THE DEVELOPMENT AND MAINTENANCE OF EXPERIMENTAL ALDOSTERONE HYPERTENSION IN THE RAT: EVIDENCE FOR A MECHANISM OTHER THAN PERIPHERAL VASCULAR AUTOREGULATION FOR THE INCREASED TOTAL PERIPHERAL VASCULAR RESISTANCE

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ABSTRACT

Peripheral vascular autoregulation at the level of the whole body has been invoked as an explanation for the increased total peripheral resistance (TPR) seen in many forms of volume dependent hypertension. An increased critical opening pressure (COP) has been found in the skin vessels in the tail of the rat made hypertensive with aldosterone. Aldosterone hypertension is believed to be a model of volume dependent hypertension. The COP is a measure of the contraction force of the vascular smooth muscle in the resistance vessels. If autoregulation is responsible for the increased resistance to flow offered by the resistance vessels, then the increase in COP seen in the rat should be part of the autoregulation process.

An experiment was conducted to test the autoregulation theory as an explanation for increased TPR in volume dependent forms of hypertension. An apparatus was designed to apply a continuous, graded increase in external pressure to the skin vessels in the tail of the rat for as long as 4 weeks while the rats became hypertensive from continuous infusion of aldosterone. This increased external pressure was applied in order to prevent increased autoregulatory stimuli (increased transmural pressure and blood flow) from acting on the tail vessels while the rat became hypertensive and went into the chronic stages of the hypertension. Provision was made to keep the tail warm while the increased external pressure was applied.

Test rats received aldosterone (dose rate about 5 µg/100g/d) in a vehicle of 15% alcohol in 5% dextrose in water and had a counterpressure of 25 to 35 mmHg applied to their tails before the hypertension developed. The counterpressure was increased as the hypertension progressed. Control rats received the vehicle only and had a counterpressure of 25-35 mmHg

applied to their tails. All 5 test rats became hypertensive, with a group mean increase in SBP of over 20%. None of the control rats became hypertensive. Their systolic blood pressures and critical opening pressures were essentially unchanged throughout the course of the experiment. It was found that the COP of the tail vessels of the hypertensive rats increased by over 100%, despite application of the increased external pressure to the tail. Elevated values for the SBP and COP in the test rats were also found after administering a ganglion blocking agent.

These results provide no evidence for peripheral vascular autoregulation as an explanation for the increase in COP of the tail vessels in aldosterone hypertension in the rat. These results also suggest that some mechanism other than autoregulation is responsible for the increased TPR in volume dependent hypertension.

The increase in SBP and COP occurs a few days after the start of excess aldosterone administration. In an experiment to investigate the influence of aldosterone dose rate on the delay of onset of increased SBP and COP, it was found that the delay was longer when low aldosterone dose rates were administered. This delay to the onset of increased SBP and COP suggests taht some new event, perhaps the release of a vasoconstrictor hormone, might be involved in the hypertension.

The delayed increase in SBP and COP may be related to renal escape from the influence of aldosterone. Release of a natriuretic hormone that is also a vasoconstrictor may be causing both events.

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INTRODUCTION AND LITERATURE REVIEW

The Peripheral Vascular Autoregulation Theory as an Explanation for Increased Total Peripheral Resistance in Volume Dependent forms of Hypertension

Introduction

Hypertension is the term applied to the sustained regulation of the arterial blood pressure at levels above some arbitrarily chosen "normal" value, usually 140-150/90. Maintenance of the blood pressure at hypertensive levels is associated with a greatly increased incidence of vascular damage to the heart, kidneys and brain, which can by itself contribute to the hypertensive condition by causing a further elevation of the blood pressure.

Hypertension itself is not a specific disease entity, it is a sign. In most cases, the pathogenesis of the hypertension is poorly understood. There are some clinical and experimental situations in which the hypertension is secondary to some defined cardiovascular, renovascular or hormonal derangement, but even in these situations, the mechanism of the sustained elevation in arterial pressure is not clear. Most human hypertension falls into the class of essential, or primary hypertension, with no clearly defined derangement save for the elevated blood pressure and an increase in the total peripheral vascular resistance.

Arterial pressure is a function of the cardiac output and the total peripheral resistance offered by the vasculature. Much of the research into hypertension has involved studying possible vasoconstrictor mechanisms and the role of Na^+ and H_2O retention in hypertension.

In some forms of hypertension, the increased TPR can be attributed to high circulating levels of known vasoconstrictors, i.e. angiotensin II. This appears to be the mechanism of the hypertension resulting from stenosis of a renal artery in man, and in the early phases of experimental one and two kidney Goldblatt hypertension. Other forms of hypertension, however, present with a normal or only slightly elevated cardiac output and an increased total peripheral resistance in the absence of high levels of known vasoconstrictors. This includes many examples of essential hypertension, the established phase of one kidney Goldblatt hypertension and the hypertension caused by excess mineralocorticoid administration. The mechanism of the increased TPR in these forms of hypertension is unclear, but an increase in exchangeable Na^+ and/or an increase in ECF volume are believed to be involved.

In this thesis, the term "volume dependent hypertension" refers to those forms of hypertension in which increased retention of Na^+ and H_2O is believed to be involved in the development or maintenance of the hypertension. This includes the following forms of hypertension:

1. that associated with excess mineralocorticoid or glucocorticoid secretion by an abnormally functioning endocrine system, ie Conn's syndrome (excess aldosterone), Cushing's syndrome (excess glucocorticoids).
2. the chronic stage of one kidney Goldblatt hypertension, where an early but transient increase in renin and angiotensin II releases aldosterone, causing the retention of Na^+ and H_2O and in some manner leading to a sustained increase in blood pressure.
3. the hypertension seen sometimes in renal failure, where renin levels appear normal in the presence of an expanded ECF volume.

4. Many examples of essential hypertension. In this broad category of hypertension, it has recently been found that there are low, normal and high renin subtypes. The low renin form of essential hypertension appears to be a volume expanded state, with levels of aldosterone being inappropriately high for the existing low levels of renin.

It is not clearly understood how an increased retention of Na^+ and H_2O can lead to a sustained elevation of arterial pressure.

Concept of Peripheral Vascular Autoregulation

Studies of blood flow in a number of tissues have shown a tendency for flow to return to its previous value, suitable for the needs of the tissue, when the rate of flow is altered by a change in perfusion pressure. This autoregulation of blood flow is independent of the vasomotor nerves.

Two mechanisms have been proposed to account for the ability of tissues to regulate their own blood flow:

1. metabolic hypothesis, whereby an increased blood flow through a tissue causes an increased washout of vasodilator metabolites, with consequent relief of their vasodilator effect and return of blood flow toward normal. No one specific metabolite has been assigned this role.
2. myogenic hypothesis, first suggested by Bayliss (1902), citing the ability of vascular smooth muscle to contract with an increase in stretch and to relax with a decrease in stretch. It is thought now that an increased perfusion pressure at the level of the resistance vessels causes these vessels to be stretched, triggering a myogenic increase in smooth muscle contraction force, resulting in a decrease in vessel lumen and return of flow toward normal, despite the increased perfusion pressure.

It is also possible that increased stretch at the level of the precapillary sphincters increases vasomotion, closing capillary beds more frequently, and reducing flow toward normal.

Whatever the mechanism, most tissues are well able to quickly (seconds to minutes) alter their resistance to blood flow in order to maintain flow at a constant level despite changes in the perfusion pressure.

Autoregulation of blood flow at the level of the whole body has been invoked as an explanation for the increased total peripheral resistance (TPR) seen in many forms of volume dependent hypertension, both human and experimental. I would like here to misquote Pickering (1968) and say that "My difficulty is that the autoregulation hypothesis, as a means to explain the increased TPR, is not one which can easily be refuted; it can always be invoked if other explanations prove wanting."

Support for this Concept as an Explanation for Increased Total Peripheral Resistance in Hypertension in which there appears to have been some Volume Expansion.

Several investigators have championed the idea that autoregulation of the peripheral vessels is a major factor in the pathogenesis of those forms of hypertension in which a high TPR cannot be attributed to high circulating levels of known vasoconstrictors. This includes most chronic forms of hypertension: the experimental and human forms of renovascular and mineralocorticoid hypertension and many essential hypertensions.

In his paper on extrarenal factors involved in the pathogenesis of hypertension, Ledingham (1956) wondered if certain pressor factors may be cardioactive rather than vasoactive. He then referred to Bayliss' (1902) concept of local myogenic activity by which arteriolar smooth muscle can contract in response to an increase in intravascular pressure, and suggested that a more forcible contraction of the heart, ie an increased

cardiac output (CO), might be primary in the genesis of hypertension, with an increase in tone of the resistance vessels occurring secondary to the increased CO, mediated through the local myogenic activity.

Ledingham (1971) has developed this idea further. He reported on a series of studies in which the mean arterial BP, CO, TPR, heart rate and stroke volume were monitored in trained conscious, one-kidney Goldblatt rats as their hypertension progressed from the acute to the chronic (25 days) stages. In these rats it was found that there was a sharp increase in mean arterial BP within 2 hours of operation and a more gradual increase as the hypertension became chronic. The cardiac output of the test rats fell below that of sham operated controls for the first 5 days, then consistently exceeded the cardiac output of the control group for the remainder of the experiment. Calculation of the TPR showed an immediate sharp increase at 2 hours and a gradual subsequent rise as the hypertension progressed. Ledingham stated that at all stages, the increased blood pressure is mainly attributable to the increased resistance. A similar series of studies, in which extracellular fluid (ECF) volume and cardiac output were found to be transiently increased in the early stages of the hypertension, has also been reported (Ledingham and Cohen, 1964). From the hemodynamic changes observed in these studies and with the knowledge that high renin levels are seen in only the first few days of one-kidney Goldblatt hypertension, Ledingham proposed the following sequence of events to explain the increased TPR seen in this experimental model of hypertension:

-increased release of renin and production of angiotensin II following renal artery constriction and removal of the contralateral

kidney.

-peripheral vasoconstriction mediated by angiotensin II, with increase in BP.

-stimulation of aldosterone secretion by angiotensin II, with increased retention of Na^+ and H_2O and an increase in ECF volume and plasma volume.

-increased plasma volume leads to increased atrial filling pressure and increased CO, which contributes to the increased BP.

-the increased CO results in perfusion of the tissues at levels beyond their metabolic needs, causing

-autoregulation of the peripheral vessels, which reduced tissue perfusion and increases the TPR.

-the increased TPR raises the blood pressure and reflexly reduces CO to normal or only slightly elevated levels.

-the increased BP also removes the stimulus for increased renin secretion, so plasma renin, angiotensin II and aldosterone levels return towards normal.

So the hypertension which originally was vasoconstrictor in origin appears, in the chronic phase, to be volume dependent, with increased TPR on the basis of autoregulation.

Guyton and his colleagues have strongly supported the hypothesis that peripheral autoregulation can account for the increased TPR seen in volume dependent hypertensions. An experiment to study total systemic autoregulation in the dog following destruction of the central nervous system was conducted by Granger & Guyton (1969) in order to determine if the whole body has quantitatively significant autoregulatory capabilities. The experiment involved making step changes in the arterial blood pressure and monitoring O_2 consumption and A-V O_2 difference in order to follow

changes in the cardiac output. Arterial blood pressure was controlled by adjusting the height of a blood filled reservoir that had been connected to the femoral artery. Adjusting the BP in this manner changed both the circulating blood volume and the CO. They found, however, that the CO would return toward its previous value and reach a new steady state in a mean of 35 minutes if the pressure increment was large (25-50 mmHg) with less time required if the pressure change was small. Granger and Guyton interpreted this response as a demonstration of whole body autoregulation that requires long periods of time in which to become established, and concluded that whole body autoregulation would significantly increase the TPR following an increase in CO.

There are difficulties in accepting this conclusion on the basis of this experiment. First, the usual autoregulation response that occurs in 1-2 minutes in individual tissues could not be demonstrated. And secondly, good flow regulation was achieved at low pressures, but not at higher, more physiologic pressures. The authors did not address themselves to these problems.

In a later paper (Guyton, Coleman & Norman, 1974), it was again suggested that autoregulation of blood flow, both short term and long term, can account for the increased TPR seen in the hypertensions where expansion of the body fluids and an increase in CO have been early events. Guyton has also speculated that with time, peripheral autoregulation may become increasingly intense, creating a tremendously increased TPR in the presence of a CO that is only slightly elevated (Guyton and Coleman, 1969). His explanation of this long term component of auto-regulation remains vague.

Despite Guyton's belief that some form of autoregulation of the peripheral vessels can account for the increased TPR in volume dependent hypertension, he feels that an increase in TPR is inconsequential as far as longterm pressure control is concerned. His systems analysis of arterial pressure regulation (Guyton and Coleman, 1969) predicts that no factor (ie increased TPR) can cause continued elevation of arterial pressure unless it in some way affects the kidneys and disturbs the body fluid volume control mechanisms. This idea was echoed a few years later by Guyton, Coleman and Norman (1974) and Tobian (1974).

Further support for the concept that some form of autoregulation can account for the increased TPR in volume dependent hypertension comes from a study on blood pressure regulation in anephric man, reported by Coleman et al (1970). They found that salt and water loading in these people, through an appropriate adjustment of the hemodialysis procedure, resulted in an increased BP and TPR and a transiently increased cardiac output. The increase in TPR occurred a few days after the CO increased and persisted when CO returned to normal levels. They believe the increase in TPR was a long-term autoregulatory response of the vessels to overperfusion of the tissues by the increased CO. They postulated that this long-term autoregulation involved either a decrease in average lumen size of the vessels, or a decrease in the total number of open vessels, but gave no mechansims for these processes.

Peer Evaluation of this Proposed Mechanism as an Explanation for Increased TPR in Volume Dependent Hypertension

In a general review, Tobian (1974) proposed a sequence very similar to Ledingham's (1971) to explain the development of a sustained elevation of arterial pressure in conditions in which some early defect results

in an inappropriately high ECF volume and a high "effective" blood volume. In Tobian's scheme, a high effective blood volume would increase venous filling pressure and CO, which would, by itself, elevate arterial pressure. Overperfusion of the tissues by this increased cardiac output is thought to cause an autoregulation of blood flow in many tissues via increased vasoconstriction of the resistance vessels, thereby increasing the TPR. In combination with the elevated cardiac output, the increased TPR raises the arterial blood pressure even more, causing a reflex reduction of the CO to about normal levels. This leaves a hypertensive condition characterized by a high TPR and nearly normal CO. Tobian then pointed out that this hypertension could be sustained only as long as the ECF volume continued to be elevated, presumably through some renal abnormality. He also mentioned that there seems to be some genetic susceptibility required before a high ECF volume can produce this hypertension.

It is generally recognized that an alteration of the salt and water balance seems to be involved in the hypertension that sometimes accompanies renal failure in man. These patients frequently show volume expanded characteristics, having a greatly increased exchangeable sodium when compared with those in renal failure who remain normotensive (Ledingham, 1971). In his paper dealing with hypertension in chronic renal failure, Schalekamp et al (1973) mentioned that an increased CO is often found in hypertensive patients in renal failure, and that peripheral resistance is usually increased in such patients as well. Schalekamp is aware of Ledingham's explanation for increased TPR on the basis of autoregulation following an increase in CO, but has reservations about applying it to the situation in man. Schalekamp cited a study by Kim et al (1972) in which

it was found that while patients in end stage renal disease tend to have a significantly elevated cardiac output, some patients remain normotensive and have a normal total peripheral resistance. On this basis, Schalekamp feels that the evidence for peripheral vascular autoregulation operating to increase the peripheral resistance in chronic renal failure in man is not as good as it is in experimental renal hypertension. He thinks instead that the rise in blood pressure in chronic renal failure is due to the vasoconstrictor effects of angiotensin II which circulates at levels inappropriately high for the prevailing exchangeable sodium.

Laragh does not consider autoregulation in his vasoconstrictor-volume analysis of hypertension (1973). He describes pure volume hypertension as being characterized by minimal vasoconstrictor activity (meaning low plasma renin activity) and suggests that the arterioles would be relatively dilated and distended in the presence of high extracellular fluid and plasma volumes. His understanding of volume expanded hypertension is that there is a renal inability to maintain sodium and water balance at lower pressures, so there results an overfilling of the available vascular space up to that pressure at which sodium and water balance can be maintained. He used this concept as an explanation for the hypertension that develops during excess mineralcorticoid administration and for the increased blood pressure seen in the established phase of one-kidney Goldblatt hypertension.

In a recent review, Davis (1977) commented on Ledingham's and Guyton's theory that autoregulation can account for the increased TPR in volume dependent hypertension. He called it an attractive theory lacking in support from a number of other studies, and pointed out that the occurrence of whole body autoregulation over an extended period of time

has not been demonstrated experimentally.

Establishing the hypothesis of whole body autoregulation as an explanation for increased TPR depends on 1) demonstrating that conditions are such that autoregulation is likely to occur, and 2) the exclusion of other vasoconstrictor mechanisms. As understated by Ledingham (1971), "the testing of this hypothesis presents physiologists with a most complex haemodynamic problem."

The Present Investigation: An Approach and Appraisal of the Hypothesis of Whole Body Autoregulation

We have been interested for some time in the cause of the increased TPR seen in volume dependent forms of hypertension. We are particularly interested in factors that might increase the contraction force of the smooth muscle in the resistance vessels. We suspect that there is some other vasoconstrictor mechanism (agent) operating in volume expanded hypertension, and that the increased resistance to flow offered by these vessels is not entirely due to autoregulation of flow.

To study factors that might influence the contraction force of the smooth muscle in the resistance vessels, one first would like to have a fairly direct method of measuring it. Burton (1951) developed the concept of critical closure of small vessels at positive perfusion pressures. The critical closing pressure (CCP) is the maximum transmural pressure against which the unstretched vessel will close by virtue of the force of contraction of the smooth muscle in its wall. The CCP measurement is an index of smooth muscle contraction force when the vessel is unstretched and is a more direct measure of vasomotor tone than the pressure-flow studies that have been used in the past.

Nichol et al (1951) found that vessels would open, as well as close, at the same critical pressure. This was confirmed by Gaskell and Krisman (1958) as they made direct visual measurements of the CCP and critical opening pressure (COP) of the vessels supplying the capillary loops of the nailfold. In practice it has been found more convenient to measure the critical opening pressure. Studies from this laboratory have involved measurements of the COP of vessels in forearm and digital skin and in the skin of the tail of the rat under a number of experimental and pathological circumstances.

As used in this laboratory, measurement of the COP is a convenient, painless, noninvasive technique for obtaining a measure of the contraction force of the smooth muscle in the resistance vessels of the skin. However, it is important to recognize one of the limitations of the COP measurement: the COP that is measured in an area of tissue is that of the vessels with the lowest COP. These vessels may not be those that provide the major proportion of the resistance to flow in that tissue, or indeed, in the whole body. The COP that is measured, then, may not reflect the tension of the smooth muscle in those resistance vessels that are the major determinants of TPR. This was mentioned by Nichol et al (1951) and was shown quite dramatically by Gaskell (1967) when he infused angiotensin II 4 ng/kg/min iv into normotensive subjects and found an increase in both the systemic blood pressure and the digital flow resistance, as expected, but at the same time there was a decrease in the measured COP of the digital skin vessels. With care, however, the COP measurement can be a useful tool in looking at the contraction force of the smooth muscle in the resistance vessels.

We have used the COP measurement to investigate changes in vascular smooth muscle contraction force in a number of what one might think are volume dependent hypertensions. We have found that the COP is elevated in the skin vessels of the tail of the rat in 1) the chronic stage of one-kidney Goldblatt hypertension (Darke, Nair and Gaskell, 1976), 2) the hypertension produced by administration of deoxycorticosterone acetate (DOCA) with 1% NaCl for drinking water (Darke et al, 1976), and 3) the hypertension produced by administration of aldosterone alone (Darke et al, 1977). In all three forms of hypertension, the COP measured after ganglion blockade is greater than normotensive blocked values, so the increase in COP cannot be attributed entirely to neurogenic influences.

The hypertension produced in the rat by administering aldosterone (continuous infusion, dose rate about 5 μg/100g/d) is particularly interesting in that the onset of the increased SBP is delayed, occurring a few days after the start of the infusion. This increase in systolic pressure is accompanied by an abrupt elevation of the COP of the resistance vessels in the skin of the tail. We believe this increase in COP is part of a generalized increase in vascular smooth muscle contraction force.

The development of mineralocorticoid hypertension is believed to involve expansion of the ECF volume. It is well know that the administration of excess aldosterone results in retention of Na^+ and H_2O up to the time that renal escape occurs. Some investigators have studied the effects of prolonged secretion or administration of excess aldosterone on the fluid volumes in man: Dustan, Bravo and Tarazi (1973) found that the plasma