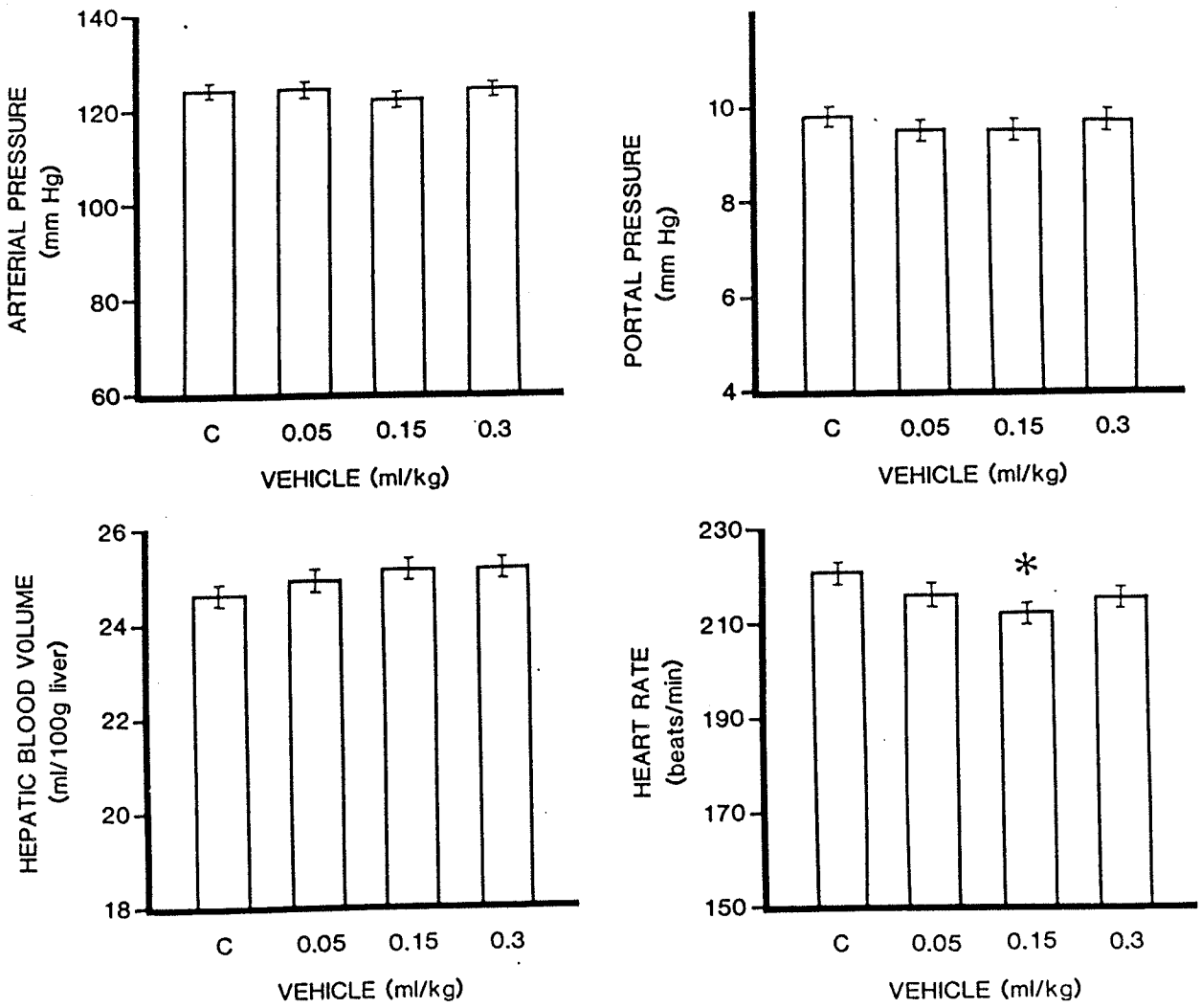

FIG. 53. Values of arterial pressure, portal pressure and hepatic blood volume during the control period (C) and heart rate during cumulative intravenous injections of vehicle for nifedipine. Means $\pm$ S.E.; n=3.

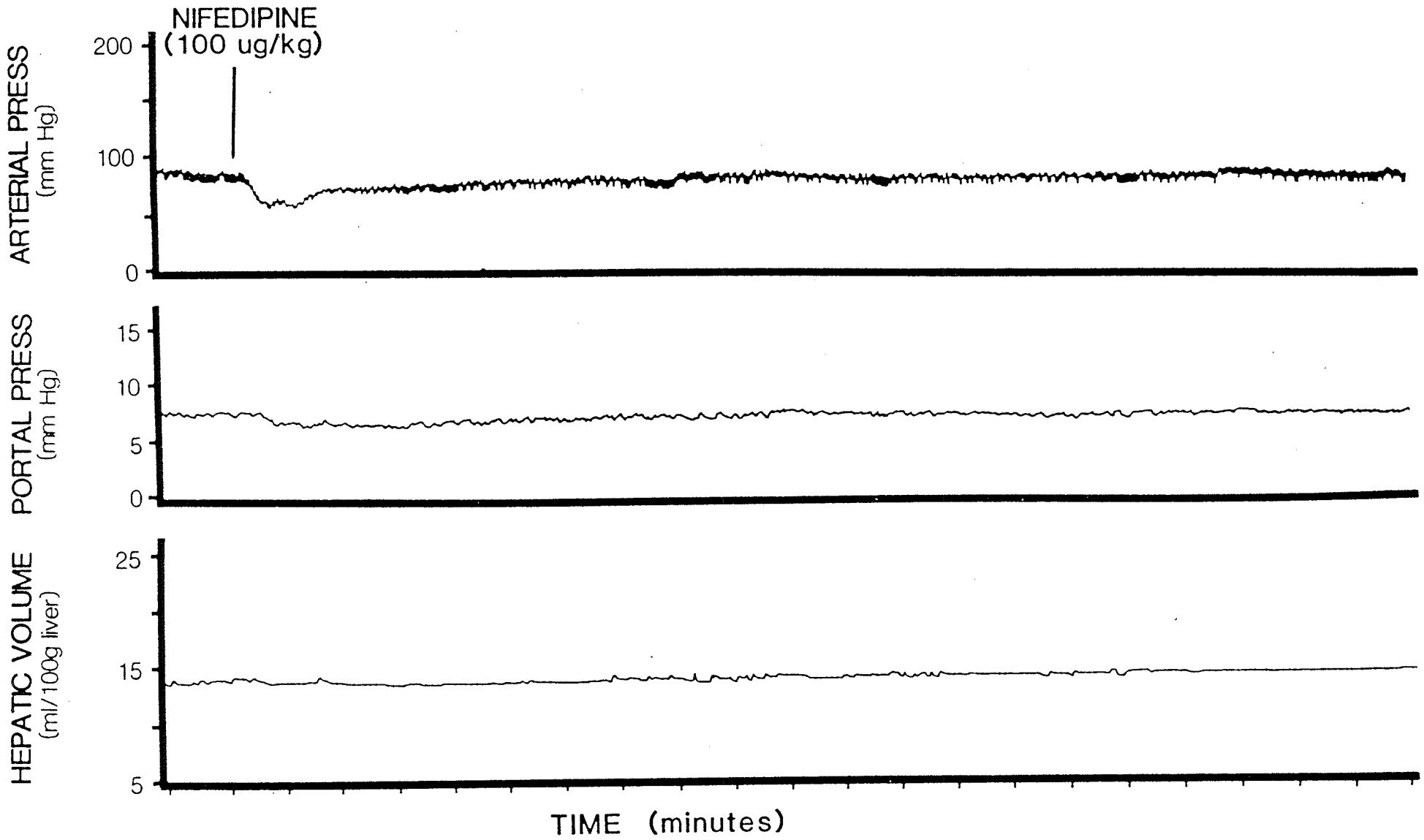

FIG. 54. Responses to intravenous nifedipine injection (100 ug/kg) in a representative cat after hepatic nerve section, adrenalectomy and nephrectomy to illustrate the protocol and recordings obtained.

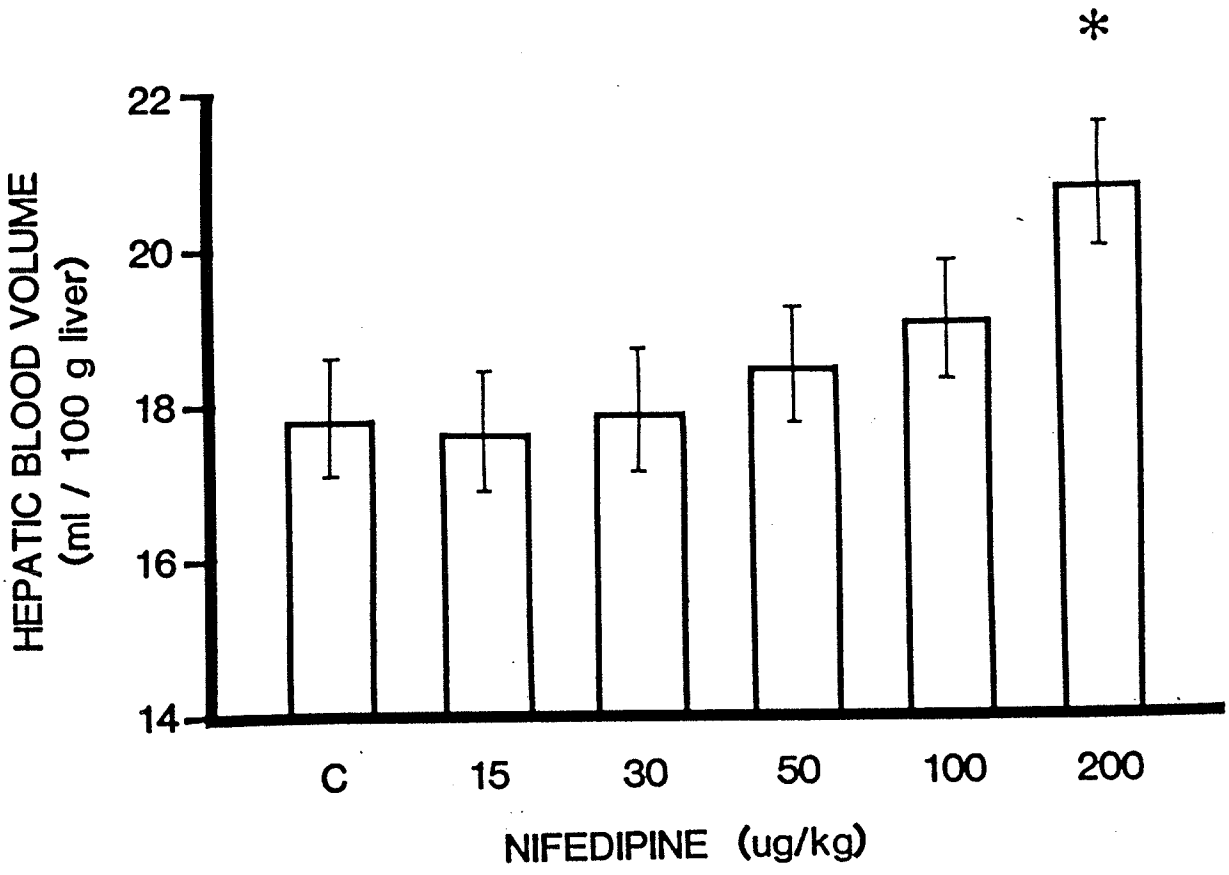

FIG. 55. Cats with hepatic nerve section, adrenalectomy and nephrectomy. Values of hepatic blood volume during the control period (C) and during cumulative doses of nifedipine. Means $\pm$ S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

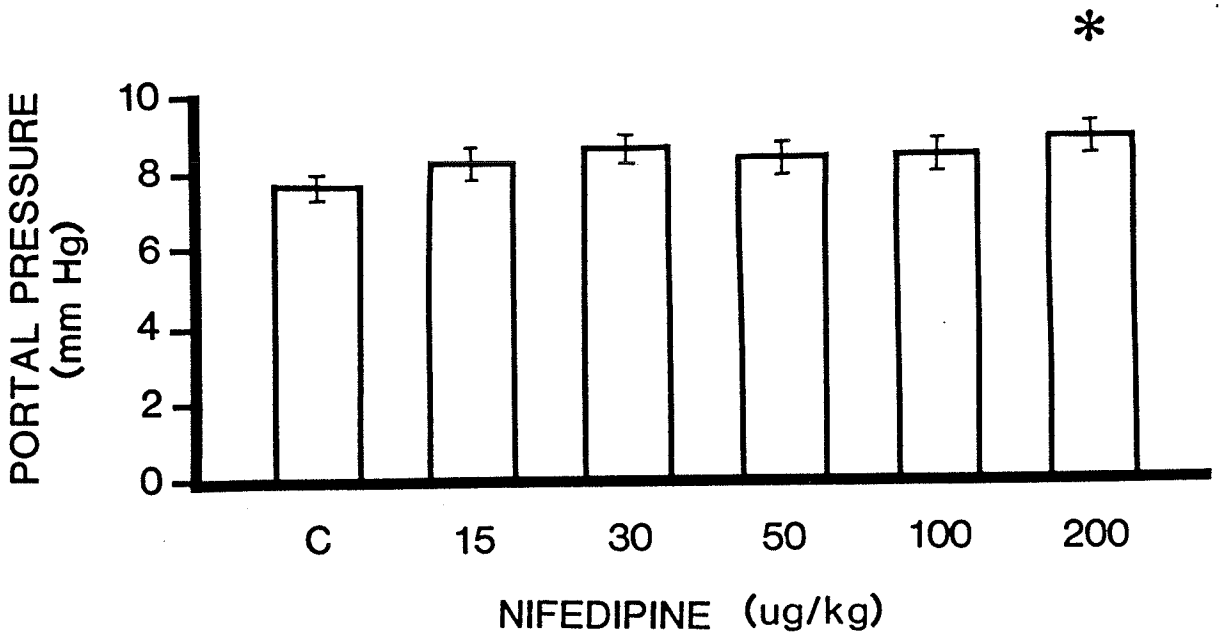

FIG. 56. Cats with hepatic nerve section, adrenalectomy and nephrectomy. Values of portal pressure during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

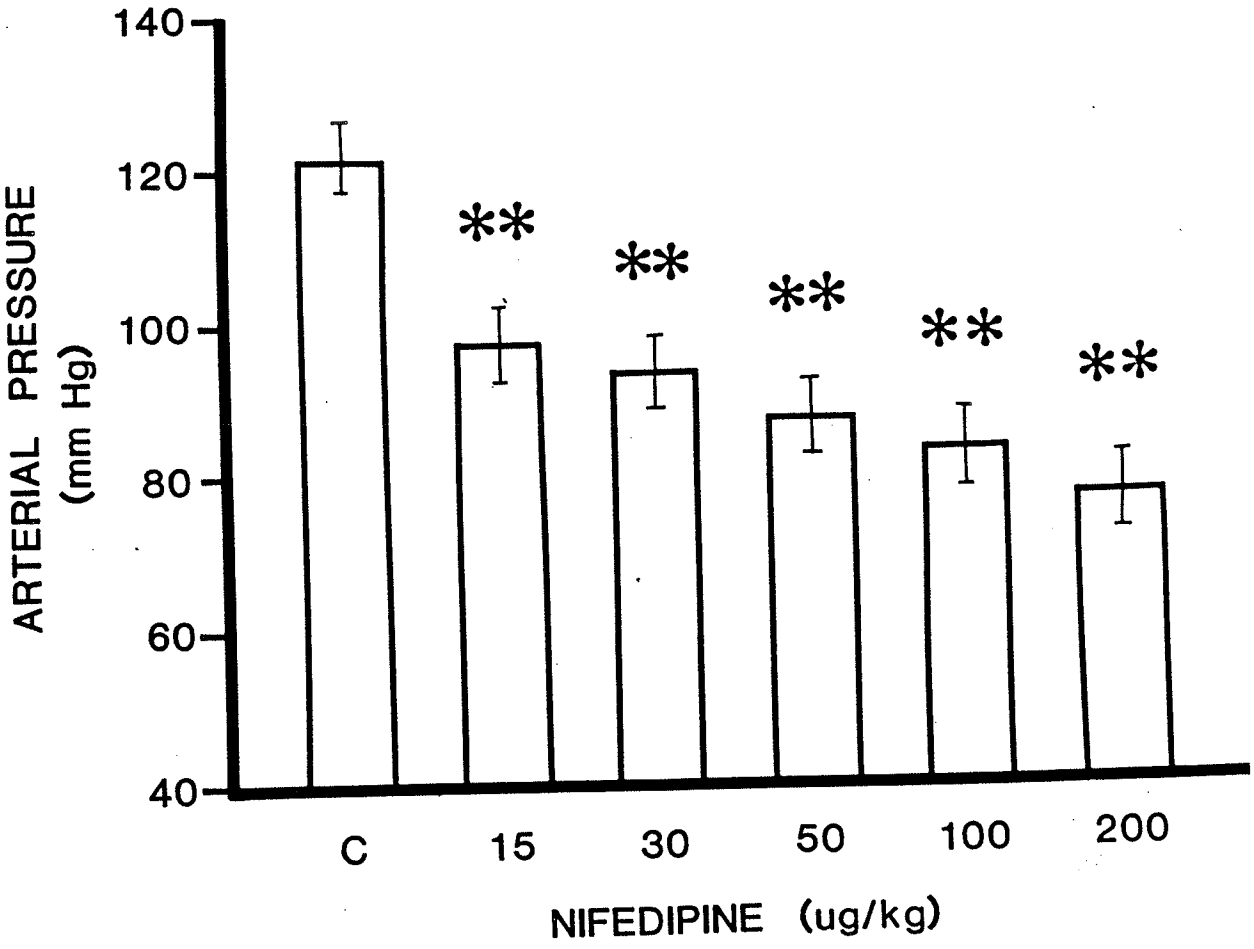

FIG. 57. Cats with hepatic nerve section, adrenalectomy and nephrectomy. Values of arterial pressure during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

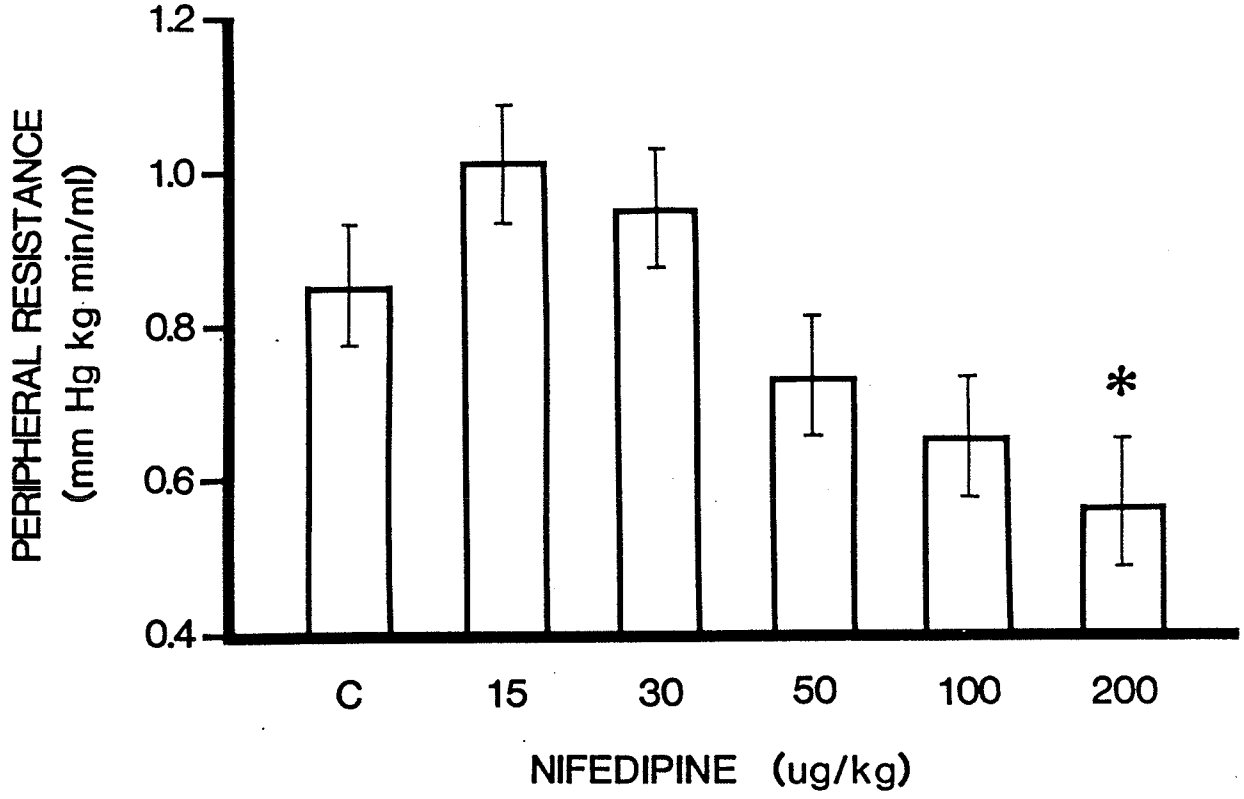

FIG. 58. Cats with hepatic nerve section, adrenalectomy and nephrectomy. Values of total peripheral resistance during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

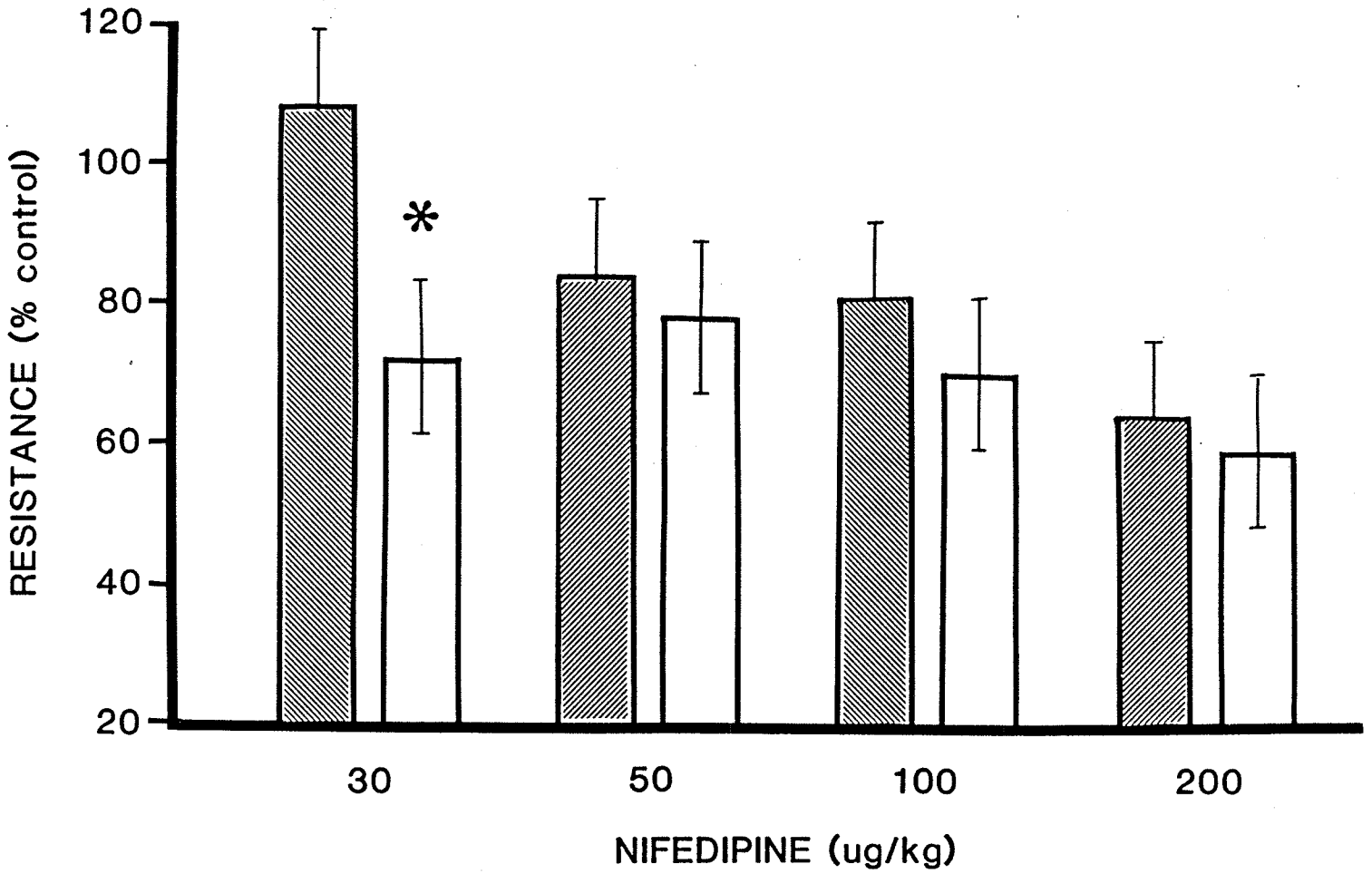

FIG. 59. Comparison of the effects of intravenous injections of nifedipine in normal cats (open bars, n=5) with the effects in cats after denervation, adrenalectomy and nephrectomy (shaded bars, n=4). Means ± S.E.; * = $P<0.05$, ** = $P<0.01$ compared with normal cats.

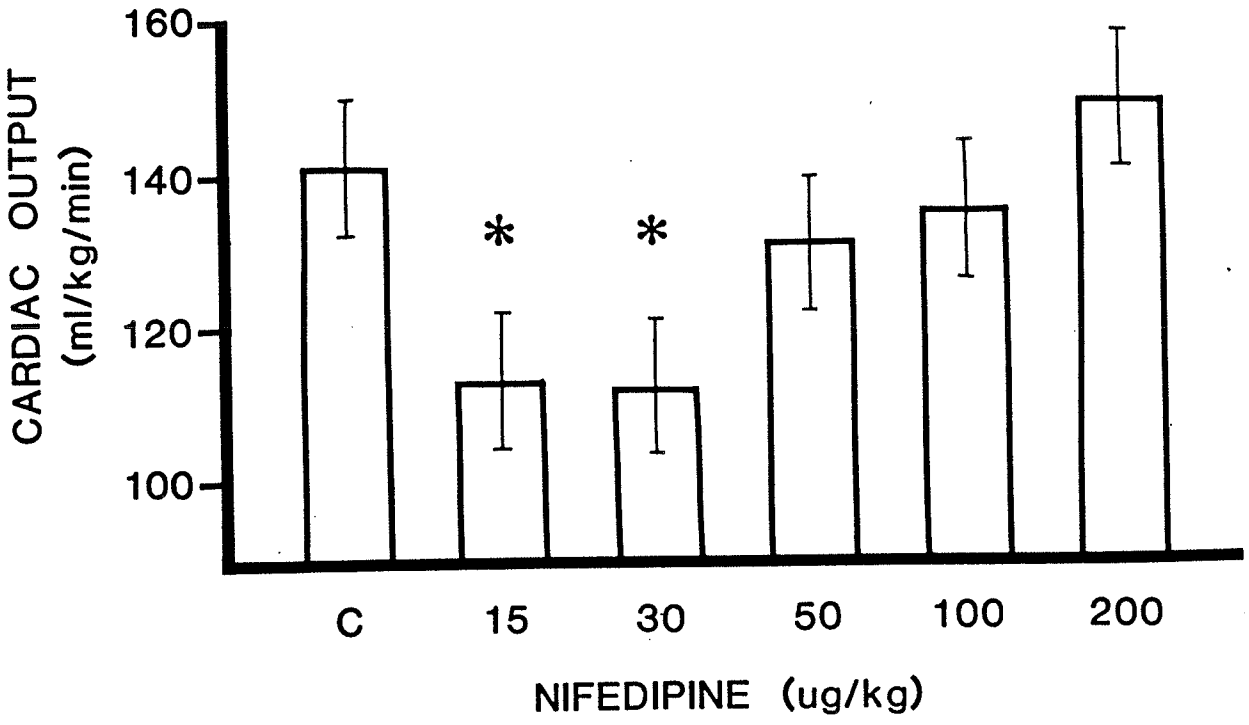

FIG. 60. Cats with hepatic nerve section, adrenalectomy and nephrectomy. Values of cardiac output during the control period (C) and during cumulative doses of nifedipine. Means ± S.E.; n=5; * = $P<0.05$, ** = $P<0.01$ compared with control.

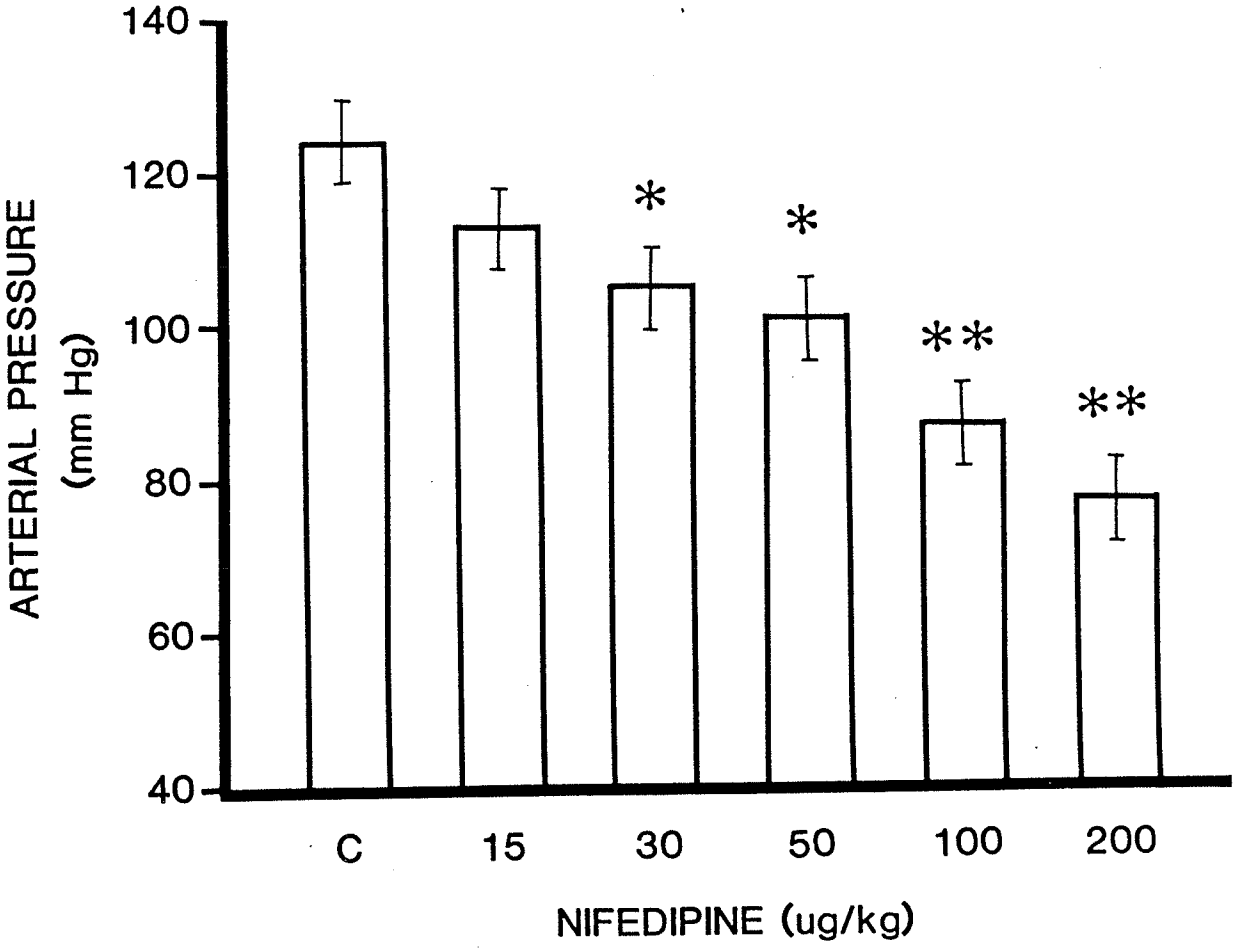

FIG. 61. Values of arterial pressure during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E.; n=4; * = $P<0.05$, ** = $P<0.01$ compared with control.

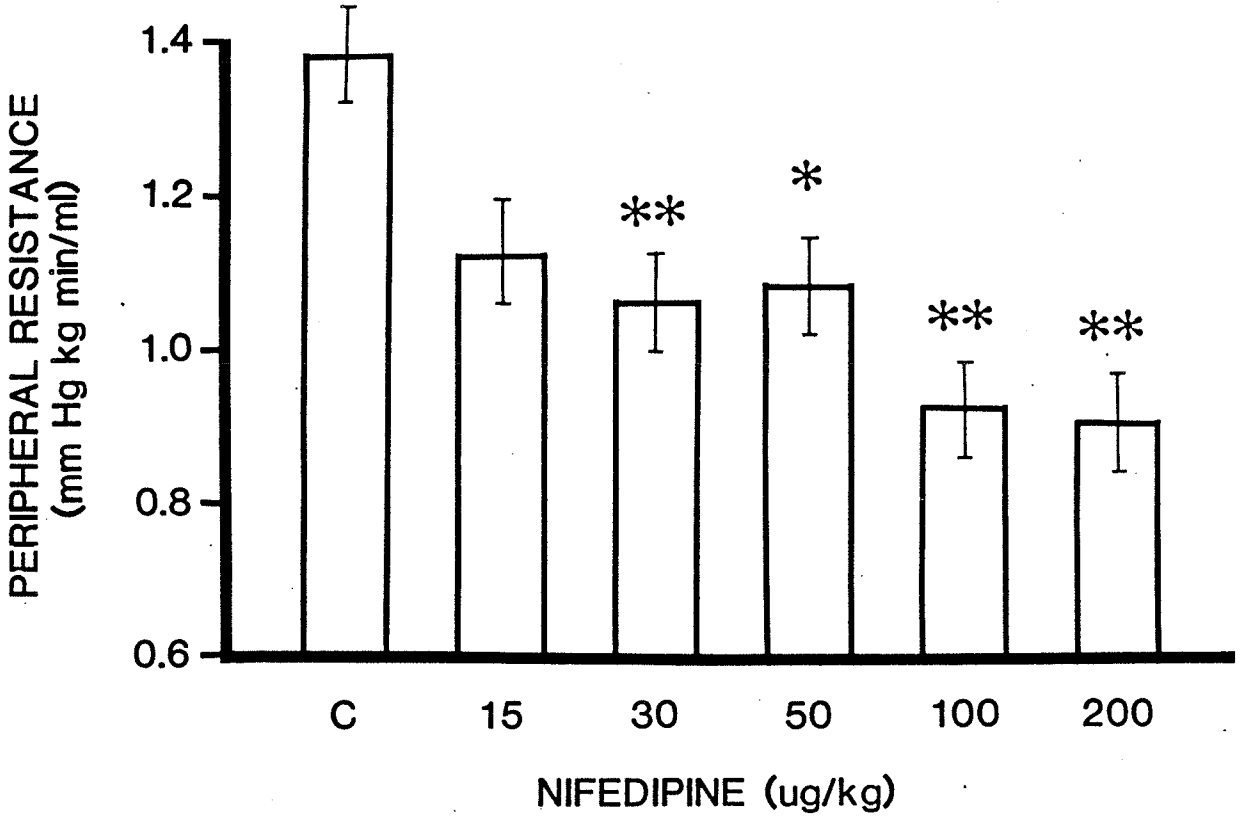

FIG. 62. Values of total peripheral resistance during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E. n=4; * = P<0.05, ** = P<0.01 compared with control.

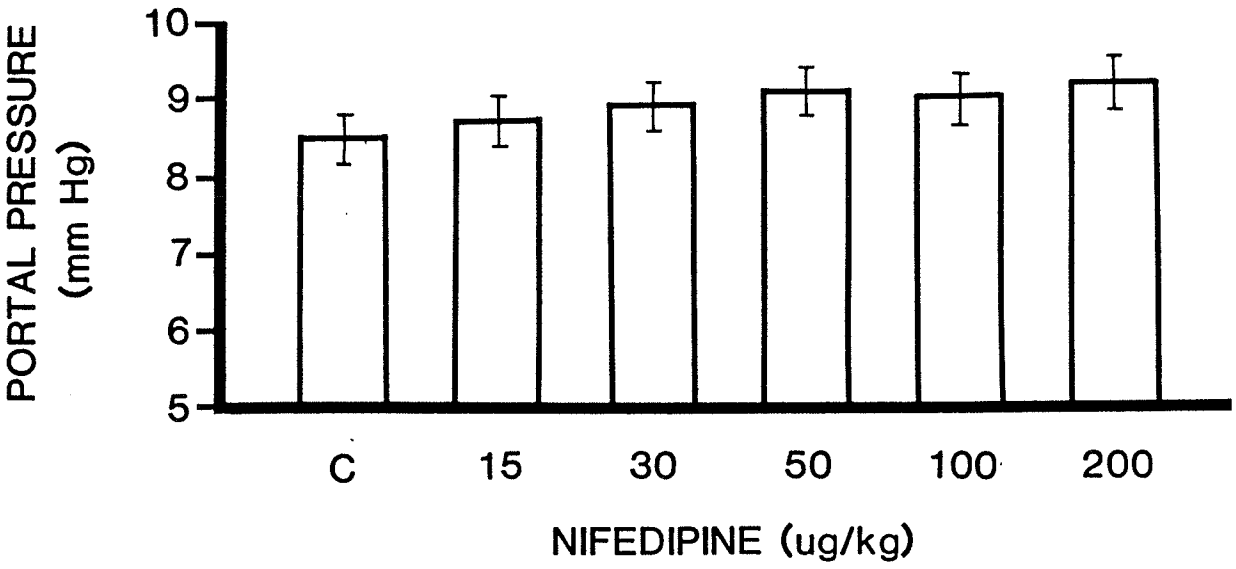

FIG. 63. Values of portal pressure during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E.; n=4; * = $P<0.05$, ** = $P<0.01$ compared with control.

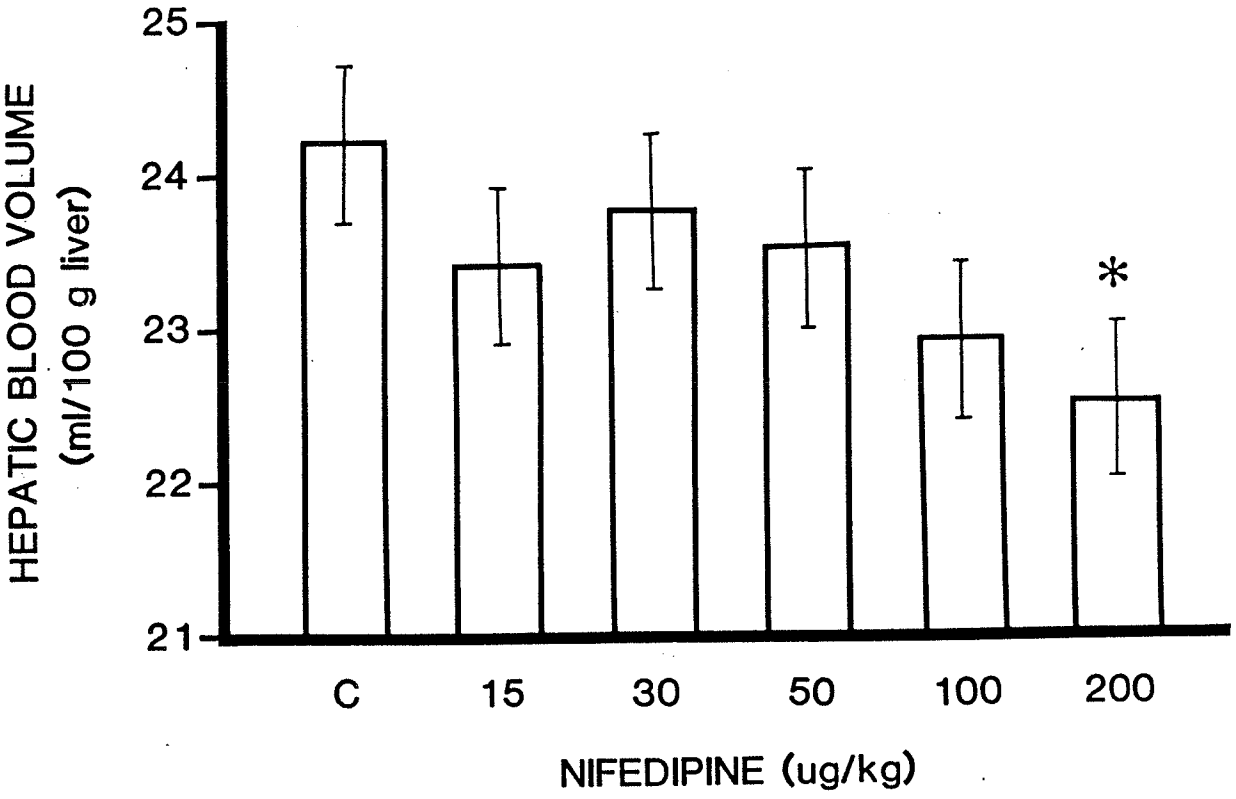

FIG. 64. Values of hepatic blood volume during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E.; n=4; * = $P<0.05$, ** = $P<0.01$ compared with control.

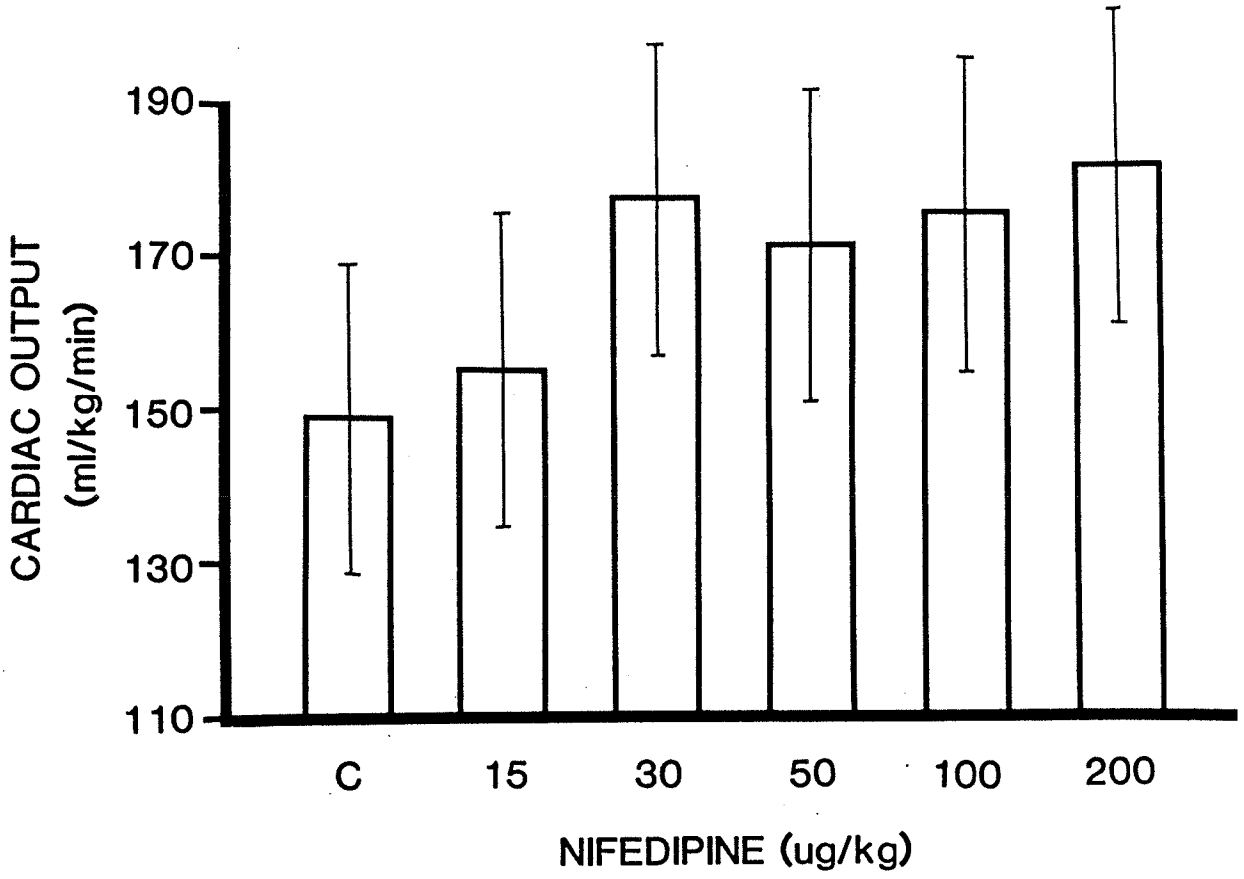

FIG. 65. Values of cardiac output during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E.; n=4; * = $P<0.05$, ** = $P<0.01$ compared with control.

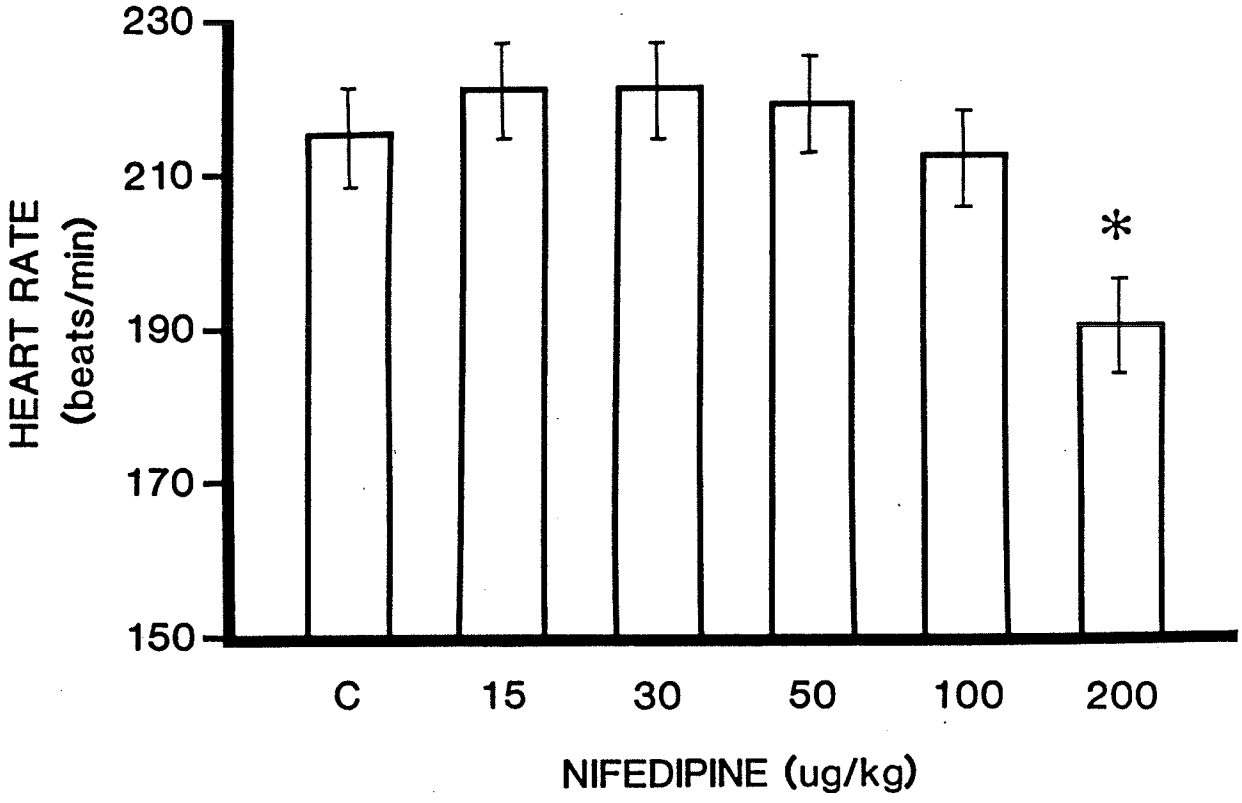

FIG. 66. Values of heart rate during the control period (C) and during cumulative doses of nifedipine at 150 min after endotoxin administration. Means ± S.E.; n=4; * = P<0.05, ** = P<0.01 compared with control.

DISCUSSION

In accord with previous studies in other species, the administration of nifedipine to healthy cats led to a decrease in peripheral resistance which was accompanied by an increase in cardiac output. The increase in cardiac output after nifedipine was not sufficient to maintain arterial pressure at pretreatment levels.

It has been concluded previously by others that the increase in cardiac output is due to peripheral vasodilation and to reflex cardiovascular compensatory mechanisms which are evoked in response to a drop in blood pressure (131, 293). It has also been concluded by others that the venous system is relatively unaffected by nifedipine (73, 131, 132, 199) and therefore should play no active role in the overall cardiovascular effects of nifedipine.

Results from experiments in this study show that nifedipine has a marked effect on the hepatic venous system. The administration of nifedipine indirectly produced a dose dependent decrease in hepatic blood volume (Fig. 48). The decrease in hepatic blood volume was not due to a decrease in hepatic venous pressure. In fact portal pressure tended to rise with increasing doses of nifedipine (Fig. 49). Therefore, the decrease in hepatic blood volume was caused by a constriction of the smooth muscle walls of the hepatic capacitance sites.

Studies with in vitro preparations have shown that nifedipine causes smooth muscle to relax. Thus it is unlikely that nifedipine directly constricted the smooth muscle of the hepatic venous system in the present experiments. A likely hypothesis for the mechanism of venous smooth muscle constriction is the indirect evoking of compensatory cardiovascular mechanisms in response to a decrease in blood pressure. As described in the Introduction, the hepatic venous bed is sensitive to circulating catcholamines, angiotensin II and hepatic nerve stimulation. Hypotension has been shown to increase catecholamine and angiotensin II blood levels and to activate the hepatic sympathetic nerves. All of these responses to hypotension would produce an active constriction of hepatic venous smooth muscle. In support of this hypothesis is the finding in this study that after the three reflex cardiovascular mechanisms were eliminated through adrenalectomy, nephrectomy and hepatic nerve section, nifedipine administration could no longer lead to constriction of hepatic venous smooth muscle (Fig. 55). The quantitative role of each of these three cardiovascular mechanisms cannot be determined from the results of this study. Although the highest dose of nifedipine produced an increase in hepatic blood volume, this was probably not the result of smooth muscle relaxation but rather the passive result of an increase in portal pressure (Fig. 56) which in turn was probably a result of an increase in hepatic blood

flow. Thus it can be concluded from these experiments that nifedipine administration causes a significant hepatic venoconstriction and that this venoconstrictor effect is indirectly caused by reflex angiotensin II formation, adrenal catecholamine release or hepatic nerve activation.

Results from these experiments also indicate that when blood is not mobilized from the hepatic venous system, nifedipine cannot produce an increase in cardiac output. In fact, from Fig. 60 it can be seen that cardiac output decreases after low doses of nifedipine. Another finding from these experiments is that after nephrectomy, adrenalectomy and hepatic nerve section, low doses of nifedipine tends to raise total peripheral resistance, although this rise is not statistically significant. In any case, low doses of nifedipine produces a significantly greater decrease in peripheral resistance in intact cats compared to cats where the three reflex cardiovascular mechanisms are eliminated (Fig. 59).

A hypothesis to explain these findings of the effects of nifedipine before and after adrenalectomy, nephrectomy and hepatic nerve section is as follows. In the intact cat, nifedipine tends to directly produce veno- and vaso-dilation which in turn lowers blood pressure through a decrease in cardiac output and peripheral resistance. The fall in blood pressure activates reflex mechanisms, that is, angiotensin II formation, adrenal catecholamine release and a generalized

sympathetic discharge. These reflex mechanisms act to constrict the hepatic venous system, i.e., to increase preload which in turn increases cardiac output. It is known that these cardiovascular reflex mechanisms can also act to constrict arterioles which would tend to to restore the fallen blood pressure. It is hypothesized that normally after nifedipine, arteriolar constriction plays only a minor role in restoring blood pressure and that blood pressure is raised primarily by an increase in cardiac output.

After adrenalectomy, nephrectomy and hepatic nerve section, nifedipine still tends to decrease blood pressure but in this case there is no accompanying reflex hepatic venoconstriction (Fig. 55), i.e., there is no compensatory increase in preload. It is hypothesized here that because the nifedipine mediated fall in blood pressure is not compensated for by an increase in preload, it is instead compensated for by a massive sympathetic discharge which produces arteriolar vasoconstriction. This proposed sympathetic discharge would be powerful enough to overcome the arteriolar relaxing effects of low doses of nifedipine but not high doses. Thus, peripheral resistance is increased at low doses of nifedipine but decreased at high doses (Fig. 58). Cardiac output decreases after low doses (Fig. 60) because of the lack of venoconstriction and the increase in peripheral resistance. Cardiac output rises to normal levels after higher doses (Fig. 52) because peripheral resistance decreases (Fig. 58).

After endotoxin, a state of extreme vasoconstriction and a relatively unresponsive hepatic venous system develop. When nifedipine is administered, the characteristic fall in blood pressure is still observed. However the hepatic venous system is now less reponsive to agonists and the venoconstriction normally observed after nifedipine is attenuated (Fig. 64). As a result of the attenuated venoconstriction, cardiac output does not rise significantly (Fig. 65).

It can be concluded that because of the lack of responsiveness of the venous system during endotoxin shock, nifedipine cannot produce hepatic venoconstriction except at high doses. Furthermore, this lack of venoconstriction could explain why nifedipine fails to increase cardiac output.

Also notable is the fact that although venous responses were attenuated during endotoxin shock, nifedipine still caused a large change in peripheral resistance (Fig. 62) which was comparable in magnitude to the change observed in healthy cats (Fig. 52). A similar situation occurred in the experiments with isoproterenol described in the previous section. In those experiments, the venous system became unresponsive to the effects of isoproterenol after endotoxin but the arterial system was relatively unaffected. It appears then that the arterial system remains responsive to various agonists and antagonists at a time when the venous system does not.

IV. GENERAL DISCUSSION

GENERAL DISCUSSION

The experiments in this study together with previous observations (107, 114) confirm and extend the hypothesis that arteriolar vasodilators can be divided into three groups with respect to their actions on the hepatic venous system, and that this classification is compatible with the observed effects of these agents on cardiac output in normals and in patients with heart failure (109). This classification is tabled below:

GROUP 1 ---	Arteriolar vasodilation with hepatic venoconstriction	-- EPINEPHRINE DOPAMINE ISOPROTERENOL HYDRALAZINE NIFEDIPINE
GROUP 2 ---	Arteriolar vasodilation with no effect on hepatic venous system	-- NITROPRUSSIDE DIAZOXIDE
GROUP 3 ---	Arteriolar vasodilation with hepatic venodilation	-- NITROGLYCERIN

Group 1 agents cause arteriolar vasodilation with hepatic venoconstriction either by direct or indirect mechanisms, and some cause cardiac stimulation. They increase cardiac output in normal humans and animals, and in heart failure patients they increase cardiac output with little relief of the pulmonary congestion. Group 2 agents cause

arteriolar vasodilation with little if any effect on the hepatic venous bed. These agents have little effect on cardiac output in normal humans or animals while in patients with with heart failure they produce a balanced effect consisting of a modest elevation in cardiac output and a decrease in pulmonary congestion. Group 3 agents cause both arteriolar dilatation and venodilation. They reduce cardiac output in normal humans and animals, and in patients with with heart failure they relieve pulmonary congestion with little change in cardiac output . Detailed documentation of these statements has been given (109). Thus the effects of these agents on the venous system appear to be an important part of their pharmacological profile. For example, it can be estimated that more than 50% of the increase in cardiac output produced by epinephrine was dependent on splanchnic venoconstriction (111).

It would appear group I drugs would be useful in the therapy of endotoxin shock because of their ability to cause venoconstriction and increase cardiac output. Their hepatic venoconstriction effect would seem to be especially important in light of the fact that, from data in this study, it can be concluded that hepatic venodilation occurs after endotoxin administration. However, the venoconstrictor effect normally produced by two drugs from group I was either completely abolished or markedly attenuated during endotoxin shock. Thus, these drugs do not affect the cardiovascular system

during endotoxin shock as might be predicted. This lack of responsiveness of the hepatic venous bed to these drugs after endotoxin is consistent with the finding from this study that endotoxin causes the hepatic venous bed to become unresponsive to other normally effective stimuli. In fact, it has been shown here that the responsiveness to the three most known potent physiological stimuli, namely, sympathetic nerve stimulation, catecholamine release and angiotensin formation, are markedly attenuated.

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