STUDIES ON THE β RECEPTOR BLOCKING AGENT NETHALIDE

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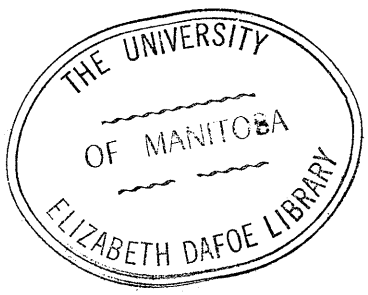

ABSTRACT

The present work shows that the β receptor blocking agent nethalide increases the heart rate of dogs depleted of catecholamines by pretreatment with reserpine, and confirms previous reports that nethalide causes bradycardia and hypotension in normal dogs. The results indicate that the bradycardia in untreated dogs is due to blockade of the cardiac actions of endogenous catecholamines, whereas the increased heart rate in reserpine-treated dogs is due to an intrinsic activity of nethalide on the heart. Both intra-arterial and intravenous injections of nethalide decreased the peripheral resistance in the dog hind limb indicating that the hypotension caused by nethalide is due to vasodilatation. Also, the latter finding shows that nethalide possesses intrinsic activity on blood vessels in skeletal muscle.

Nethalide markedly inhibited the cardioaccelerator responses to isoproterenol. However, ouabain, partially restored the positive chronotropic action of isoproterenol after establishment of β receptor blockade by nethalide. In the absence of β receptor blockade, ouabain antagonized the positive chronotropic action of isoproterenol. The data show that ouabain opposes both the stimulating action of isoproterenol and the blocking action of nethalide in the heart.

A cardiac action of ouabain is antagonized by nethalide. Arrhythmias induced with toxic doses of ouabain were converted to sinus rhythm by single intravenous injections of nethalide. However, sinus rhythm persists for only short periods of time before re-emergence of the arrhythmia, although β receptor blockade was still present. This finding indicates that blockade of the cardiac actions of endogenous catecholamines is not

responsible for the antagonism of digitalis-induced arrhythmias. When constant intravenous infusions of nethalide were given the heart was maintained in sinus rhythm as long as the infusion was continued. Furthermore, constant intravenous infusions of nethalide significantly increased the survival time of dogs given lethal doses of ouabain. Experiments in which the blood pressure was controlled show that the antiarrhythmic effect of nethalide is independent of its peripheral vascular actions.

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HISTORICAL REVIEW

Development of the concept of α and β receptors.

Before the work of Ahlquist (1948) adrenaline receptors were classified simply as excitatory and inhibitory. This classification stemmed from the early experiments of Dale (1906) showing that ergot blocked the excitatory actions of adrenaline on smooth muscle, but had no effect on the inhibitory action. This is an appealing classification because it describes the two types of responses elicited by adrenergic drugs. However, as a classification of adrenaline receptors it is inadequate because it fails to explain the actions of the classical adrenergic blocking agents in different tissues.

Blocking agents such as ergot, phentolamine, Dibenamine, and phenoxybenzamine inhibit the excitatory action of adrenaline on smooth muscle, but do essentially nothing to the excitatory action of adrenaline on cardiac muscle (Moran and Perkins, 1961; Nickerson and Chan, 1961). Since the original work of Dale another discrepancy in this classification has become apparent. Nanda (1931) and Rothlin, Konzett, and Cerletti (1954) have shown that ergot blocks the inhibitory action of adrenaline on the intestine. The classification of adrenaline receptors proposed by Ahlquist (1948) is more in line with the experimental observations.

Ahlquist found that a series of six sympathomimetic amines had one order of potency on vasoconstriction, excitation of the uterus and ureters, contraction of the nictitating membrane, dilatation of the pupil and inhibition of the gut. In contrast the same series of amines had an entirely different order of potency on vasodilatation, inhibition of the uterus, and myocardial stimulation. Ahlquist concluded that the difference in order of potency was due to differences in the receptors involved.

He called the first kind of receptor the "α adrenotropic receptor" and the second kind the "β adrenotropic receptor".

According to Ahlquist (1948) the α receptor is associated with most of the excitatory functions such as vasoconstriction, and stimulation of the uterus, nictitating membrane, ureter, and radial muscle of the iris. It is associated with one inhibitory function also, i.e. relaxation of the intestine. The β receptor is associated with most of the inhibitory functions, e.g. vasodilatation of certain vessels, inhibition of the uterine and bronchial musculature. However, in cardiac muscle the β receptor is excitatory; its stimulation leads to an increased rate and force of contraction of the heart. It follows that sympathomimetic drugs acting on β receptors with marked cardiac stimulating action will have marked inhibitory action on smooth muscle containing β receptors. Conversely, drugs with strong smooth muscle excitatory action will be weaker cardiac stimulants. Ahlquist showed this to be experimentally true. Isoproterenol was the most potent cardiac stimulant and smooth muscle relaxant. Noradrenaline, on the other hand, was a potent smooth muscle stimulant, but had less effect on cardiac muscle than isoproterenol.

Ariens and Simonis (1960) showed that the length of the chain substituted on the nitrogen of phenethylamine derivatives was a factor determining specificity of these compounds with the type of adrenergic receptor stimulated. In general, increasing the chain length of the derivative increased its reactivity with β receptors more than with α receptors. This same principle holds for catecholamines. Noradrenaline is a primary amine and acts chiefly on α receptors. Adrenaline is a methylated secondary amine with actions on both α and β receptors, and isoproterenol is an isopropyl substituted secondary amine that acts

predominantly on β receptors. All the actions associated with the α receptors are blocked by the classical adrenergic blocking agents, ergot alkaloids, benzodioxanes, imadazolines, and β-haloalkylamines. However, a specific blocking agent for the β receptors was not reported until ten years after Ahlquist proposed the α and β classification of adrenaline receptors.

Powell and Slater (1958) introduced dichloroisoproterenol* (DCI) as a specific blocking agent of inhibitory adrenaline receptors. Surprisingly, these authors made no mention of Ahlquist nor did they attempt to show that DCI antagonized the cardiac excitatory actions of adrenergic drugs. However in the same year, Moran and Perkins (1958) showed that DCI selectively blocked the positive inotropic and chronotropic effects of adrenergic stimuli. They also confirmed the results of Powell and Slater (1958). On the basis of the specific blockade caused by DCI of the cardiac excitatory action and smooth muscle inhibitory action of adrenergic stimuli, Moran and Perkins concluded that smooth muscle inhibitory receptors and cardiac excitatory receptors are functionally homologous. This strongly supported the classification of adrenaline receptors proposed by Ahlquist.

Cardiovascular pharmacology of DCI.

Moran and Perkins (1958) showed that DCI blocks the positive chronotropic and inotropic effects of adrenergic stimuli in dogs with intact circulatory systems and in isolated rabbit hearts. In dogs the positive inotropic effects of adrenaline, noradrenaline, and isoproterenol, and of supramaximal stimulation of the cardiac sympathetic nerves

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* 1-(3', 4'-dichlorophenyl)-2-isopropylaminoethanol

were completely blocked with cumulative doses of DCI of 3 mg/kg and higher. Depression of contractile force was frequently observed in response to sympathomimetic amines and to sympathetic nerve stimulation after the administration of large doses of DCI. The positive inotropic effects of theophylline, digoxin, and calcium chloride were not altered by treatment with DCI. They found that small doses of DCI initially stimulated the heart, but that subsequent doses depressed the heart.

Using cat papillary muscle preparations, Dresel (1960) found that small doses of DCI increased the strength of contraction and that larger doses antagonized the positive inotropic effect of adrenaline. Furchgott, de Gubareff, and Grossman (1959) used the same preparation and isolated guinea pig atrium to confirm that DCI antagonized the positive inotropic effect of both adrenaline and noradrenaline.

Fleming and Hawkins (1960) showed that DCI in small doses had sympathomimetic actions in dog heart-lung preparations and isolated guinea pig atria; large doses had a negative chronotropic and inotropic effect in both preparations. Doses of DCI smaller than those which demonstrated sympathomimetic actions had no effect on responses to adrenaline and noradrenaline. The writers concluded that the interaction of DCI with adrenaline and noradrenaline was properly described by the term "competitive dualism" according to the principles advanced by Ariens (1954). Therefore according to the terminology proposed by Stephenson (1956) DCI is a "partial agonist".

Several investigators have shown that DCI protects the heart against a variety of experimentally induced arrhythmias. Gilbert, Lang, and Brooks (1959) demonstrated an action of DCI in preventing arrhythmias induced by successive ventricular electrical stimuli during noradrenaline

administration. Moore and Swain (1960) showed that DCI prevented ventricular fibrillation in dogs given adrenaline after sensitization of the heart with various agents. Lucchesi and Hardman (1961) concluded that DCI possessed antiarrhythmic action which was independent of its ability to block β receptors. This conclusion was based on experiments in isolated rabbit hearts and intact dogs. DCI temporarily restored sinus rhythm in these preparations after arrhythmias had been established with toxic doses of ouabain and acetylstrophanthidin. However, the arrhythmias reappeared although β receptor blockade was still present. Furthermore these authors showed that an analogue of DCI which possessed no β receptor blocking activity also antagonized the arrhythmia.

Pharmacology of nethalide.

Black and Stephenson (1962) introduced a new β receptor blocking agent, nethalide* (2-isopropylamino-1-(2-naphthyl) ethanol hydrochloride, Alderlin). The drug was studied by Black and Stephenson in isolated tissues and in intact cats and dogs. Nethalide blocked the stimulating action of adrenaline on isolated cat and guinea pig papillary muscle, and on Langendorff preparations made from guinea pig hearts. Nethalide also blocked adrenaline-induced relaxation of the guinea pig tracheal chain, fowl caecum, and rabbit uterus. On the other hand, nethalide did not block the contraction of the rabbit uterus and constriction of the perfused rabbit ear. In short nethalide blocked only those responses which are elicited by excitation of β receptors.

In cats nethalide inhibited the positive chronotropic action of isoproterenol. Nethalide also inhibited the positive inotropic

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* In recent publications nethalide has been referred to as pronethalol.

response to stellate ganglion stimulation in cats and dogs. Dornhorst and Robinson (1962) confirmed the results of Black and Stephenson by experiments on man. Their experiments showed that after infusion of nethalide into the brachial artery the usual increase in blood flow in the forearm caused by intra-arterial injections of isoproterenol was blocked. The cardioaccelerator action of isoproterenol was completely inhibited by nethalide given intravenously or orally. Black and Stephenson emphasized that nethalide differed from DCI in that it showed no intrinsic sympathomimetic activity.

General preparation of dog.

Dogs of either sex weighing 4 to 17 kg were anaesthetized with intravenous pentobarbital sodium (35 mg/kg), and maintained on positive pressure respiration with a Palmer Ideal Pump delivering room air (20 ml/kg) at sixteen strokes per minute through a tracheal cannula or endotracheal tube. Both vagi were cut, and the femoral vein and artery in the right or left leg were exposed for measurement of blood pressure and injection of drugs. Femoral arterial pressure was measured with a Statham P-23 AC pressure transducer connected to a polyethylene catheter filled with a 4% solution of heparin in 0.9% saline. The Lead II electrocardiogram was recorded on a Grass polygraph. Rectal temperature was maintained at 37° with a heating lamp.

Administration of drugs.

All drugs except reserpine were given intravenously in 0.9% solution of NaCl; lyophilized reserpine (Serpasil, Ciba) was injected intraperitoneally. Doses of isoproterenol are expressed in micrograms of the free base, whereas doses of nethalide (2-isopropylamino-1-(2-naphthyl) ethanol hydrochloride, 'Alderlin' I.C.I. 38,174) are expressed in micrograms or milligrams of the salt. Isoproterenol in volumes varying from 0.1 to 1.0 ml and ouabain in volumes varying from 1.0 to 5.0 ml were injected into a catheter in the right or left femoral vein, and flushed in with 1.0 to 5.0 ml of 0.9% saline. These volumes of saline given alone had no effect on heart rate or blood pressure. The time taken for an injection of isoproterenol was less than five seconds; ouabain was injected over a one minute period. Nethalide was given either by rapid injection or by a Harvard constant infusion pump at rates varying from 190 to 333

μg/kg/min. The total volume infused varied from 10 to 50 ml.

Measurement of heart rate and blood pressure.

The heart rate was counted from the electrocardiogram for one minute immediately before each injection of isoproterenol and for one minute after completing the injection. The maximum heart rate was always reached within the first minute of the injection. When the heart rate data were used to construct dose-response curves, the increases in heart rate were plotted both as the percentage increase from the basal rate and as the increase in beats per minute. The blood pressure was recorded on the Grass polygraph as the integrated mean pressure; this was obtained by reducing the frequency response of the polygraph to 0.1 cycle per second. The blood pressure was noted immediately before each injection of isoproterenol, and noted again at the lowest point reached after the injection. The lowest point always occurred within the first minute after the injection. The blood pressure data were plotted as the percentage decrease from the basal pressure, and as the decrease in mm Hg. Repeated injections of isoproterenol were spaced at ten minute intervals. During infusions of nethalide the heart rate and blood pressure were recorded every five minutes.

Perfusion of the denervated vascularly isolated hind limb.

Perfusion experiments were done on either hind limb. The sciatic and perivascular nerves were cut to avoid reflex changes in vascular resistance. All large blood vessels except the femoral artery and vein were ligated. Thirty minutes were allowed for bleeding to stop, after completing the dissection, before administering heparin (6.0 mg/kg i.v.). Ten minutes later, the femoral artery was ligated just beneath the

inguinal ligament and a polyethylene catheter inserted just distal to the ligature. The leg was immediately perfused with blood from the left carotid artery. The perfusing catheter was threaded through a DeBakey constant flow pump; thus the blood flow was maintained constant regardless of vascular resistance. Flow was estimated from calibrations on the DeBakey pump. The flow varied in different experiments from 35 to 50 ml/min. Perfusion pressure was measured by connecting a Statham pressure transducer with a Y tube to the perfusing catheter 5 cm above the entry of the catheter into the artery.

Injections were made into the limb with a 27 gauge needle through a rubber joint in the perfusing catheter 5 cm above the entry of the catheter into the artery. Control injections of 0.1 ml normal saline had no effect on the perfusion pressure. Therefore all injections of nethalide into the limb were made in 0.1 ml normal saline. Ten minutes were allowed between injections for the perfusion pressure to return to control level.

Procedure for control of the systemic arterial pressure.

In some experiments the systemic arterial pressure was prevented from falling after injections of nethalide by giving intra-arterial infusions of blood under pressure. The pressure regulator consisted of a 25-litre air filled tank connected to a 2-litre reservoir primed with donor blood. The reservoir was connected to the arterial system of the animal by a catheter leading through the femoral artery into the abdominal aorta. A clamp was placed on the catheter between the animal and reservoir. The pressure in the air filled tank, measured with a mercury manometer, was raised to 100 mm Hg higher than the control level of systemic arterial pressure. Immediately after injecting nethalide

the clamp was removed, and the systemic arterial pressure increased by 10 to 20 mm Hg. The pressure in the reservoir was immediately lowered to the level of the original control systemic arterial pressure by allowing air to escape through a valve. The systemic arterial pressure was thus maintained at the original control level throughout the duration of the experiment.

SECTION I

THE EFFECT OF NETHALIDE ON HEART RATE AND BLOOD PRESSURE

Introduction

Black and Stephenson (1962) showed that nethalide given intravenously caused bradycardia and hypotension in vagotomized or atropine pretreated cats and dogs. They found that rapid single injections of nethalide given intravenously, reduced both cardiac rate and contractility. Although bradycardia still resulted, there was only a slight decrease in contractility when nethalide was given by slow intravenous infusion (200 μg/kg/min). Other experiments showed that the bradycardia was less marked but still significant after acute bilateral stellate ganglionectomy.

On the basis of these results Black and Stephenson suggested that the bradycardia caused by nethalide was due to an inhibition of "sympathetic drive" to the heart. They attributed the hypotension occurring with slow intravenous infusions of nethalide to peripheral vasodilatation. But they suggested that decreased cardiac output was responsible for the hypotension associated with rapid single injections of nethalide. The purpose of the present experiments was to delineate more clearly the cause of bradycardia and hypotension produced by nethalide.

A. Bradycardia due to nethalide.

Experiments were done on four vagotomized dogs to determine whether blockade of the cardiac actions of endogenous catecholamines was the only cause of bradycardia induced by nethalide. The dogs were treated with reserpine (0.5 mg/kg/day) for two consecutive days prior to their

use on the third day. Paasonen and Krayer (1958) showed that this treatment is adequate to deplete the dog heart of all but negligible amounts of catecholamines. Before starting each experiment the carotid arteries were occluded for thirty seconds. This procedure caused no change in heart rate or blood pressure in any of the dogs, thus showing that the response to reflex activity of the sympathetic nervous system was absent. It was therefore assumed that the reserpine pretreatment had been effective in each dog. Nethalide was given by slow intravenous infusion because Black and Stephenson (1962) believed that rapid single injections caused myocardial depression.

Nethalide infused at a rate of 190 μg/kg/min (three dogs) or 333 μg/kg/min (one dog) caused in each dog an immediate and marked increase in the heart rate. The maximum increase in heart rate ranged from 32 to 40 beats per minute in the dogs given nethalide (190 μg/kg/min). The maximum increase in heart rate of the dog given nethalide (333 μg/kg/min) was 34 beats per minute. In all four dogs the heart rate remained above control level throughout the infusion (45 to 60 minutes), but declined toward the control level near the end of the infusion. In addition to the increase in heart rate nethalide caused a fall in the systemic arterial pressure in each dog. Figure 1 illustrates data typical of these experiments.

The increase in heart rate caused by nethalide in the dogs pretreated with reserpine is in marked contrast to the bradycardia caused by nethalide in normal dogs. Black and Stephenson (1962) found that nethalide invariably caused bradycardia in anaesthetized cats and dogs. The present work confirms these findings. Nethalide (333 μg/kg/min) caused bradycardia in each of seventeen vagotomized dogs. The decrease in heart

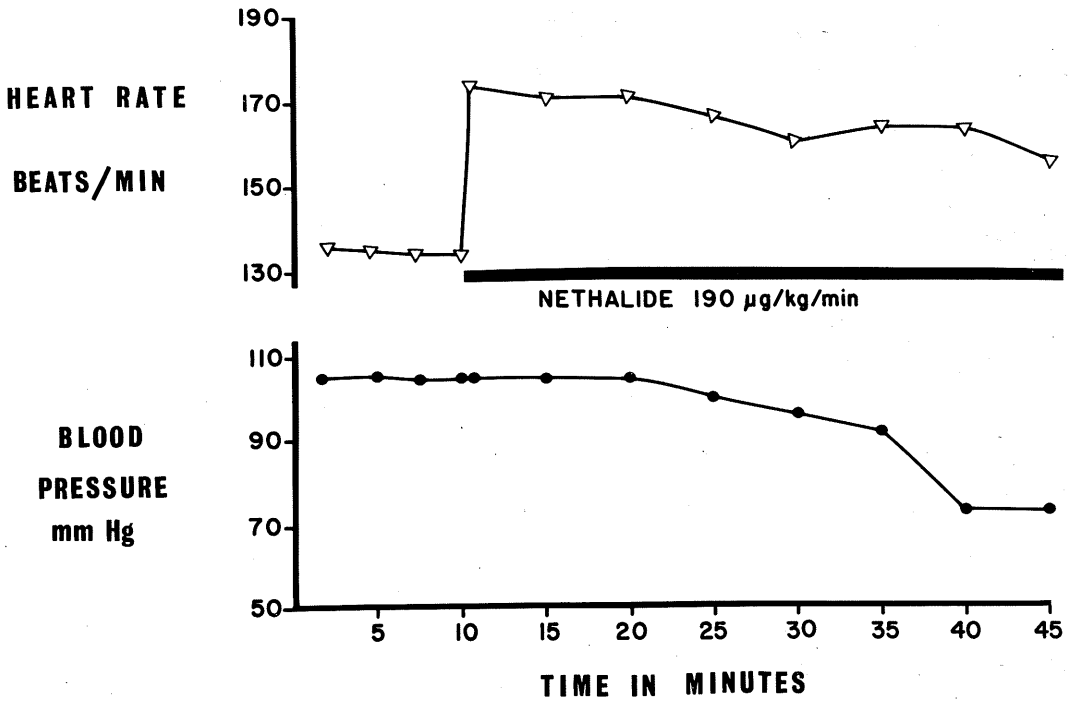

Figure 1. Effect of intravenous infusion of nethalide on heart rate and blood pressure in a dog (17 kg) treated with reserpine (0.5 mg/kg 48 and 24 hours before experiment). Black bar indicates nethalide infusion (190 µg/kg/min).

rate ranged from fifteen to forty beats per minute. The experiments done on these dogs are reported in Section II of the results. Figure 2 shows the effects of nethalide (333 μg/kg/min) on heart rate and blood pressure in a dog pretreated with reserpine and an untreated dog. The data indicate that the bradycardia caused by nethalide in normal animals is due to blockade of the positive chronotropic action of endogenous catecholamines; the increase in heart rate caused by nethalide in dogs pretreated with reserpine is due to a sympathomimetic action.

B. Hypotension due to nethalide.

The above results exclude bradycardia as the cause of hypotension, since nethalide caused a fall in blood pressure concomitant with an increase in the heart rate in dogs pretreated with reserpine (Fig. 1). However, the hypotension may be due to reduced cardiac output or reduced peripheral resistance or both. Experiments were done on the denervated, blood perfused hind limb in four dogs to determine the effect of nethalide on peripheral resistance. Nethalide was given intra-arterially by injection into the perfusing catheter. Changes in perfusion pressure were taken as an index of changes in vascular resistance in the limb. This was valid because the flow to the limb was constant regardless of changes in resistance. At the start of each experiment the flow was adjusted so that the perfusion pressure equalled the systemic arterial pressure. This varied from 90 to 120 mm Hg in different experiments, but was constant throughout any one experiment.

Intra-arterial injections of nethalide (0.1 to 2.0 mg) caused no change in the systemic arterial pressure. The perfusion pressure, however, fell markedly in all four dogs after each injection. The dose-response curve shown in Figure 3 is typical of these four experiments.

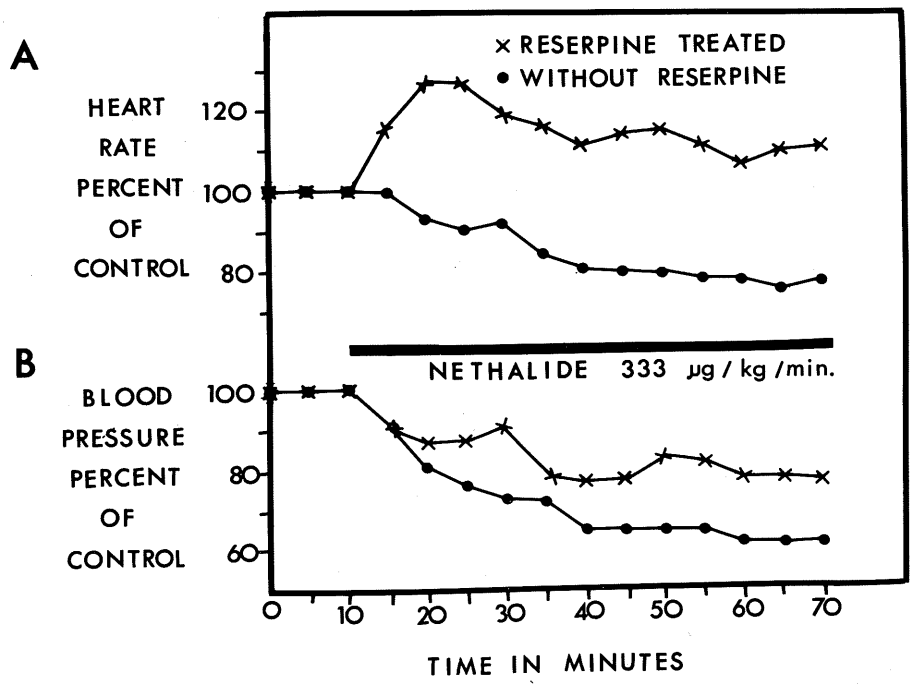

Figure 2. Contrasting effects of intravenous infusions of nethalide on a reserpine (0.5 mg/kg 48 and 24 hours before the experiment) treated dog and dog without reserpine. A, heart rate as percentage of the basal rate existing before start of nethalide infusion. B, systemic arterial pressure as percentage of the basal pressure existing before start of nethalide infusion. Black bar indicates nethalide infusion (333 µg/kg/min). Crosses, reserpine pretreated dog; solid circles, without reserpine. Control heart rate and arterial pressure of treated dog are 126 beats/min and 115 mm Hg. Control heart rate and arterial pressure of dog without reserpine are 136 beats/min and 120 mm Hg.

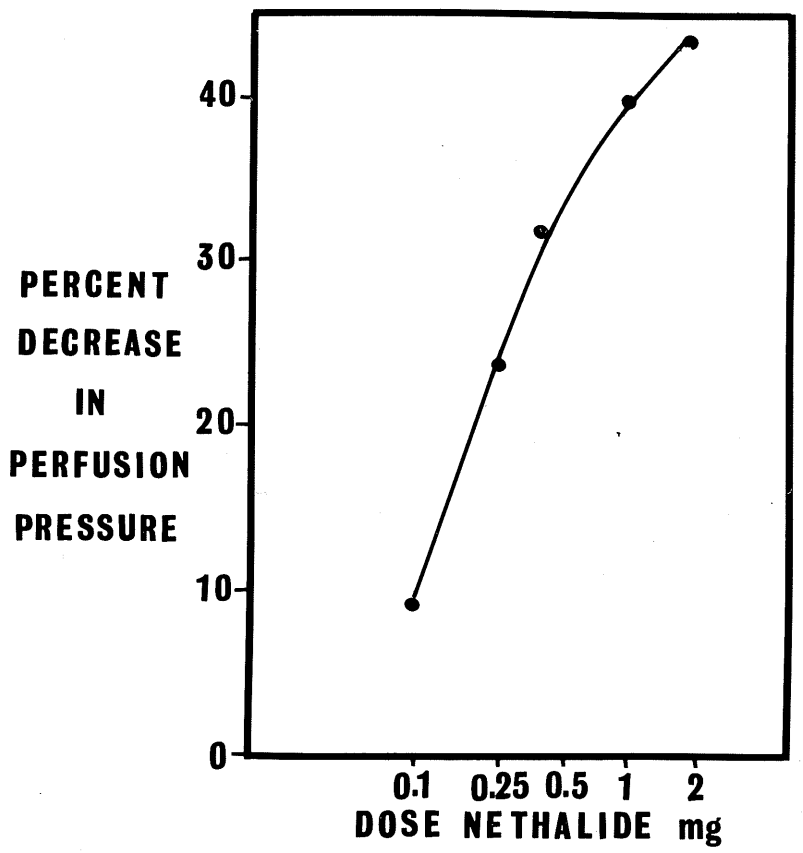

Figure 3. Effect of intra-arterial injections of nethalide on the perfusion pressure of a denervated, blood-perfused hind limb. Abscissa, dose of nethalide. Ordinate, percent decrease in perfusing pressure. Control perfusion pressure equals 100 mm Hg.

The fall in perfusion pressure ranged from 10 to 50 mm Hg depending on the dose. The depressor response occurred immediately after the injection, but the perfusion pressure always returned to control level within ten minutes. These results indicate that nethalide is a potent vasodilator. The magnitude of the decreases in perfusion pressure was great enough to suggest that the hypotension caused by nethalide is due principally to a decrease in peripheral resistance. This suggestion was supported by an additional observation in two of these same animals. Fifteen minutes after giving the last intra-arterial injection, nethalide (10 mg/kg) was rapidly injected intravenously. This was done so that the fall in the systemic arterial pressure could be compared to the fall in the perfusion pressure. In one dog the systemic arterial pressure fell by 50 mm Hg while the perfusion pressure fell by 30 mm Hg. In the other dog, however, both the systemic arterial pressure and the perfusion pressure decreased by 75 mm Hg; the record from this dog is shown in Figure 4.

If a decreased cardiac output were chiefly responsible for the hypotension, the fall in systemic arterial pressure would have been much greater than the fall in perfusion pressure, since the flow to the limb was constant. Since this was not the case, these observations support the suggestion that decreased peripheral resistance is the chief cause of nethalide induced hypotension. However, the observation that the systemic arterial pressure fell 20 mm Hg more than the perfusion pressure in one animal indicates that reduced cardiac output may have contributed to the hypotension.

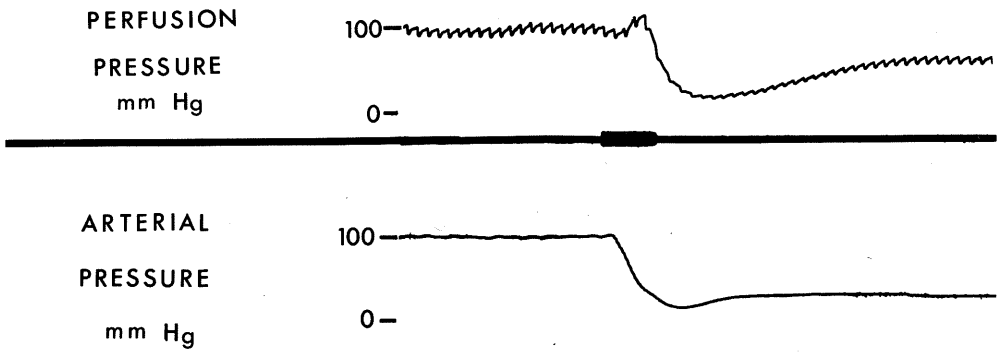

Figure 4. Effect of intravenous injection of nethalide (10 mg/kg) on the perfusion pressure of the denervated, blood-perfused hind limb and on the systemic arterial pressure. Signal indicates injection of nethalide.

SECTION II

THE EFFECT OF OUABAIN ON BLOCKADE OF β RECEPTORS BY NETHALIDE

Introduction

Mendez, Aceves, and Mendez (1961) showed that the cardiac glycoside acetyldigitoxin inhibited the cardioaccelerator action of adrenaline on the heart. It was the purpose of the present work to determine whether the cardiac glycoside ouabain modified the blockade of β receptors caused by nethalide both in the heart and in the blood vessels. The cardioaccelerator and vasodepressor responses to isoproterenol were taken as an index of activity of sympathomimetic amines acting on β receptors.

Two series of experiments were carried out on vagotomized dogs. In the first series the degree of β receptor blockade due to nethalide was tested before and after ouabain by giving graded doses of isoproterenol intravenously and constructing dose response curves. In the second series blockade was tested before and after ouabain with repeated injections of a fixed dose of isoproterenol.

A. The effect of ouabain on β receptor blockade in the heart as tested with graded doses of isoproterenol.

In this series of experiments on nine dogs, increases in the heart rate due to five doses of isoproterenol (0.25 to 4.0 μg/kg) were recorded before other drugs were given. In order to eliminate the risk of arrhythmias doses of isoproterenol larger than 4.0 μg/kg were not given. Nethalide (20 mg/kg) was then infused over 60 minutes. After nethalide, the responses to five more graded doses of isoproterenol (4.0 to 64 μg/kg) were measured. The high doses of isoproterenol after

nethalide were necessary to cause increases in the heart rate comparable to those in the control period. The same high doses were repeated 20 minutes after giving ouabain (50 μg/kg), and the attendant increases in heart rate measured. Higher doses of ouabain could not be used because of the frequent occurrence of arrhythmias. Four animals developed arrhythmias with the ouabain dosage at 50 μg/kg. These animals are not included in the series.

Nethalide greatly reduced the cardioaccelerator responses to isoproterenol and the responses were partially restored after ouabain in all nine dogs. The results were similar whether the heart rate was expressed as an absolute increase (beats/min) or as a percentage of the basal rate recorded just before each injection of isoproterenol. Figure 5 illustrates a typical experiment.

B. The effect of ouabain on β receptor blockade in the heart as tested with a fixed dose of isoproterenol.

The first nine experiments of this section showed that the inhibiting effect of nethalide on the cardioaccelerator responses to isoproterenol was less marked after giving ouabain. There are three possible explanations for these results:

1. The β receptor blockade caused by nethalide may have lessened during the course of the experiment.
2. Ouabain may have sensitized the heart so that it was more responsive to isoproterenol regardless of nethalide blockade.
3. Ouabain may have attenuated the β receptor blockade caused by nethalide.

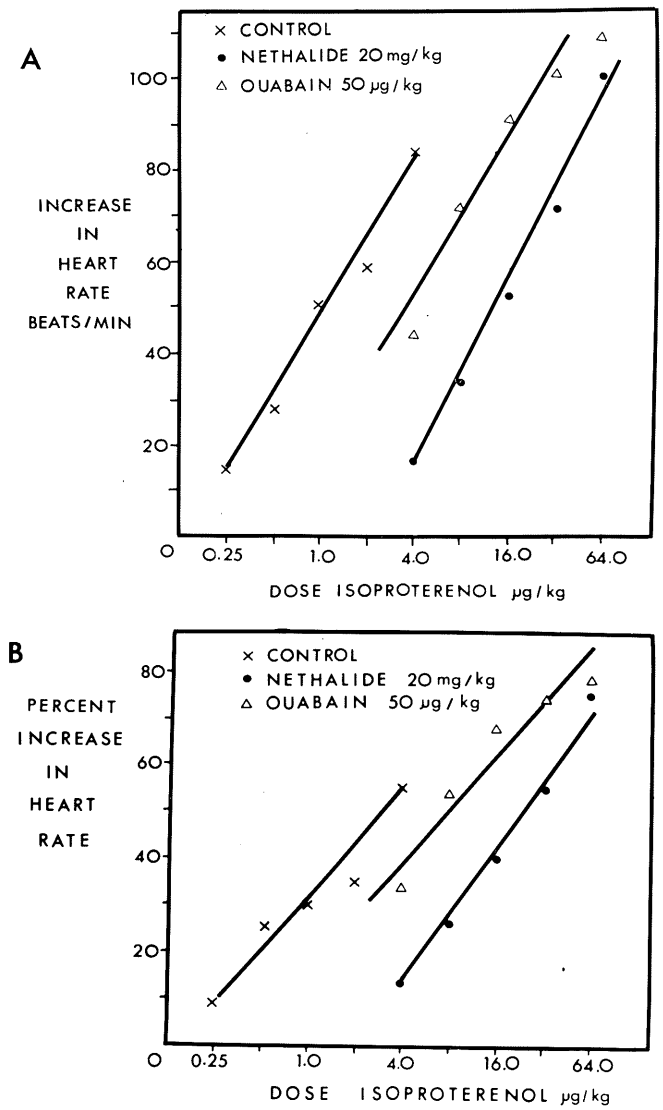

Figure 5. Inhibition of the cardioaccelerator responses to isoproterenol by nethalide, and partial recovery of the response after ouabain. A, increase in heart rate (beats/min). B, the same increases in heart rate expressed as percentage of the basal rate. Crosses indicate responses during the control period; dots indicate responses after completion of nethalide infusion; triangles indicate responses after the nethalide infusion and ouabain (50 µg/kg).

Experiments on 16 vagotomized dogs were carried out to determine which of these explanations applied. The experiments were divided into four groups with four dogs in each group. To facilitate statistical comparisons a fixed dose of isoproterenol was used in these experiments rather than graded doses.

1. The effect of ouabain on the cardioaccelerator responses to isoproterenol after nethalide.

Control responses to isoproterenol (2.0 μg/kg) were obtained. Nethalide (20 mg/kg) was then infused over a 60 minute period. Beginning 20 minutes after the infusion, the degree of β receptor blockade was tested with five injections of isoproterenol (2.0 μg/kg) at ten minute intervals. Ouabain (50 μg/kg) was given ten minutes after the last injection of isoproterenol. Twenty minutes were allowed for the onset of ouabain action, and the degree of blockade was tested a second time with five injections of isoproterenol (2.0 μg/kg) at ten minute intervals. The mean increases in heart rate caused by isoproterenol before and after ouabain were compared in each experiment (Table 1a).

The cardioaccelerator responses to isoproterenol were increased after ouabain in all four dogs. The mean difference in the responses before and after ouabain was 19.5 beats per minute (Table 1a). This increase was statistically significant by the "t" test for paired data ($P < 0.01$). The mean basal heart rate (the rate just before each injection of isoproterenol) was not significantly different after ouabain (Table 1b).

2. The effect of time on the cardioaccelerator responses to isoproterenol after nethalide.

TABLE 1

THE EFFECT OF OUABAIN ON THE CARDIOACCELERATOR RESPONSES TO ISOPROTERENOL AFTER NETHALIDE

a) Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2.0 μg/kg) before and after ouabain (50 μg/kg).

Dog No.	A Increase in H.R. before ouabain	B Increase in H.R. after ouabain	Difference (B-A)
1	11	32	21
2	9	23	14
3	8	34	26
4	6	23	17
Mean	8.5	28.0	19.5
			S.E. ± 2.6
			t = 7.5
			P < 0.01

b) Mean basal heart rate (mean of rates just before injection of isoproterenol) before and after ouabain (50 μg/kg).

Dog No.	A Basal H.R. before ouabain	B Basal H.R. after ouabain	Difference (B-A)
1	145	141	-4
2	163	170	7
3	180	208	28
4	139	142	3
Mean	156.8	165.3	8.5
			S.E. ± 6.9
			t = 1.2
			P > 0.30

Control experiments were done in a second group of four dogs to determine whether the above results could be explained by the natural decay of β receptor blockade during the course of the experiments. The experimental procedure was identical to that used in the preceding group except that an injection of 0.9% saline (5.0 ml) was substituted for the injection of ouabain. The time taken for an experiment in each group was two hours after the completion of the nethalide infusion. A statistically significant increase in the cardioaccelerator responses occurred after saline; the mean increase was 10.7 beats per minute (Table 2a). The mean basal heart rate was not significantly different after saline (Table 2b).

These results indicate that the passage of time is a contributing factor in the partial recovery of the cardioaccelerator response to isoproterenol. However, comparison of the four control dogs with the four dogs treated with ouabain showed the mean increase in the cardioaccelerator response was 8.8 beats per minute greater in animals given ouabain than the corresponding increase in animals given only saline. This was a statistically significant difference by Student's "t" test ($P < 0.05$). Therefore ouabain was a factor in the partial restoration of the positive chronotropic action of isoproterenol after establishment of β receptor blockade by nethalide. The four animals treated with ouabain are contrasted to the four control animals in Figure 6.

3. <u>The effect of ouabain on the cardioaccelerator responses to isoproterenol in the absence of nethalide</u>.

Experiments were carried out in a third group of four dogs to determine whether the enhanced action of isoproterenol observed after ouabain was due to a sensitizing effect of ouabain on the heart. Five

TABLE 2

THE EFFECT OF TIME ON THE CARDIOACCELERATOR RESPONSES TO ISOPROTERENOL AFTER NETHALIDE

a) Mean increase in heart rate (beats/min) due to five injections of isoproterenol (2.0 μg/kg) before and after saline.

Dog No.	A Increase in H.R. before saline	B Increase in H.R. after saline	Difference (B-A)
5	8	19	11
6	1Ø	26	16
7	11	20	9
8	6	13	7
Mean	8.8	19.5	10.7
			S.E. ± 1.7 t = 5.5 P < 0.02

b) Mean basal heart rates (mean of rates just before injection of isoproterenol) before and after saline.

Dog No.	A Basal H.R. before saline	B Basal H.R. after saline	Difference (B-A)
5	131	143	12
6	17O	172	2
7	133	144	11
8	117	119	2
Mean	137.8	144.6	6.8
			S.E. ± 2.75 t = 2.47 P > 0.05

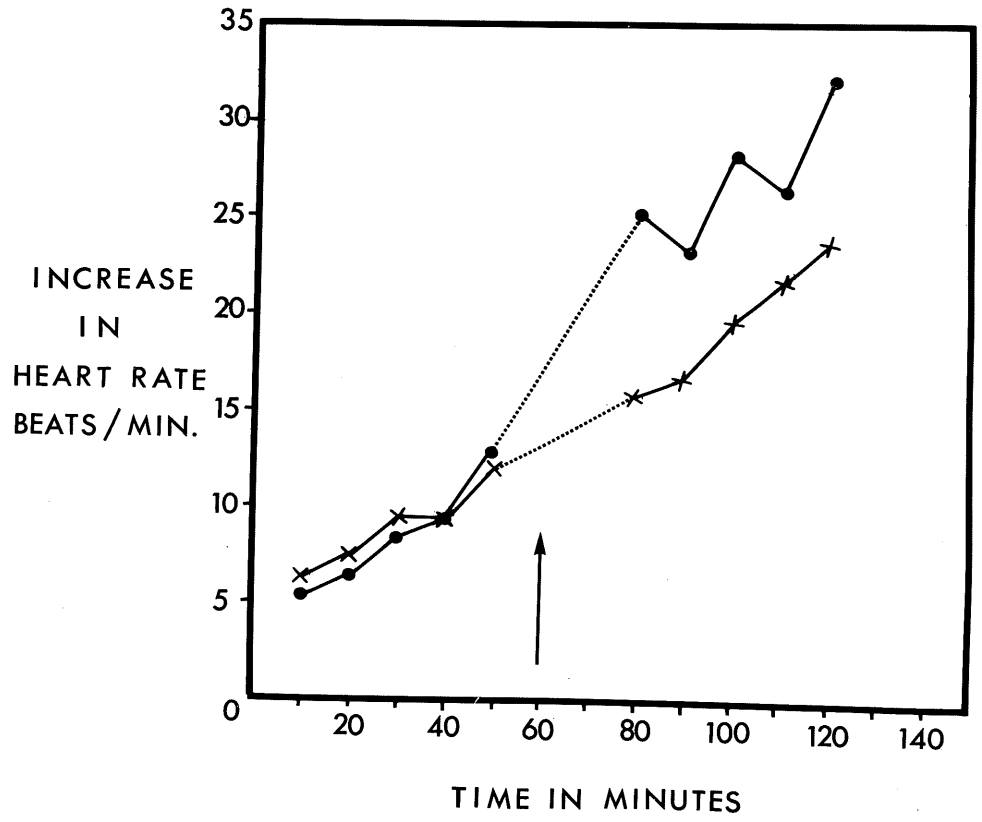

Figure 6. The increasing chronotropic responses of the heart to isoproterenol with time after β receptor blockade by nethalide (20 mg/kg). Nethalide infusion was complete at time 0. Each point represents the mean increase in heart rate (beats/min) caused by intravenous injections of isoproterenol (2.0 μg/kg) in four dogs. Dots, dogs given ouabain (50 μg/kg) at the arrow. Crosses, control dogs given saline (5 ml) at the arrow.

injections of isoproterenol (2.0 μg/kg) were given at ten minute intervals before and after ouabain (50 μg/kg). Twenty minutes were allowed for the onset of ouabain action before beginning the second series of injections. The mean increases in heart rate were compared as in the first two groups.

The cardioaccelerator responses were less after ouabain in all four dogs. The mean decrease was 11.5 beats per minute (Table 3a). This decrease was statistically significant by the "t" test for paired data ($P < 0.05$). There was no consistent change in the mean basal heart rate after ouabain (Table 3b).

4. The cardioaccelerator response to isoproterenol before and after saline.

Control experiments were done in a fourth group of four dogs to determine whether the decreased response to isoproterenol after ouabain was due to tachyphylaxis. The experimental procedure was identical to that in the third group of experiments except that an injection of 0.9% (5.0 ml) saline was substituted for the injection of ouabain. There was no statistically significant difference in either the cardioaccelerator response to isoproterenol or in the mean basal heart rate after saline (Table 4a and b). Therefore it is apparent that ouabain antagonized the cardioaccelerator responses to isoproterenol.

The increased responsiveness of the heart to isoproterenol after ouabain in A and B-1 could not be due to a sensitization by ouabain, since ouabain was shown to inhibit the positive chronotropic action of isoproterenol. Therefore it is probably due to an attenuation by ouabain of the β receptor blockade caused by nethalide.

TABLE 3

THE EFFECT OF OUABAIN ON THE CARDIOACCELERATOR RESPONSES

TO ISOPROTERENOL IN THE ABSENCE OF NETHALIDE

a) Mean increase in heart rate (beats per minute) due to five injections of isoproterenol (2 μg/kg) before and after ouabain (50 μg/kg).

Dog No.	A Increase in H.R. before ouabain	B Increase in H.R. after ouabain	Difference (A-B)
9	59	40	19
10	75	67	8
11	76	62	14
12	60	55	5
Mean	67.5	56.0	11.5
			S.E. ± 3.43
			t = 3.35
			P < 0.05

b) Mean basal heart rate (mean of rates just before injection of isoproterenol) before and after ouabain (50 μg/kg).

Dog No.	A Basal H.R. before ouabain	B Basal H.R. after ouabain	Difference (B-A)
9	164	169	5
10	153	165	12
11	173	166	-7
12	164	154	-10
Mean	163	163	0

TABLE 4

THE CARDIOACCELERATOR RESPONSES TO ISOPROTERENOL

BEFORE AND AFTER SALINE

a) Mean increase in heart rate (beats per minute) due to five injections of isoproterenol (2.0 μg/kg) before and after saline.

Dog No.	A Increase in H.R. before saline	B Increase in H.R. after saline	Difference (A-B)
13	56	52	4
14	84	83	1
15	37	35	2
16	62	63	-1
Mean	59.7	58.2	1.5

b) Mean basal heart rate (mean of rates just before injection of isoproterenol before and after saline.

Dog No.	A Basal H.R. before saline	B Basal H.R. after saline	Difference (B-A)
13	181	182	1
14	132	150	18
15	182	181	-1
16	152	162	10
Mean	162	169	7.0
			S.E. ± 4.37
			t = 1.6
			P > 0.20

C. The effect of ouabain on β receptor blockade in the blood vessels as tested with graded doses of isoproterenol.

To determine whether ouabain affected β receptor blockade in the blood vessels, decreases in the blood pressure in response to graded doses of isoproterenol were recorded at the same time as were the increases in heart rate in the nine dogs reported in Section II A. Nethalide inhibited the vasodepressor responses to isoproterenol, and the responses were partially restored after the subsequent administration of ouabain in all nine dogs. The results were similar whether the change in blood pressure was expressed as the absolute decrease (mm Hg) or as the percentage of the basal pressure existing just before each injection of isoproterenol. The results illustrated in Figure 7 are typical of these experiments. However, for reasons similar to those stated in Section II B conclusions could not be drawn from these first nine experiments.

D. The effect of ouabain on β receptor blockade in the blood vessels as tested with a fixed dose of isoproterenol.

Decreases in the blood pressure in response to isoproterenol (2.0 μg/kg) were recorded at the same time as were the changes in heart rate in all sixteen dogs reported in Section II B 1-4. The mean decreases in blood pressure (mm Hg) were compared in the same way as were the increases in heart rate.

1. The effect of ouabain on the vasodepressor responses to isoproterenol after nethalide.

Analysis of the blood pressure data from the first group of four dogs (B-1) showed that the vasodepressor responses to isoproterenol

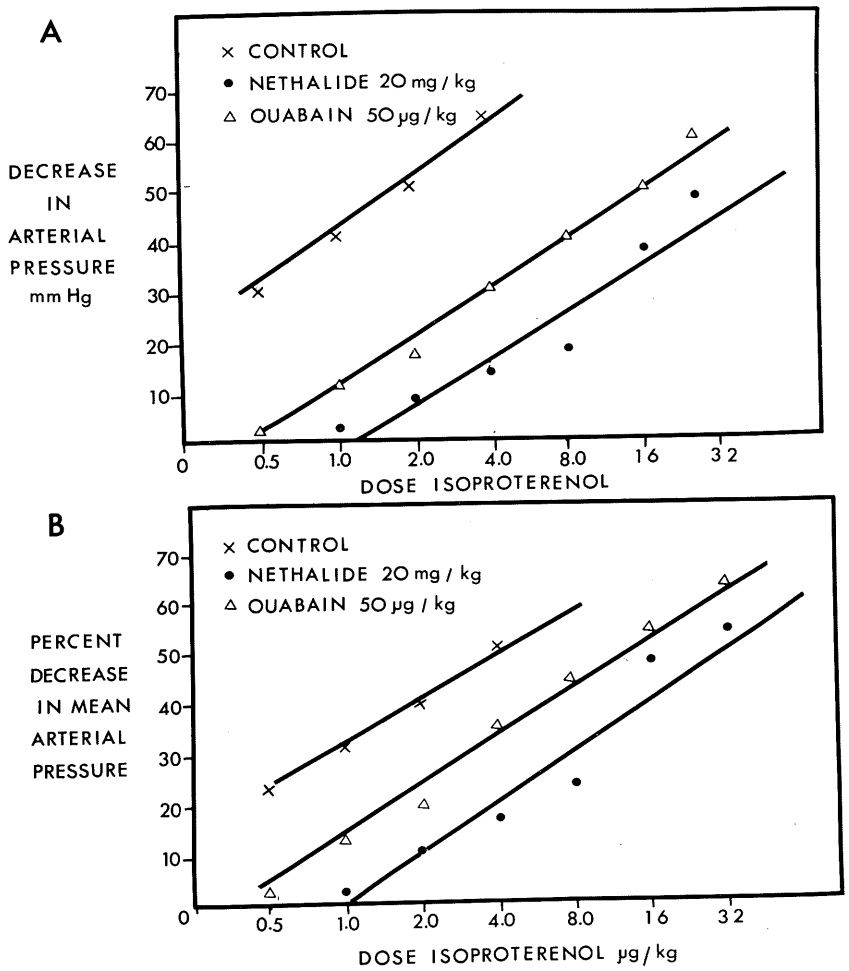

Figure 7. Inhibition of the vasodepressor responses to isoproterenol by nethalide, and partial recovery of the response after ouabain. A, decreases in mean arterial pressure (mm Hg). B, the same decreases expressed as a percentage of the basal pressure. Crosses indicate responses during control period; dots indicate responses after completion of nethalide infusion (20 mg/kg); triangles indicate responses after the nethalide infusion and ouabain (50 µg/kg).

TABLE 5

THE EFFECT OF OUABAIN ON THE VASODEPRESSOR RESPONSES TO ISOPROTERENOL AFTER NETHALIDE

a) Mean decrease in mean blood pressure (mm Hg) due to five injections of isoproterenol (2.0 μg/kg) before and after ouabain (50 μg/kg).

Dog No.	A Decrease in B.P. before ouabain	B Decrease in B.P. after ouabain	Difference (B-A)
1	23	30	7
2	18	33	15
3	16	35	19
4	17	21	4
Mean	18.5	29.8	11.3
			S.E. ± 3.5 t = 3.22 P < 0.05

b) Mean basal blood pressure (mean of pressures just before injection of isoproterenol) before and after ouabain (50 μg/kg).

Dog No.	A Basal B.P. before ouabain	B Basal B.P. after ouabain	Difference (B-A)
1	100	105	5
2	94	130	36
3	120	121	1
4	71	83	12
Mean	96.3	109.8	13.5
			S.E. ± 7.8 t = 1.7 P > 0.10

were increased after ouabain in all four dogs (Table 5a). The mean difference between the responses before and after ouabain was 11.3 mm Hg. This difference was statistically significant by the "t" test for paired data ($P < 0.05$). There was no statistically significant change in the mean basal blood pressure (the mean of the blood pressures just before each injection of isoproterenol) after ouabain (Table 5b).

2. The effect of time on the vasodepressor responses to isoproterenol after nethalide.

The control experiments on the second group of four dogs (B-2) showed that the vasodepressor responses increased by a mean of 7.5 mm Hg after saline (Table 6a). This increase was not statistically significant by the "t" test for paired data ($P > 0.05$). The basal blood pressure was not significantly changed after saline (Table 6b).

The mean vasodepressor responses to isoproterenol was 3.8 mm Hg greater in dogs given ouabain (group D-1) than in dogs given saline (group D-2). However, this difference was not statistically significant by Student's "t" test ($P > 0.60$).

3. The effect of ouabain on the vasodepressor responses to isoproterenol in the absence of nethalide.

The experiments carried out in the third group of four dogs (B-3) showed that the vasodepressor response to isoproterenol was 4.0 mm Hg less after ouabain in animals not treated with nethalide. However, this difference was not statistically significant (Table 7a). The mean basal blood pressure was 4.7 mm Hg less after ouabain (Table 7b). This slight decrease was statistically significant by the "t" test for paired data ($P < 0.05$).

TABLE 6

THE EFFECT OF TIME ON THE VASODEPRESSOR RESPONSES TO ISOPROTERENOL AFTER NETHALIDE

a) Mean decrease in mean blood pressure (mm Hg) due to five injections of isoproterenol (2.0 μg/kg) before and after saline.

Dog No.	A Decrease in B.P. before saline	B Decrease in B.P. after saline	Difference (B-A)
5	16	30	14
6	29	36	7
7	23	25	2
8	6	13	7
Mean	18.5	26	7.5
			S.E. ± 2.5 t = 3.0 P > 0.05

b) Mean basal blood pressure (mean of pressures just before injection of isoproterenol) before and after saline.

Dog No.	A Basal B.P. before saline	B Basal B.P. after saline	Difference (B-A)
5	79	94	15
6	84	94	10
7	100	91	-9
8	55	69	14
Mean	79.5	87.0	7.5
			S.E. ± 5.6 t = 1.3 P > 0.20

TABLE 7

THE EFFECT OF OUABAIN ON THE VASODEPRESSOR RESPONSES

TO ISOPROTERENOL IN THE ABSENCE OF NETHALIDE

a) Mean decrease in mean blood pressure (mm Hg) due to five injections of isoproterenol (2.0 μg/kg) before and after ouabain (50 μg/kg).

Dog No.	A Decrease B.P. before ouabain	B Decrease B.P. after ouabain	Difference (A-B)
9	44	33	11
10	59	56	3
11	48	45	3
12	44	45	-1
Mean	48.75	44.75	4.0
			S.E. ± 2.51 t = 1.59 P > 0.20

b) Mean basal blood pressure (mean of pressures just before injection of isoproterenol) before and after ouabain (50 μg/kg).

Dog No.	A Decrease B.P. before ouabain	B Decrease B.P. after ouabain	Difference (A-B)
9	92	84	8
10	101	96	5
11	92	90	2
12	79	75	4
Mean	91	86.2	4.7
			S.E. ± 1.25 t = 3.76 P < 0.05

4. The vasodepressor response to isoproterenol before and after saline.

The control experiments in the fourth group of dogs showed that there was no statistically significant change in either the magnitude of the vasodepressor responses to isoproterenol or in the basal blood pressure after saline in dogs not treated with nethalide (Table 8a).

TABLE 8

THE VASODEPRESSOR RESPONSE TO ISOPROTERENOL BEFORE AND AFTER SALINE

a) Mean decrease in mean blood pressure (mm Hg) due to five injections of isoproterenol (2.0 μg/kg) before and after saline.

Dog No.	A Decrease in B.P. before saline	B Decrease in B.P. after saline	Difference (A-B)
13	66	56	10
14	64	58	6
15	58	68	-10
16	61	58	3
Mean	62.2	60	2.2
			S.E. ± 4.33 t = 0.51 P > 0.60

b) Mean basal blood pressure (mean of pressures just before injection of isoproterenol) before and after saline.

Dog No.	A Basal B.P. before saline	B Basal B.P. after saline	Difference (A-B)
13	120	104	16
14	145	130	15
15	88	99	-11
16	98	96	2
Mean	113	107	5.5
			S.E. ± 6.36 t = 0.86 P > 0.40

SECTION III

THE EFFECT OF NETHALIDE ON OUABAIN-INDUCED ARRHYTHMIAS

Introduction

The results in section II showed that ouabain lessened the β receptor blockade caused by nethalide. It was also of interest to find whether the antagonism was mutual, i.e. if nethalide had any influence on the effect of ouabain. Experiments were therefore carried out to determine whether nethalide altered arrhythmias induced by high doses of ouabain.

A. Temporary restoration of sinus rhythm.

Arrhythmias varying in severity, A-V nodal rhythm, bigeminal rhythm, and ventricular tachycardia, were induced with toxic doses of ouabain (75 to 1ØØ μg/kg) in seven vagotomized dogs. In each dog several injections of nethalide were made during a period of two to five hours after the arrhythmia first appeared. Arrhythmia was allowed to persist for a period of five to twenty minutes before each injection of nethalide. Rapid (less than three seconds) intravenous injections of nethalide (5.0 mg/kg) temporarily restored sinus rhythm on each of several trials in all seven dogs. Sinus rhythm appeared within thirty seconds after injecting nethalide and persisted from one to twelve minutes before re-emergence of the arrhythmia. Figure 8 shows Lead II electrocardiograms from one of these experiments. The data are presented in Table 9.

B. Maintenance of sinus rhythm.

Experiments were done in four vagotomized dogs to determine

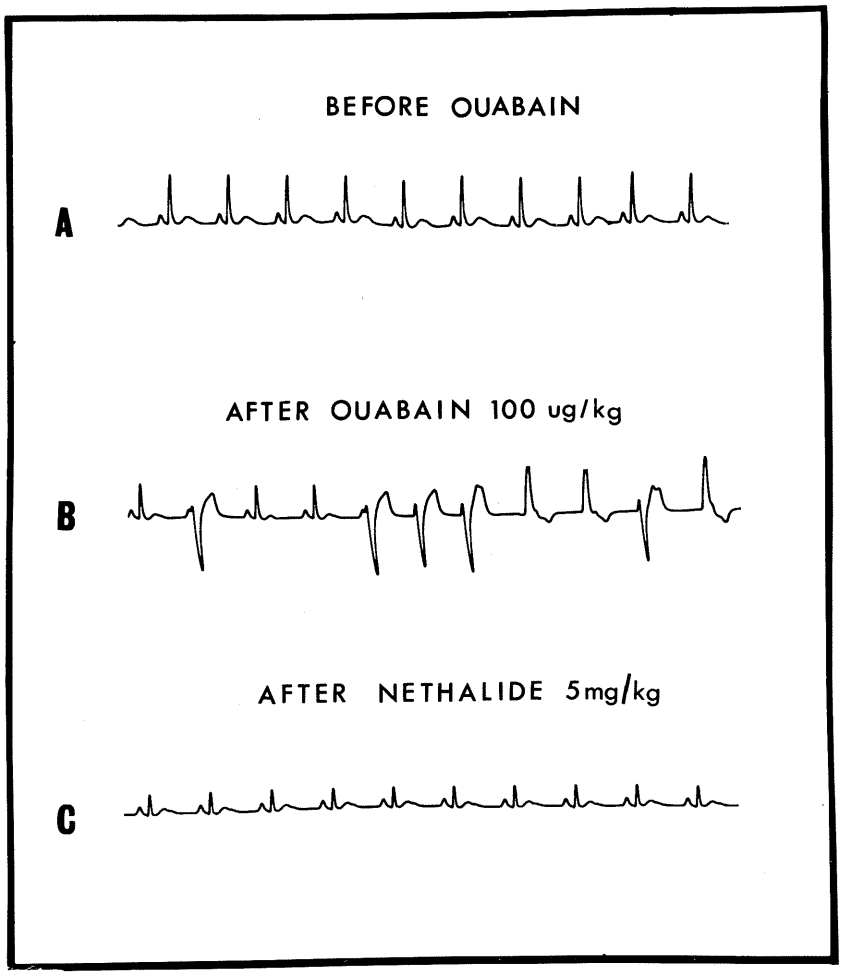

Figure 8. Lead II ECG tracing: A, sinus rhythm before ouabain; B, arrhythmia after ouabain (100 μg/kg); C, sinus rhythm after nethalide (5.0 mg/kg). Paper speed of recorder, 25 mm/sec.

TABLE 9

CONVERSION TO NORMAL SINUS RHYTHM OF OUABAIN-INDUCED ARRHYTHMIAS BY NETHALIDE

Dog No.	Dose (μg/kg) Ouabain	Type of Arrhythmia	No. of Trials	No. of Conversions	Duration of Sinus Rhythm
1	75	Nodal Rhythm	3	3	4-12 min
2	85	Nodal Rhythm	4	4	6-11 min
3	85	Bigeminal Rhythm	5	5	2-8 min
4	85	Ventricular Tachycardia	4	4	7-11 min
5	85	Ventricular Tachycardia	5	5	1-3 min
6	85	Ventricular Tachycardia	4	4	1-3 min
7	100	Ventricular Tachycardia	9	9	1-9 min

whether sinus rhythm could be maintained by a constant intravenous infusion of nethalide. Ventricular tachycardia appeared in all four dogs within thirty minutes of administering ouabain (86 μg/kg). The arrhythmias was immediately abolished by a single injection of nethalide (5.0 mg/kg). As soon as normal sinus rhythm was restored a constant infusion of nethalide (220 μg/kg/min) was started. Sinus rhythm was maintained by the infusion for the duration of the experiment (five hours).

Control experiments in three dogs were done to determine how long the arrhythmia would last if left untreated. Ventricular tachycardia persisted for over five hours in two dogs. The third died of ventricular fibrillation ninety minutes after being given ouabain (85 μg/kg).

Experiments with a constant infusion of nethalide (220 μg/kg/min) were carried out in two dogs in which each dog served as its own control on different days. Since the dogs were to be kept alive for seven days between the first and second experiment the vagi were left intact. A nodal rhythm was induced in the first dog with ouabain (85 μg/kg). The arrhythmia was abolished, and the heart maintained in sinus rhythm by nethalide given as in the previous experiments. Sinus rhythm was still present when the experiment was ended and the dog returned to its cage five hours later. Seven days after the first experiment the dog was treated a second time with ouabain (85 μg/kg), but nethalide was withheld. The animal died of ventricular fibrillation within ninety minutes after the administration of ouabain.

In the second dog ouabain (85 μg/kg) was given, causing a ventricular tachycardia which was still present two hours later when the animal was returned to its cage. No nethalide was given at this stage of the experiment. Seven days later ventricular tachycardia was induced a second

time with ouabain (85 μg/kg). The arrhythmia was abolished and sinus rhythm maintained for two hours by nethalide in contrast to the persistence of the arrhythmia for an equal period when nethalide had been withheld.

C. Exclusion of the depressor effect of nethalide as the mechanism of arrhythmia antagonism.

The administration of ouabain (50 μg/kg and over) always resulted in a prompt rise in the arterial pressure. The increase in pressure varied from 10 to 125 mm Hg in different experiments and occurred within two minutes of giving the ouabain. The onset of the arrhythmia was within 20 minutes of administering the ouabain; the pressor response caused by ouabain was still present at this time. Conversion of the arrhythmia to sinus rhythm was always preceded by a sharp fall in the pressure (20 to 60 mm Hg) due to the vasodepressor effect of nethalide. Therefore five experiments were carried out to determine whether the depressor response to nethalide was related to the antagonism of the arrhythmia.

Arrhythmias were induced with ouabain (85 μg/kg). Rapid lowering of the blood pressure (50 mm Hg in 15 sec) by haemorrhage had no effect on the arrhythmia in three dogs. The rate of fall in blood pressure caused by the haemorrhage was comparable to the rate of fall caused by nethalide. Furthermore, a rapid injection of nethalide (5.0 mg/kg) was still effective in converting the arrhythmia to sinus rhythm when the depressor response was prevented by intra-arterial infusions of blood under pressure in the other two dogs.

D. Effect of nethalide on survival time after lethal doses of ouabain

A lethal dose of ouabain (130 to 150 μg/kg) was injected intravenously into each of eight dogs. Nethalide infusion (220 μg/kg/min) was

begun immediately after the ouabain in four dogs. The other four dogs served as controls, and were given infusions of 0.9% saline instead of nethalide. The data from these experiments are presented in Table 10. Nethalide did not prevent the development of the arrhythmias, but the survival time (the time from the administration of ouabain to death) was 138 minutes longer in animals given nethalide than in the controls. This difference was statistically significant by Student's "t" test ($P < 0.01$).

TABLE 10

EFFECTS OF NETHALIDE ON SURVIVAL TIME AFTER

LETHAL DOSES OF OUABAIN

DOSE OUABAIN µg/kg	MINUTES TO DEATH CONTROLS	MINUTES TO DEATH NETHALIDE
130	50	420*
130	30	150
150	5	56
150	29	42
MEANS	28.5	167.0
Difference = 138.5 ($P < 0.01$)		

* Still alive after 7 hours.

DISCUSSION

THE EFFECT OF NETHALIDE ON HEART RATE AND BLOOD PRESSURE

It is concluded that the bradycardia caused by nethalide is due to blockade of the positive chronotropic action of endogenous catecholamines. This conclusion is based on the observation that nethalide increased rather than decreased the heart rate of dogs depleted of catecholamines by pretreatment with reserpine. These results add to the findings of Black and Stephenson (1962) who found that the degree of bradycardia caused by nethalide was reduced but still present after acute bilateral stellate ganglionectomy. Their work indicates that only part of the bradycardia is due to blockade of noradrenaline released from the cardio-accelerator nerves arising from the stellate ganglia. Cannon, Lewis, and Britton (1926) demonstrated in cats the presence of accessory accelerator fibres which came from sympathetic ganglia located below the stellate ganglia. Therefore the work of Black and Stephenson does not exclude the possibility that the adrenergic blocking action of nethalide on the heart is the entire cause of bradycardia.

In recent years evidence from experiments on animals depleted of catecholamines by reserpine has accumulated showing that catecholamines released from myocardial stores influence the heart rate. Innes and Krayer (1958) suggested the catecholamines released from stores near the pacemaker were a factor in the regulation of the heart rate. Roberts and Stadter (1961) showed both atrial and ventricular rhythmicity were in part dependent on catecholamine stores. Lee and Shideman (1959) have concluded that there is a constant release of catecholamines from myocardial stores which are important in the regulation of both rate and contractility.

The positive chronotropic action of nethalide in dogs pretreated

with reserpine shows that nethalide has intrinsic sympathomimetic activity in the heart. The intrinsic activity is apparently masked by its adrenergic blocking action in normal animals, and bradycardia is the result of a competition between nethalide and endogenous catecholamines for the same receptor sites. Therefore the negative chronotropic effect of nethalide must be secondary to adrenergic blockade.

Although nethalide stimulated the heart in reserpine pretreated dogs, as judged from increases in heart rate, it still caused hypotension. This observation suggests that the hypotension is due chiefly to a peripheral vascular effect rather than a direct depressant action on the heart. It is clear from the experiments on the perfused hind limb that nethalide causes vasodilatation. The marked decreases in perfusion pressure occurring after intra-arterial injections of nethalide can be accounted for only by a decrease in resistance, since the blood flow to the limb was held constant. The vasodilatating effect of nethalide is most likely due to a direct action on the resistance vessels. Changes in resistance due to reflex activity of the autonomic nervous system were excluded by denervation of the limb. The results obtained from the dog hind limb are consistent with the findings of Dornhorst and Robinson (1962) who showed that the blood flow in the forearm of man was increased by intra-arterial injections of nethalide.

Black and Stephenson (1962) presented ample evidence that nethalide blocks both the positive chronotropic and inotropic actions of catecholamines. Therefore the administration of nethalide will result in a decrease in contractility if the degree of contractility under the experimental conditions depends on endogenous catecholamines. Lee and Shideman

(1959) have suggested that there is a continuous release of small amounts of catecholamines which serve to regulate normal cardiac function. Their conclusions are based on experiments showing that there is a significant reduction in contractility of papillary muscles taken from cat hearts depleted of catecholamines either by pretreatment with reserpine or sympathectomy. If nethalide decreases myocardial contractility, decreased cardiac output would be expected to result. A reduced cardiac output could also result from a loss of venous tone, but at present there is no evidence to show whether nethalide has an effect on veins.

The experiments on the perfused hind limb indicate that a reduction in cardiac output makes at most a minor contribution to the hypotension induced by nethalide, and that the principal cause of hypotension is peripheral vasodilatation. Support for this conclusion comes from the observation that nethalide given in a single intravenous injection caused a fall in the perfusion pressure of the hind limb which was comparable in magnitude to the fall in the systemic arterial pressure. If decreased cardiac output were chiefly responsible for the hypotension, the fall in the systemic arterial pressure would have been much greater than the fall in perfusion pressure since the flow to the hind limb was kept constant.

The latter results are not entirely in agreement with the conclusions of Black and Stephenson (1962). They attributed the hypotension occurring with slow intravenous infusions of nethalide to peripheral vasodilatation. On the other hand, they suggested that reduced cardiac output was responsible for the hypotension associated with rapid single injections of nethalide. They implied that reduced cardiac output resulted from myocardial depression. However, the conclusions made by Black and Stephenson were based on indirect measurements. Reduced cardiac output

was assumed to be a factor because rapid single injections of nethalide decreased myocardial contractility. Contractility was measured with a Cushny myocardiograph in cats, and in dogs with either a ballistocardiograph or strain gauge arch. Clearly, these measurements are inadequate to permit valid conclusions about cardiac output. The evidence that decreased peripheral resistance was involved when slow intravenous infusions of nethalide were given was based on inconsistent results, i.e. they found that the aortic blood flow was reduced in only one of four dogs.

The present results do not support the statement of Black and Stephenson (1962) that nethalide has no intrinsic activity. Although the intrinsic activity of nethalide in the heart is apparent only in animals depleted of catecholamines, the intrinsic activity of nethalide in the blood vessels is apparent in normal as well as catecholamine-depleted animals.

THE EFFECT OF OUABAIN ON BLOCKADE OF β RECEPTORS BY NETHALIDE

The data presented indicate that ouabain antagonizes the adrenergic blocking action of nethalide on the heart. A preliminary series of experiments showed that nethalide inhibited the cardioaccelerator responses to graded doses of isoproterenol as indicated by a parallel shift in the dose-response curve to the right. This confirmed the work of Black and Stephenson (1962) showing that nethalide is a potent β receptor blocking agent. Ouabain, on the other hand, appeared to enhance the positive chronotropic action of isoproterenol after establishment of β receptor blockade by nethalide. This was reflected in the dose-response curve by a parallel shift to the left.

Definite conclusions could not be drawn from these experiments because it was not known to what extent β receptor blockade diminished during the course of the experiments. Also, there was no way of knowing whether the shift in the dose-response curve toward the left after ouabain was due to a lessening of blockade, or to a non-specific sensitization of the heart by ouabain to the action of isoproterenol. It was for these reasons that control experiments with a fixed dose of isoproterenol were carried out. Fixed doses of isoproterenol were used rather than graded doses because this design gives a smaller statistical variance in the data.

These experiments showed that the shift in the dose-response curve to the left could be accounted for only in part by a diminution of β receptor blockade during the course of the experiments. Ouabain was definitely a factor. Furthermore, ouabain did not sensitize the heart to the action of isoproterenol. In the absence of nethalide ouabain inhibited the cardioaccelerator action of isoproterenol. This was in contrast

to the enhancing action of ouabain in the presence of nethalide. Therefore, it is concluded that ouabain has a double effect in this regard. Whether the effect is enhancement or inhibition depends on the presence or absence of β receptor blockade.

The outstanding feature of this study is that ouabain was shown to oppose both the action of the agonist (isoproterenol) and the antagonist (nethalide). Ariens (1954) and Stephenson (1956) have studied cases of drug antagonism in which a drug of low intrinsic activity opposed the stimulating action of drugs with high intrinsic activity and also the blocking action of the antagonist. Stephenson called such drugs "partial agonists". At present, however, the principles advanced by Ariens and Stephenson cannot be used to explain the double antagonistic effect of ouabain. In the cases cited by Ariens and Stephenson there was evidence to indicate that the "partial agonist", the agonist, and the antagonist competed for the same receptor sites. First, data from dose-response studies on isolated tissues indicated that the antagonism was competitive. Secondly, the "partial agonist", the agonist and the antagonist were usually part of the same homologous series so that there was a close structural similarity between all three drugs. There is no previous evidence to show that ouabain acts on the same receptors as isoproterenol and nethalide.

Although this is the first report of a cardiac glycoside antagonizing the action of an adrenergic blocking agent, other workers have shown that cardiac glycosides inhibit the action of adrenergic stimuli on the heart. Nadeau and James (1963) showed in dogs that acetylstrophanthidin inhibited the chronotropic responses of the heart to adrenaline injected directly into a catheter perfusing the sinus node artery at a

constant flow. Mendez, Aceves, and Mendez (1961) showed that acetyldigitoxin inhibited the cardioaccelerator action of adrenaline and sympathetic nerve stimulation in dogs. The degree of inhibition produced by a given dose of acetyldigitoxin was independent of the dose of adrenaline or the frequency of nerve stimulation. The maximum response after the glycoside was lower than the maximum response before the glycoside. On the basis of these findings, they rightly concluded that the inhibiting action of acetyldigitoxin was not due to competitive antagonism.

It is unlikely that metabolic or distributional changes can account for the two effects (inhibition and enhancement) of ouabain on the cardioaccelerator responses to isoproterenol. Increased destruction of isoproterenol with attendant reduction in its effect should not be altered by the presence of β receptor blockade. Similarly, if isoproterenol is distributed differently after ouabain because of hemodynamic changes, it is also unlikely that the effect would be reversed in the presence of β receptor blockade. Moreover, the experimental technique employed by Nadeau and James (1963) shows that inhibition of the positive chronotropic action of sympathomimetics by cardiac glycosides cannot be explained on a hemodynamic basis.

The data from the preliminary experiments carried out with graded doses of isoproterenol suggested the possibility that ouabain antagonized the adrenergic blocking action of nethalide in the blood vessels. The dose-response curves for the vasodepressor responses to isoproterenol were similar to the curves for the cardioaccelerator responses. Nethalide inhibited the vasodepressor action of isoproterenol. This was shown by a shift in the dose-response curve to the right. As in the case of the cardioaccelerator responses, the vasodepressor responses were less inhibited

after giving ouabain. This was reflected in the dose-response curve by a shift to the left.

The control experiments carried out with a fixed dose of isoproterenol failed to show whether ouabain was the cause of the shift in the dose-response curve to the left. These experiments showed that there was a statistically significant increase in the vasodepressor response to a fixed dose of isoproterenol, after ouabain, in dogs treated with nethalide. There was no significant change in the response in control dogs given saline instead of ouabain. However, it was not possible to conclude from these data whether ouabain antagonized the blocking action of nethalide in the blood vessels. A "t" test between the dogs given ouabain and the control dogs given saline showed that there was no statistically significant difference between the two groups of experiments.

These experiments were designed to determine whether ouabain affected β receptor blockade in the heart. The vasodepressor responses were recorded concurrently with the cardioaccelerator responses and analysed because they were readily available data in the same animals, not because they were considered the best index of events occurring at the receptor level. Clearly interpretation of changes in blood pressure is complicated by many variables. For example the action of ouabain in the heart may have increased the cardiac output enough to mask its effects on the blood vessels. Since ouabain enhances the positive chronotropic action of isoproterenol after establishment of β receptor blockade, the positive inotropic action also is likely to be enhanced, although this point has not yet been tested. Enhancement of the positive inotropic action would lead to an increase in cardiac output which would tend to oppose the vasodepressor response.

More conclusive results could be obtained from a simpler test system such as the isolated perfused limb used in Section I. This system would be free of variations due to the heart. Also, other difficulties could be circumvented with this preparation. For example, in the experiments on the whole animal the dose of ouabain was limited by its cardiac toxicity. In the vascularly isolated limb ouabain could be restricted to the bed under study, thereby allowing much higher concentrations to be used. Furthermore, there would be the advantage of studying one bed known to contain β receptors rather than the total vasculature of the animal.

THE EFFECT OF NETHALIDE ON OUABAIN-INDUCED ARRHYTHMIAS

The results clearly indicate that nethalide alleviates arrhythmias caused by ouabain (75 to 100 μg/kg). Single injections of nethalide converted ouabain-induced arrhythmias to sinus rhythm. However, sinus rhythm persisted for only short periods of time before re-emergence of the arrhythmia. On the other hand, constant intravenous infusions of nethalide kept the heart in sinus rhythm for the duration of the infusion. Nethalide was equally effective in dogs with vagi intact or cut. Moreover, the effectiveness of nethalide was unaltered when the vasodepressor action of the drug was prevented by intra-arterial infusions of blood. This observation was reinforced by experiments showing that rapid lowering of the blood pressure by haemorrhage had no effect on the arrhythmias. In addition to these observations nethalide prolonged the survival time of dogs given lethal doses of ouabain.

Vaughan Williams (1963) recently showed that nethalide antagonized ouabain-induced arrhythmias in guinea pigs. He concluded that the anti-arrhythmia effect of nethalide was due to blockade of endogenous catecholamines, but he presented no evidence to support this conclusion. The present results indicate that antagonism of cardiac glycoside-induced arrhythmias by nethalide is not due to blockade of catecholamines. This conclusion is based on the observation that single injections of nethalide repeated throughout a five hour period of ouabain-induced arrhythmia suppressed the arrhythmia for periods no greater than twelve minutes. However, the results in Section II show that β receptor blockade persists for at least ninety minutes after infusing nethalide. Black and Stephenson (1962) showed that single doses of nethalide, (only half as large as

that used to convert the arrhythmias in this study) inhibited for at least thirty minutes the increase in heart rate due to stellate ganglion stimulation or injected isoproterenol. Lucchesi and Hardman (1961) used the same argument in concluding that antagonism of acetylstrophanthidin-induced arrhythmias by DCI was not due to β receptor blockade. Moreover, they showed that analogues of DCI without β receptor blocking action also antagonized the arrhythmia.

In recent years there has been disagreement as to whether endogenous catecholamines play a role in digitalis-induced arrhythmias. Erlij and Mendez (1963) concluded that digitoxin's action in increasing ventricular automaticity depended on the presence of catecholamines. They based this conclusion on the observations that treatment with nethalide, pretreatment with reserpine, or removal of the thoracic sympathetic and adrenals suppressed the ability of digitoxin to precipitate ventricular premature contractions. On the other hand, Yelnosky, and Ervin (1961) found no difference between the doses of ouabain required to induce ventricular tachycardia in normal and reserpine pretreated dogs.

In a brief report Cairoli, Reilly and Roberts (1961) said that ouabain induced spontaneous beating in papillary muscles taken from normal cats, but not in papillary muscles taken from cats pretreated with reserpine. They therefore suggested that the action of ouabain on cardiac rhythmicity may be due to catecholamine release. Cairoli et al., (1962) later concluded that catecholamine release was not an important factor in ventricular fibrillation caused by digitalis. Their conclusion was based on experiments showing that large doses of acetylstrophanthidin caused ventricular fibrillation in both normal and reserpine pretreated dogs.

Roberts, Ito, Reilly and Cairoli (1963) recently suggested that digitalis-induced arrhythmias are related only in part to catecholamine release. In electrically driven papillary muscles from cats pretreated with reserpine the incidence of ouabain-induced extra beats was reduced, and in intact cats the dose of acetylstrophanthidin necessary to induce ventricular arrhythmia in combination with vagal stimulation was increased by pretreatment with reserpine. However, pretreatment did not effect arrhythmias induced by high doses of acetylstrophanthidin.

It cannot be assumed that the protection afforded by reserpine against arrhythmias caused by digitalis is due to catecholamine depletion. Morrow, Gaffney, and Braunwald (1963) showed that dog hearts depleted of catecholamines by chronic cardiac denervation were no more resistant to ouabain-induced premature contractions than control dogs. The mean concentration of atrial noradrenaline was 1.97 μg/gram for the control dogs and 0.03 μg/gram for the denervated dogs. Boyajy and Nash (1963) showed in dogs that protection comparable to reserpine was provided against ouabain toxicity by the Rauwolfia alkaloid ajmaline which does not deplete tissues of catecholamines. Innes and Krayer (1958) found that reserpine had a depressant action on the heart which was independent of catecholamine depletion. When reserpine was injected acutely into heart-lung preparations made from dogs previously depleted of catecholamines by chronic treatment with reserpine it produced a negative chronotropic and inotropic effect. Withrington and Zaimis (1961) found that twenty-four hours after the administration of 1 mg/kg reserpine to cats the hearts were in failure.

These findings suggest that reserpine may oppose digitalis induced-arrhythmias by causing a non-specific depression of the heart.

A depressant action would be expected to attenuate the increased ventricular automaticity caused by toxic doses of digitalis. In contrast, the antagonism of ouabain-induced arrhythmias by nethalide cannot be explained on this basis. The results from section I showed that in dogs pretreated with reserpine nethalide stimulates rather than depresses the heart.

A possible explanation for the antiarrhythmic action of nethalide is suggested by the work of Regan, London, Binak and Hellems (1962). These authors showed in dogs that arrhythmias induced by acetylstrophanthidin are associated with a loss of potassium from the myocardium. Moreover, they showed that adrenaline and methoxamine reduced the net loss of potassium, and this change was correlated with antagonism of the arrhythmia. At present, however, there is no work indicating whether nethalide affects myocardial potassium fluxes.

The present study shows that nethalide antagonizes one of the actions of digitalis on the heart, i.e., induction of cardiac arrhythmias. Whether nethalide also antagonizes the positive inotropic action of digitalis on the failing heart is a question of therapeutic interest. If nethalide antagonizes only the arrhythmias, it may be possible to separate the beneficial and toxic effects of digitalis on the heart. Investigation of this possibility may prove to be a fruitful line of research.

SUMMARY AND CONCLUSIONS

1. Nethalide invariably decreased the heart rate and blood pressure in vagotomized dogs anaesthetized with pentobarbital. In contrast, nethalide consistently increased the heart rate of dogs depleted of catecholamines by pretreatment with reserpine. However, hypotension still resulted. These observations indicate that the bradycardia caused by nethalide in normal animals is due to blockade of the positive chronotropic action of endogenous catecholamines. Also, it is apparent from these experiments that nethalide has a slight intrinsic activity on the heart and vessels.

2. Intra-arterial injections of nethalide in small doses into the denervated dog hind limb which was artificially perfused with the dog's own blood at a constant flow, caused marked decreases in the perfusion pressure. Intravenous injections of nethalide in large doses produced comparable falls in the systemic arterial pressure and the perfusion pressure. These results show that nethalide is a potent vasodilator. It is concluded that vasodilatation is the principal cause of hypotension induced by nethalide. If a decreased cardiac output contributes to the hypotension it is probably due to blockade of the positive inotropic action of endogenous catecholamines.

3. Nethalide markedly inhibited the cardioaccelerator responses to isoproterenol in dogs. Ouabain enhanced the positive chronotropic action of isoproterenol after establishment of β receptor blockade by nethalide. In the absence of β receptor blockade ouabain antagonized the positive chronotropic action of isoproterenol. It is concluded that ouabain opposes both the stimulating action of isoproterenol and the blocking action of nethalide in the heart. The vasodepressor responses to isoproterenol were recorded concurrently with the cardioaccelerator responses,

but these data failed to show whether ouabain antagonized the adrenergic blocking action of nethalide in the vessels.

4. Cardiac arrhythmias induced by toxic doses of ouabain were invariably converted to sinus rhythm by intravenous injections of nethalide. However, sinus rhythm lasted for only short periods of time before the arrhythmia reappeared. The heart was kept in sinus rhythm only when constant intravenous infusions of nethalide were maintained. Prevention of the hypotensive response to nethalide by giving intra-arterial infusions of blood under pressure did not alter the effectiveness of nethalide in converting ouabain-induced arrhythmias. Furthermore, the arrhythmias were unaffected by rapid lowering of the blood pressure by haemorrhage. Constant intravenous infusions of nethalide increased the survival time of dogs given lethal doses of ouabain. It is concluded that nethalide is an effective agent against arrhythmias caused by cardiac glycosides, and that the antiarrhythmic action of nethalide is independent of its peripheral vascular actions. The evidence does not support the hypothesis that blockade of the cardiac actions of endogenous catecholamines is responsible for the antagonism of digitalis-induced arrhythmias by nethalide.

BIBLIOGRAPHY

Ahlquist, R.P. A study of the adrenotropic receptors. Amer. J. Physiol. 153:586, 1948.

Ariens, E.J. Affinity and intrinsic activity in the theory of competitive inhibition. Arch. Intern. Pharmacodyn. 99:32, 1954.

Ariens, E.J. and Simonis, A.M. Autonomic drugs and their receptors. Arch. Intern. Pharmacodyn. 127:479, 1960.

Black, J.W. and Stephenson, J.S. Pharmacology of a new adrenergic beta receptor blocking compound (nethalide). Lancet. ii:311, 1962.

Boyajy, L.D. and Nash, C.B. Influence of reserpine on fibrillatory and positive inotropic responses to ouabain. Federation Proc. 22: 184, 1963.

Cairoli, V., Reilly, J., Ito, R. and Roberts, J. The relation of digitalis-induced arrhythmias to catecholamine release. Federation Proc. 21:127, 1962.

Cairoli, V., Reilly, J. and Roberts, J. The effect of reserpine pretreatment on the positive inotropic action of ephedrine and ouabain. Federation Proc. 20:122, 1961.

Cannon, W.B., Lewis, J.T. and Britton, S.W. A lasting preparation of the denervated heart for detecting internal secretion with evidence for accessory accelerator fibers from the thoracic sympathetic chain. Amer. J. Physiol. 77:326, 1926.

Dale, H.H. On some of the physiological actions of ergot. J. Physiol. 34:163, 1906.

Dornhorst, A.C. and Robinson, B.F. Clinical Pharmacology of a beta-adrenergic blocking agent (nethalide). Lancet ii:314, 1962.

Dresel, P.E. Blockade of some cardiac actions of adrenaline by dichloroisoproterenol. Can. J. Biochem. and Physiol. 38:375, 1960.

Erlij, D. and Mendez, R. Modification of digitoxin intoxication by exclusion of the sympathetic system. Federation Proc. 22:184, 1963.

Fleming, W.W. and Hawkins, D.F. The actions of dichloroisoproterenol in the dog heart-lung preparation and the isolated guinea-pig atrium. J. Pharmacol. Exptl. Therap. 129:1, 1960.

Furchgott, R.F., de Gubareff, T. and Grossman, A. Release of autonomic mediators in cardiac tissue by suprathreshold stimulation. Science 129:328, 1959.

Gilbert, J.L., Lang, B.A. and Brooks, C. McC. Influence of sympathomimetic pressor drugs on arrhythmias caused by multiple stimuli. Circulation Res. 7:417, 1959.

Innes, I.R. and Krayer, O. Studies on veratrum alkaloids. XXVII. The negative chronotropic action of veratramine and reserpine in the heart depleted of catecholamines. J. Pharmacol. Exptl. Therap. 124:245, 1958.

James, T.N. and Nadeau, R.A. The chronotropic effect of digitalis studied by direct perfusion of the sinus node. J. Pharmacol. Exptl. Therap. 130:42, 1963.

Lee, W.C. and Shideman, F.C. The role of myocardial catecholamines in cardiac contractility. Science 129:967, 1959.

Lucchesi, B.R. and Hardman, H.F. The influence of dichloroisoproterenol (DCI) and related compounds upon ouabain and acetylstrophanthidin induced cardiac arrhythmias. J. Pharmacol. Exptl. Therap. 132:372, 1961.

Mendez, C., Aceves, J. and Mendez, R. Inhibition of cardiac acceleration by cardiac glycosides. J. Pharmacol. Exptl. Therap. 131:191, 1961.

Moore, J.I. and Swain, H.H. Sensitization to ventricular fibrillation I. Sensitization by a substituted propiophenone U-0882. J. Pharmacol. Exptl. Therap. 128:243, 1960.

Moran, N.C. and Perkins, M.E. Blockade of adrenergic stimulation of the heart by 1-(3',4'-dichlorophenyl)-2-isopropylaminoethanol. J. Pharmacol. Exptl. Therap. 122:55A, 1958.

Moran, N.C. and Perkins, M.E. Adrenergic blockade of the mammalian heart by a dichloro analog of isoproterenol. J. Pharmacol. Exptl. Therap. 124:223, 1958.

Moran, N.C. and Perkins, M.E. An evaluation of adrenergic blockade of the mammalian heart. J. Pharmacol. Exptl. Therap. 133:192, 1961.

Morrow, D.H., Gaffney, T.E. and Braunwald, E. Studies on digitalis. VIII. Effect of autonomic innervation and of myocardial catecholamine stores upon the cardiac action of ouabain. J. Pharmacol. Exptl. Therap. 140:236, 1963.

Nadeau, Reginald A. and James, Thomas N. Antagonistic effects on the sinus node of acetylstrophanthidin and adrenergic stimulation. Circulation Res. 13:388, 1963.

Nanda, T.C. The action of ergotamine on the response of the rabbit's gut to adrenaline. J. Pharmacol. 42:9, 1931.

Nickerson, M. and Chan, G. C-M. Blockade of isolated myocardium to epinephrine. J. Pharmacol. Exptl. Therap. 133:186, 1961.

Paasonen, M.K. and Krayer, O. The release of norepinephrine from the mammalian heart by reserpine. J. Pharmacol. Exptl. Therap. 123:153, 1958.

Powell, C.E. and Slater, I.H. Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. J. Pharmacol. Exptl. Therap. 122:48Ø, 1958.

Regan, T.J., London, B.L., Binak, K. and Hellems, K. Sympathomimetics as antagonists of strophanthidin's ionic and arrhythmic effects. Circulation Res. 11:17, 1962.

Roberts, J., Iro, R., Reilly, J. and Cairoli, V.J. Influence of reserpine and β TM 1Ø on digitalis-induced ventricular arrhythmias. Circulation Res. 13:149, 1963.

Roberts, J. and Stadter, R.P. Pharmacological evidence for the importance of catecholamines in cardiac rhythmicity. Circulation Res. 9: 171, 1961.

Rothlin, E., Konzett, H. and Cerletti, A. The antagonism of ergot alkaloids towards the inhibitory response of the isolated rabbit intestine to epinephrine and norepinephrine. J. Pharmacol. Exptl. Therap. 112:185, 1954.

Stephenson, R.P. A modification of receptor theory. Brit. J. Pharmacol. 11:379, 1956.

Vaughan Williams, E.M. Prevention of arrhythmias due to cardiac glycosides by block of sympathetic β receptors. Lancet. i:42O, 1963.

Withrington, P. and Zaimis, E. The reserpine-treated cat. Brit. J. Pharmacol. 17:38O, 1961.

Yelnosky, J. and Ervin, R. The effect of ouabain on cardiac automaticity in reserpine-pretreated dogs. Amer. Heart. J. 62:687, 1961.