CIRCULATORY CONTROL IN HYPERTENSION

STUDIES IN EXPERIMENTAL RENAL HYPERTENSION AND SOME ASPECTS OF HUMAN ESSENTIAL HYPERTENSION

by

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SUMMARY

CIRCULATORY CONTROL IN HYPERTENSION. STUDIES IN EXPERIMENTAL RENAL HYPERTENSION AND SOME ASPECTS OF HUMAN ESSENTIAL HYPERTENSION

WEST, M.J. (1974) Ph.D. Thesis, University of Sydney

Reflex function and the role of autonomic and non-autonomic factors in circulatory haemodynamics have been studied in unanaesthetized rabbits with renal hypertension which was produced by bilateral wrapping of the kidneys with cellophane. During the development of renal hypertension there is a steady rise in blood pressure and total peripheral resistance which depends on non-autonomic factors. The cardiac output is not raised above control levels from 10 to 33 days after the induction of hypertension. In established hypertension when the experimental conditions are carefully controlled there is an increased non-autonomic hindlimb resistance, however the autonomic component of hindlimb resistance is the same as in the sham-operated preparation. A significant fall in non-autonomic hindlimb resistance occurs in the blocked hypertensive animal following reduction in blood pressure with bleeding. The greater vascular resistance of the hindlimb bed in the hypertensive animal after full dilation with glyceryl trinitrate suggests

the presence of narrowing of the vessel lumen independently of muscle tone after 4-6 weeks of hypertension. The open loop vascular responsiveness to pressor agents and the responsiveness to sympathetic constrictor stimuli in the hindlimb of the hypertensive animal are both greater than that in the normotensive animal in the ratio of 1.9 to 1. The enhanced responsiveness is not uniform in all beds since the rise is less in the total vascular bed.

The steady state properties of the baroreceptor-heart rate and constrictor reflexes were assessed by the construction of S-shaped curves relating the changes in mean arterial pressure to the changes in heart period (pulse interval) or hindlimb vascular resistance. There is impairment of the baroreceptor-heart rate reflex in hypertensive compared to normotensive animals. Resetting occurs about a higher pressure which is probably due to adaptation of the baroreceptors. These changes in renal hypertension are almost identical to those in patients with essential hypertension. Arterial hypoxia was used to alter the properties of the mean arterial pressure-heart period curves. Interactions between the baroreceptor system and other afferent inputs on the one hand and baroreflex independent effects on the other

contributed to the shift in curves. The study suggested a means of assessing the excitability of the central autonomic motoneurone pool. The range of the hindlimb constrictor response mediated through the baroreceptor system was greater in hypertensive than in normotensive animals. This appears to be due to the enhanced responsiveness of the hypertensive vascular bed to constrictor stimuli.

The work presented in this thesis was carried out in the Department of Medicine, Sydney University and the Hallstrom Institute of Cardiology, Royal Prince Alfred Hospital, Sydney, during the years 1971, 1972 and 1973. The work relating to the baroreceptor-heart rate reflex described in Chapters 2 and 5 has been published in two papers: -

Korner, P.I., Shaw, J., West, M.J. and Oliver, J.R. (1972). Central nervous control of baroreceptor-heart rate reflexes in the rabbit. Circulation Res., 31, 637-652.

Korner, P.I., Shaw, J., West, M.J., Oliver, J.R. and Hilder, R.B. (1973). Integrative reflex control of heart rate in the rabbit during hypoxia and hyperventilation. Circulation Res., 33, 63-73.

All the data reported in this thesis, unless otherwise specifically stated, are the results of experiments conducted by myself in collaboration with and under the supervision of Professor P.I. Korner and with the assistance of the other co-authors.

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ABSTRACT

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CHAPTER 1

INTRODUCTION

Most investigators believe that a number of factors are involved in the pathogenesis of hypertension including disturbances of autonomic function, humoral disturbances and abnormalities in structure and function of the vascular wall. The multifactorial nature of the cause of hypertension was first emphasized by Page (1949) in his "mosaic theory". A primary causal factor can often be identified in certain types of human hypertension and when this is removed blood pressure is restored to normal (e.g. renal artery stenosis, coarctation of the aorta, pheochromocytoma, primary aldosteronism). However the cause of the hypertension cannot be found in many instances and the hypertension is then termed "essential hypertension".

One of the problems in investigating the pathogenesis is related to the lack of adequate methods for quantifying the various circulatory control mechanisms in the intact animal, for example, autonomic reflex function. Recently Guyton and colleagues have stressed that the kidney probably plays a central role in the maintenance of every type of hypertension irrespective of primary causation (Fig. 1; Guyton and

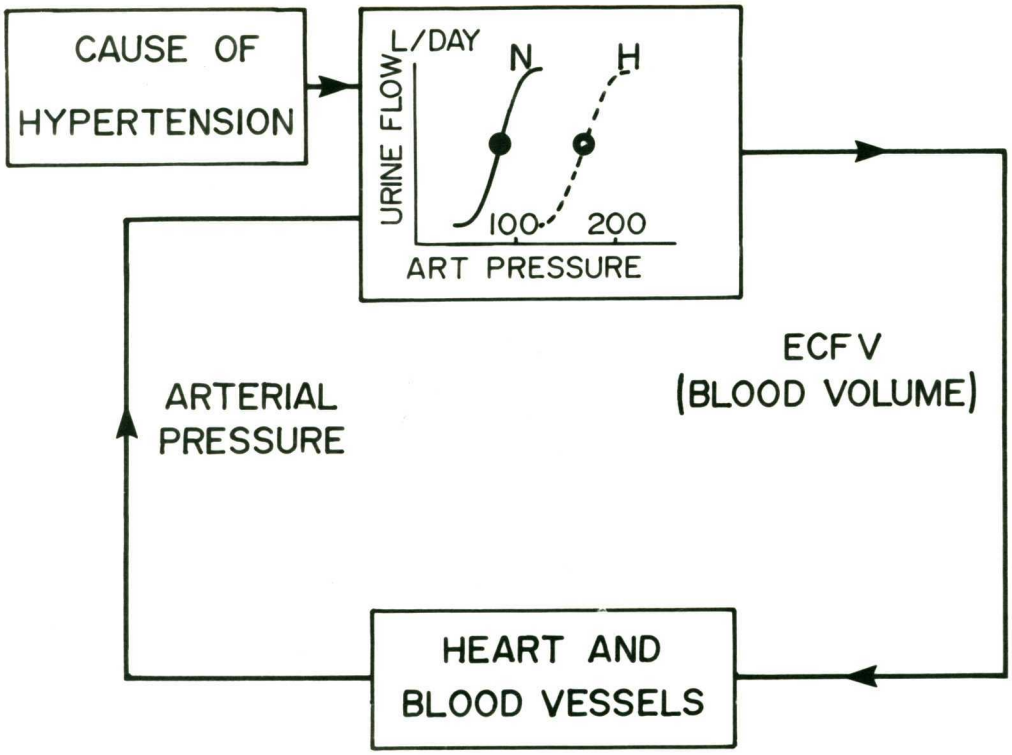

FIGURE 1

Extracellular fluid volume (ECFV) circulatory control system where the arterial pressure-urine flow relationship within the kidney (upper box) occupies the central role in the control of arterial pressure. It is envisaged that disturbances which result in hypertension act by shifting the normal arterial pressure-urine flow relationship (N) to the right (H). Arterial pressure becomes raised in order to maintain normal urine flow.

Coleman, 1967; Guyton, Coleman, Cowley, Scheel, Manning and Norman, 1972). They have examined the properties of those parts of the circulatory control system involved in the control of arterial pressure, blood volume and cardiac output and have suggested that the different types of hypertension are due to a disturbance of the arterial pressure-urine flow relationship in the kidney. As a result, normal urine flow can be maintained only in the presence of a raised arterial pressure. Such a change can be envisaged for renal artery stenosis, increased (neurogenic) afferent arteriolar constriction, increased (aldosterone mediated) tubular reabsorption and in those conditions where there is decreased renal mass due to various types of renal disease. The rise in blood pressure can be regarded as a compensatory mechanism which increases urine flow towards normal levels after the alteration in renal function. These workers further suggest that in the early stages of many types of hypertension the blood pressure is raised as a result of an increase in cardiac output and later more as a result of peripheral resistance.

Plan of Study

Most of the work reported in this thesis deals with experimental renal hypertension in the rabbit. Many believe

that once renal hypertension becomes established secondary mechanisms come into play which alter autonomic function (Ferrario, Gildenberg and McCubbin, 1972). In this work a method for investigating baroreflex function in established hypertension is described and that data was compared with that derived in human essential hypertension. In addition the changes occurring in cardiac output and total peripheral resistance are considered during the development of this type of hypertension. Lastly, a method was developed for estimating the autonomic and nonautonomic components of resistance and for examining properties of the blood vessels under open-loop conditions.

AUTONOMIC REFLEX FUNCTION IN HYPERTENSION

It is not known to what extent autonomic function plays a role in human essential hypertension. According to Folkow and colleagues and Brod and coworkers long continued stress may lead to elevation of blood pressure through a mechanism akin to the defence-alarm reaction (Folkow and Neil, 1971; Brod, 1972). It has been suggested that with enhanced excitability of central autonomic mechanisms there may be enhancement of responsivenss to any type of afferent stimulus arising from cardiorespiratory or somatic receptor groups

(e.g. cold pressor test). Alternatively the abnormality may be a vascular one where because of altered properties of the blood vessels a given sympathetic constrictor stimulus will lead to greater vasoconstriction than in subjects with normal vessels. One problem has been the difficulty of providing quantitative data about reflex autonomic function both in intact man and in animals. A quantitative method for the assessment of baroreflex sensitivity has recently been developed by Sleight and colleagues (Sleight, 1972). With their method sensitivity is determined from the heart period responses during small rapid changes in blood pressure. In the present thesis a 'steady-state' method for quantitative evaluation of the baroreceptor-heart rate reflex was developed. In the first part of the work the reflex was compared in patients with essential hypertension and in rabbits with relatively brief periods of renal hypertension. In human essential hypertension the properties of the baroreceptor-heart rate reflex differ from those of normotensive subjects of the same age (Sleight, 1972). It is not known whether these changes are due to a primary disorder of autonomic function in essential hypertension or whether they are merely secondary consequences of chronic elevation of arterial pressure. In the latter case one would expect that in other types of

hypertension (e.g. renal hypertension) there would be similar changes in baroreflex properties.

The effect of severe arterial hypoxia on the baroreceptor-heart rate reflex was studied in normotensive rabbits to determine the magnitude of baroreceptor interaction within the central nervous system with other inputs. The method suggested a means by which possible changes in the level of excitation of central cardiac motoneurones in renal hypertension might be theoretically examined, though this was not actually carried out.

HAEMODYNAMICS IN HYPERTENSION

In the second part of the work, the haemodynamic changes during the development of renal hypertension have been studied. Data is available from other species which suggests that following the induction of renal hypertension by renal arterial constriction or encapsulation of the kidney, there is fluid retention which results in increased arterial pressure predominantly due to a raised cardiac output (Ledingham and Cohen, 1964; Ferrario, Page and McCubbin, 1970). Ledingham and Cohen (1963) first suggested that there was over-perfusion of the tissues due to the rise in cardiac output and that as a result local 'autoregulatory' vascular processes occur which lead to generalised vasoconstriction and return of cardiac

output towards normal levels. The results of Guyton and Coleman (1967), in a systems analysis of the circulation during the onset of renal hypertension, were in agreement with these experimental findings. Their computer model also indicated that vasoconstriction of the renal bed alone was sufficient to produce the experimentally observed circulatory changes. The role of the nervous system in the production and maintenance of renal hypertension is regarded as being trivial according to this theory, although secondary involvement due to for example interaction with the renin-angiotensin system cannot be excluded.

An examination of the extent to which autonomic and nonautonomic mechanisms contributed to the haemodynamic pattern during the development and in the established phase of renal hypertension was one additional aspect of this work.

VASCULAR PROPERTIES

Many consider that in hypertension there is either a fundamental abnormality of vascular smooth muscle or that there are secondary changes evoked by the rise in blood pressure. In any case vascular responsiveness to a variety of stimuli is altered (Somlyo and Somlyo, 1970; Page and

McCubbin, 1968). In man the problem has been studied by Doyle and Fraser (1961) who showed increased responsivness to pressor stimuli in the forearm vessels of subjects with essential hypertension compared to normotensive individuals. In the anaesthetized rat with renal and genetic hypertension Folkow, Hallbäck, Lindgren, and Weis (1970, a and b) demonstrated an increased vascular reactivity which was a mechanical consequence of thicker vascular walls at maximal vasodilation. The results are reported of changes in vascular responsiveness in an 'open loop' preparation of the unanaesthetized renal hypertensive rabbit where the effects of reflex changes in sympathetic constrictor stimuli have been removed.

In the last part of the work a new method was developed to assess baroreflex function in the hindlimb bed of the renal hypertensive and normotensive rabbit. The changes in baroreflex function in the hindlimb were then related to the altered vascular responsiveness in hypertension.

Each chapter in this thesis deals with a self-contained problem and the general perspective of the work is given in Chapter 8.

CHAPTER 2

METHODS - PART 1

ANIMALS

Commercially obtained cage bred rabbits crossbred with the New Zealand white strain were used in these experiments. They were 3 to 4 months old and weighed between 2 and 3.5 kg. The animals were housed separately in cages and fed with commercial food pellets (Allied medicated rabbit and guinea pig pellets).

ANAESTHESIA

For major operative procedures anaesthesia was induced with propanidid (Epontol, Bayer) 75 mg i.v. followed by tracheal intubation using a Foregger size 12 endotracheal tube and an infant laryngoscope. Anaesthesia was maintained with open circuit halothane (Fluothane, I.C.I.) using a Goldman vaporizer connected to a one way respiratory flap valve. The depth of anaesthesia was kept as light as possible during operations but was increased to produce adequate relaxation during abdominal procedures. Animals maintained for one to two hours on this regime recovered quickly postoperatively and were moving around within thirty minutes without apparent ill effect. All animals received 35 mg oxytetracycline i.m.

(Terramycin, Pfizer) post-operatively as a prophylactic procedure.

PRELIMINARY PROCEDURES

Preliminary operative procedures involved the implantation of inflatable balloons and a Doppler ultrasonic flow transducer and the production of hypertension (Fig. 2). These were usually performed at one operation and with negligible blood loss.

(i) Implantation of Inflatable Balloons

At laparotomy small inflatable balloons were placed around the inferior vena cava above the right renal vein and the abdominal aorta below the diaphragm. The connecting tubes were taken out through the abdominal flanks and were passed subcutaneously to the middle of the back where they could be later located prior to experiments. In some instances the venous balloon was placed around the inferior vena cava within the thorax. This allowed a greater reduction in intravascular pressure to be induced than could be obtained with the abdominally placed balloon.

Construction of Inflatable Balloon

The inferior vena caval balloon (10 mm internal diameter) and the aortic balloon (6 mm in diameter) were constructed using

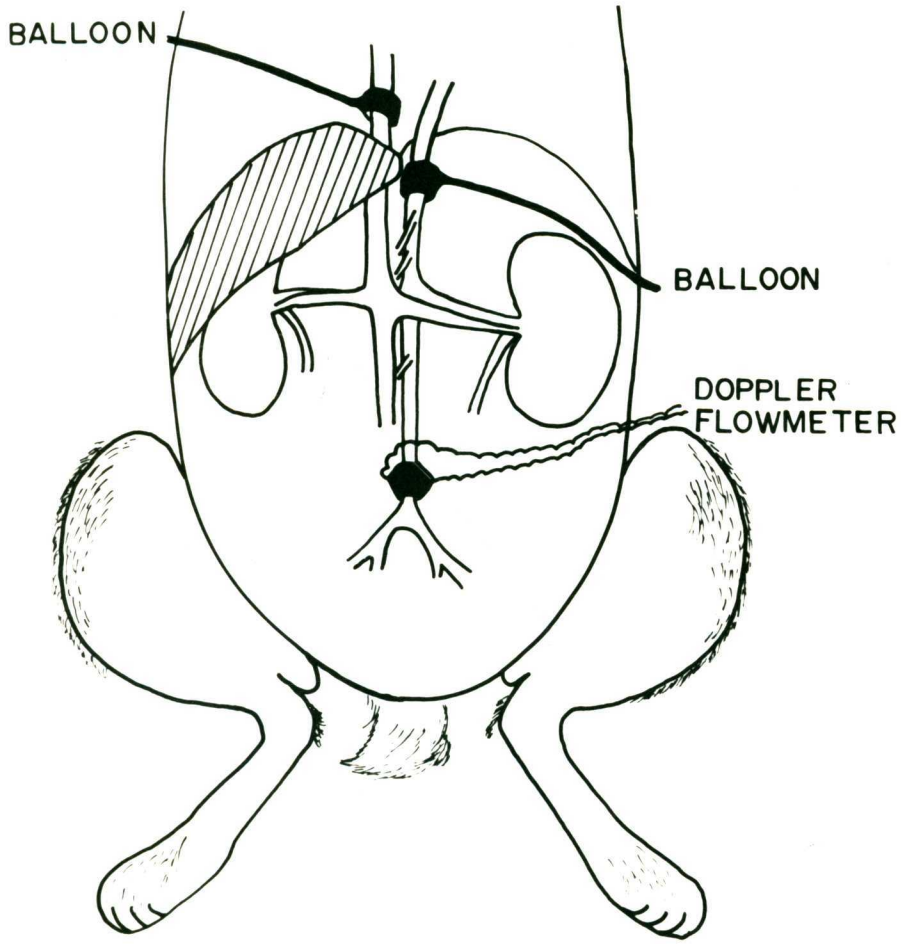

FIGURE 2A

Diagrammatic illustration of the rabbit preparation used for the construction of baroreflex stimulus response curves and for the measurement of hindlimb haemodynamics.

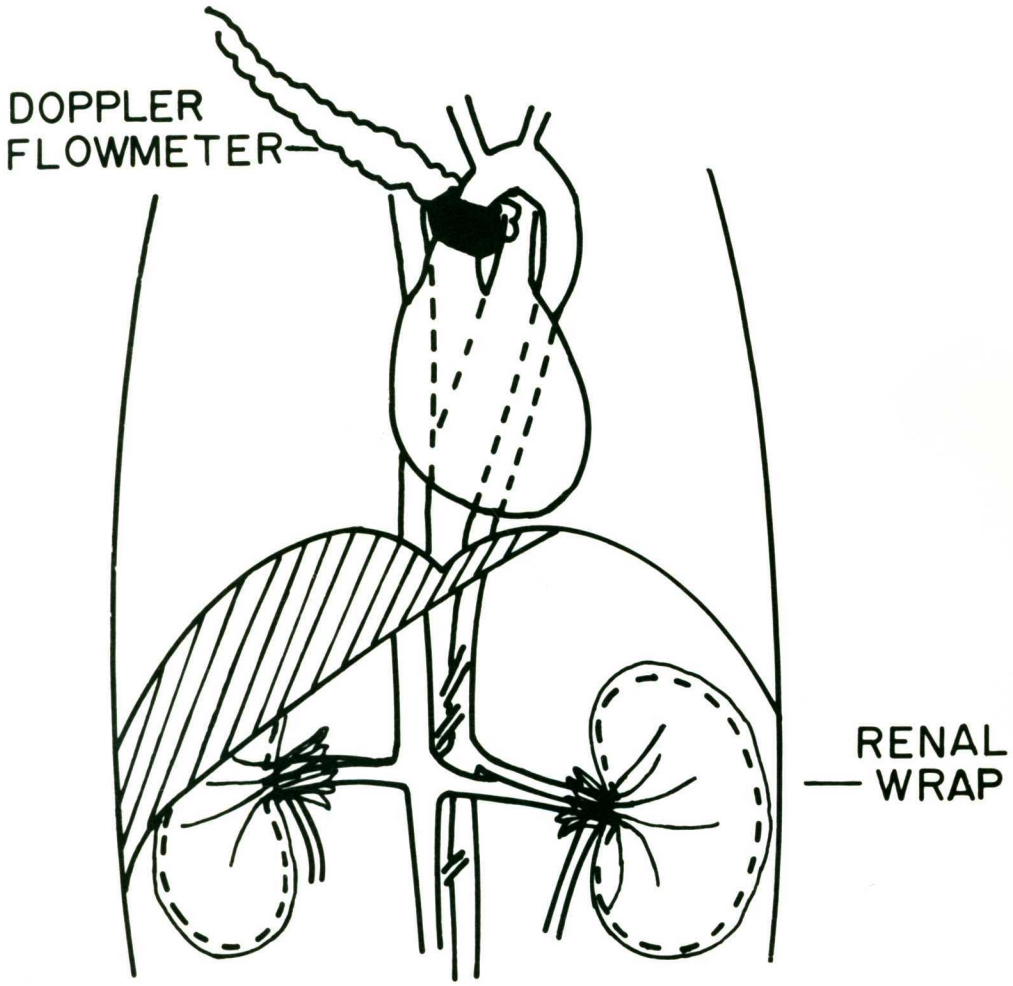

FIGURE 2B

Diagrammatic illustration of the rabbit preparation used for the measurement of circulatory haemodynamics during the development of hypertension.

a short length of silicone rubber tubing (Silastic, Dow Corning, 3.18 mm o.d. and 2.41 mm o.d. respectively) fixed to a backing of polyvinyl chloride (P.V.C.) (Fig. 3). This was connected to a length of P.V.C. tubing (1.6 mm o.d.) which was used to inflate the balloon.

(ii) The Production of Renal Hypertension

Renal hypertension was produced by the method of Page (1939). The kidneys were exposed extra-peritoneally through longitudinal incisions in the loin. A small sheet of cellophane (0.03 mm thickness) was placed around each kidney and tied loosely at the hilum. The wrapped kidneys were then replaced in the perinephric space and the wound closed.

Wrapping the kidneys in this manner resulted in 90% of animals developing hypertension. The hypertension developed slowly and continuously over the following 4 to 6 weeks at which time serum electrolytes and blood urea nitrogen were similar to sham operated animals. Renal failure as indicated by a rising blood urea nitrogen, was usually the cause of death in those animals which died less than four weeks after renal wrapping.

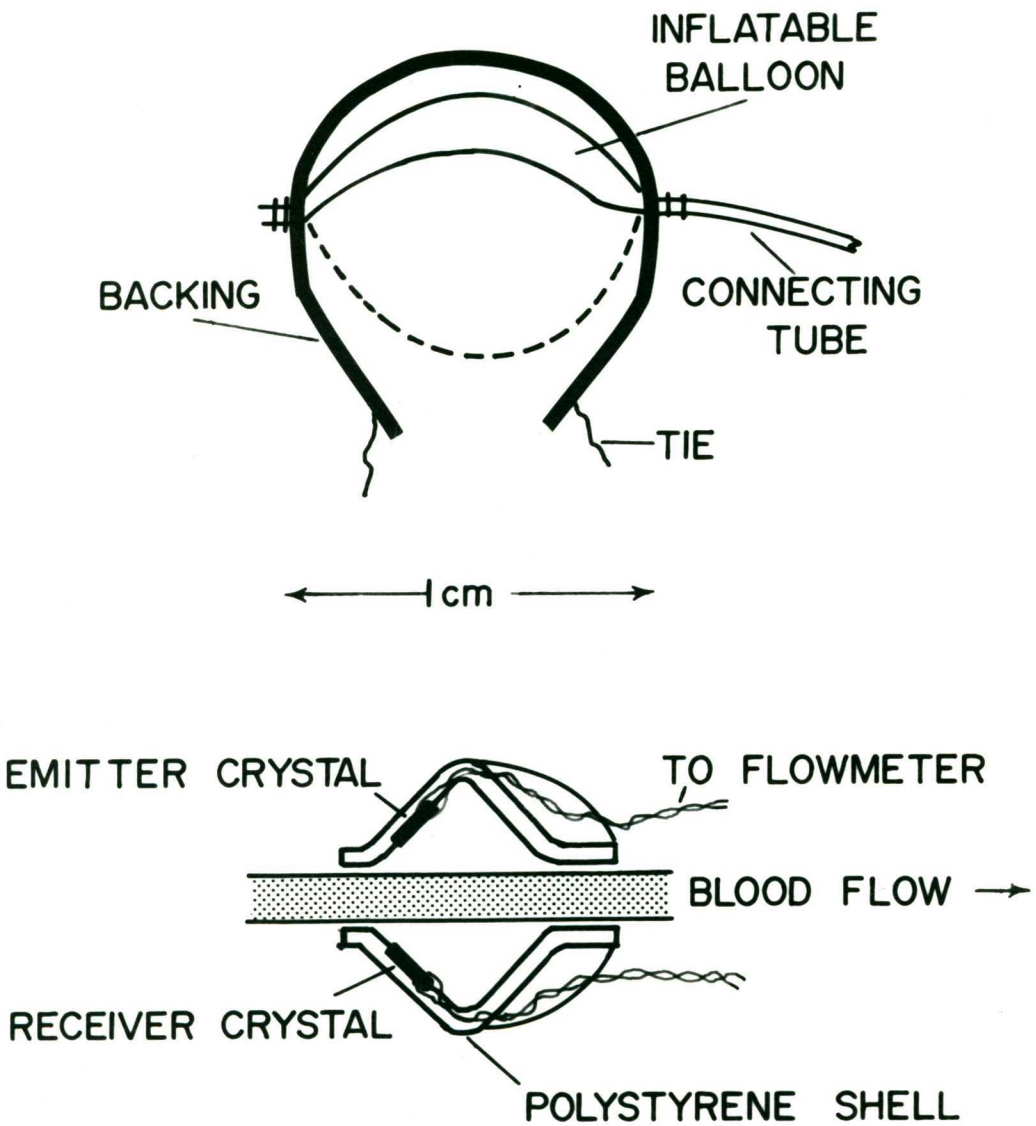

FIGURE 3

Diagrammatic illustration of inflatable balloon (TOP) and Doppler ultrasonic flow transducer (BOTTOM).

In animals subjected to a sham procedure the kidneys were exposed through the loin and partly freed from the renal bed. Anaesthesia was maintained for the same time interval as for the wrapping procedure - usually about 30 minutes.

Autopsy Appearances

At post mortem examination of the hypertensive animals following experiments,the kidneys were smaller in size than the sham operated kidneys. The capsule was thickened and the parenchyma narrowed in width. Light microscopy revealed occasional focal scars and various minor non-specific changes. There were no abnormalities of the juxtaglomerular cells of the macula densa region or of the intrarenal vessels. The heart was enlarged due to hypertrophy and other organs showed no abnormality on light microscopy. In occasional rabbits dying from malignant hypertension, widespread haemorrhagic infarcts were found in the brain, lungs, small bowel and kidneys.

(iii) Implantation of Doppler Ultrasonic Flow Transducers

Lower abdominal aorta

The operation was carried out through a lower midline abdominal incision. The lower abdominal aorta was freed

for a distance extending 2 cm above the iliac bifurcation to 1 cm below, along both iliac vessels. The sympathetic chain could usually be identified and was not disturbed. The flow transducer was placed in position around the aorta just above the iliac bifurcation and the P.V.C. covered transducer wires were passed through the abdominal wall to be buried subcutaneously on the back.

Aortic Root

With the animal on a respiratory pump the chest was opened on the right side anteriorly between the second and third rib. The pericardium was opened to expose the ascending aorta which was freed from the underlying pulmonary artery. The transducer was placed in position around the aorta close to the aortic root and following reinflation of the lungs, the thoracotomy was closed using a temporary underwater drain.

Construction of Doppler Ultrasonic Flow Transducers

P.V.C. coated multistrand copper wire (30 cm) was soldered to each side of the silver surface of small piezoelectric lead titanate zirconate crystals (LZT-5, Transducer Products Inc. Torrington, Conneticut) and the crystals were coated with a thin layer of silicone

insulating fluid (Midland Silicones Ltd., England). The crystals were then mounted in small moulded polystyrene shells and two shells were hinged together with a piece of nylon fabric to form a cuff 5 mm i.d. for the aortic root transducer and 4 mm i.d. for the hindlimb transducer (Fig. 3).

MINOR OPERATIVE PROCEDURES

These operations consisted of cannulation of the central ear artery, vein and right atrium, the production of a tracheotomy and the exposure of subcutaneous leads and tubing. With the use of 1 to 5 ml of local 0.5% lignocaine anaesthetic (Zylocaine, Astra) infiltrated into the operation sites, the animals rested quietly with little restraint during the procedures. The ear artery and vein were cannulated with 0.9 mm o.d. thin walled P.V.C. tubing. The natural frequency of the catheter manometer system was about 20 c.p.s. so that in rabbits with a pulse rate of 250 beats per minute records of the ear artery pressure pulse faithful up to about the second harmonic could be obtained (Yanof, 1965).

For right atrial cannulation and the production of a tracheotomy a midline longitudinal incision in the neck

was made while the rabbit was held firmly wrapped in a hand towel. The atrial catheter (1.5 mm o.d. thin walled P.V.C. tubing) was passed 7.5 cm down the right jugular vein from the junction with the internal jugular vein. This technique placed the catheter tip in the rabbit's right atrium. For a tracheotomy a small T-shaped tracheotomy tube was placed in the trachea below the thyroid cartilage and tied in position. The tracheotomy tube was connected to a one way respiratory flap valve fixed to the animal's back. The artificial dead space produced by the tracheotomy tube, connecting tube and flap valve approximated the physiological dead space.

NEURAL ABLATION OPERATIONS

These operations were carried out as described previously from this laboratory (Korner, Uther and White, 1969). On the day of the experiment the animal was anaesthetized and placed in a sling with its head fixed in a rabbit neurophysiological head clamp (David Kopf Instruments).

(i) Sham-operated animals

The dura was exposed through a bilateral fronto-parietal craniotomy. After removal of 5 ml of blood anaesthesia was continued for 45 minutes before the wound

was closed. In this way blood loss (3-8 ml (Uther, 1970)) and length of operations were similar in sham-operated and pontine animals.

(ii) Pontine animals

In these animals following craniotomy the dura was incised and areas of brain removed using a powerful sucker (20 lb per sq inch, Wade Pumps, Aust. VP 240F). All structures above the tentorium cerebelli were removed (Fig. 4) making a plane of transection from the lower margin of the inferior colliculus to the anterior pontine border near the point of emergence of the oculomotor nerves (Monnier and Gangloff, 1961). The operation was carried out with less than 5 ml blood loss due to early coagulation of the middle cerebral artery. Distortion of the brain stem during the operation was kept to a minimum. Exposed brain at the end of the operation was covered with moist gelatin sponge (Sterispon, Allen and Hanbury Ltd.) and the wound closed. Post operatively the animals were given 5.5% dextrose in distilled water, 10 ml i.v. hourly and the pontine animals had their body temperature controlled using an electric blanket. Pontine animals had decerebrate rigidity and could not maintain an upright posture. They recovered

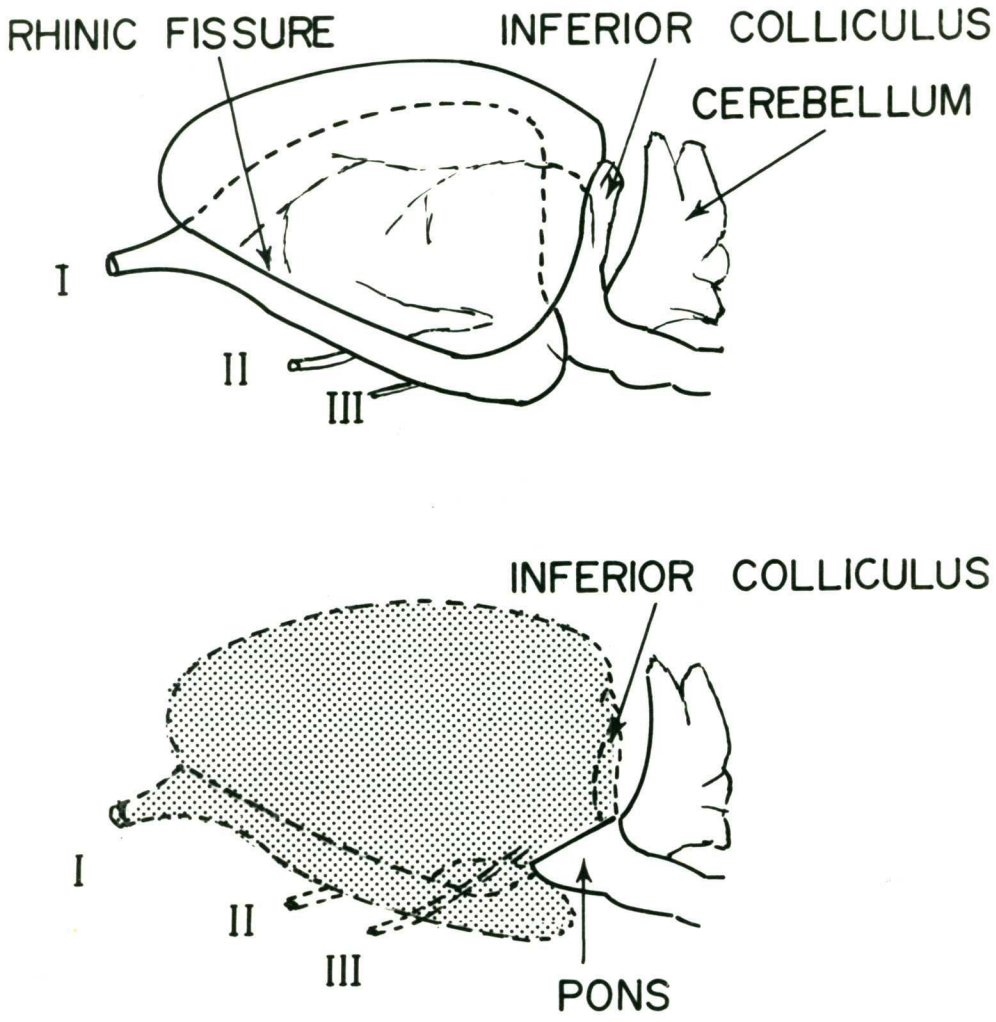

FIGURE 4

Diagrammatic illustration of a normal rabbit brain (TOP) and a pontine preparation (BOTTOM). The shaded area represents regions removed during the neural ablation operation.

on their sides and were placed in a special holder just prior to the experiment to maintain normal posture.

Autopsy appearances following neural ablation operations

At the end of experiments the operation site was examined for intracranial haemorrhage or excessive oedema of brain tissue. The results of experiments in animals where this occured were not used. The changes were gross and usually could be predicted from the animal's behaviour during the experiment. It has been shown previously that haemorrhage and oedema does not extend for greater than 500 microns in depth from the surface following ablation procedures and that the parts of the brain covered with pia mater were not damaged (Uther, 1970).

SECTION OF THE CAROTID SINUS AND AORTIC NERVES

This operation was carried out under general anaesthesia as described by Korner (1965a). The completeness of chemoreceptor and baroreceptor denervation was tested during the experiments using the responses to severe arterial hypoxia and pressor stimuli respectively (Korner, 1965a; Chalmers, Korner and White, 1967a).

VAGOTOMY

The vagus was isolated on both sides of the neck under general anaesthesia and either sectioned before the experiments began or during the course of the experiments after further application of local anaesthetic to the region.

AUTONOMIC BLOCKING DRUGS

(i) Atropine sulphate (Lancet Pharmaceuticals Pty. Ltd., Sydney)

This drug was used to block the vagal effects on the heart and to block cholinergic vasodilator effects on the peripheral circulation. The drug was given at an initial dose of 1 mg/kg i.v. followed by a continuous infusion of 0.1 mg/kg per minute, i.v. This dose is effective in blocking the heart rate changes due to alteration in vagal tone induced either reflexly or by electrical stimulation of the vagus (Korner, Langsford, Starr, Uther, Ward and White, 1968; Korner, Uther and White, 1969). The high dose of atropine used has no apparent toxic effect on the animal (Korner, Uther and White, 1968) and was needed because the drug is rapidly inactivated by an atropine esterase (Goodman and Gilman, 1970). In some rabbits much higher doses of atropine were necessary to produce cholinergic blockade. The results of experiments in these animals were not used.

(ii) Propranolol hydrochloride (Inderal, I.C.I.)

This drug was used to produce β-adrenergic block in the heart and in the vascular bed of skeletal muscle. An initial dose of 0.3 mg/kg i.v. was given followed by an infusion of 0.03 mg/kg per minute i.v. The absence of heart rate changes following β-stimulation with isoprenalin 4 µg i.v. was used to test the potency of the block at the heart. Although the effectiveness was not tested in these experiments, the same dose of propranolol has been previously shown to block the peripheral β-stimulant effects of low levels of circulating adrenaline from the adrenal medulla (Celander, 1954; Chalmers, Korner and White, 1966). The direct depressant effects of propranolol on smooth muscle are small at the dose used and have been neglected (Naylor, Chipperfield and Lowe, 1969).

(iii) Phenoxybenzamine hydrochloride (Dibenzyline; Smith, Kline and French)

Phenoxybenzamine was used to produce postsynaptic α-adrenergic nerve block following presynaptic nerve blockade with guanethidine. An initial dose of 6 mg/kg i.v. over 20 minutes was given followed by booster doses of 1 mg/kg per 30 minutes i.v. Thirty minutes after administration, the potency of the α-adrenergic block was tested by the absence of a constrictor response to noradrenaline, 4 µg i.v. (Fig. 24)

The drug has a slow onset of action and forms a stable bonding with α-receptors. Its mode of action is complex. As well as its blocking effect on α-receptors, the mechanism by which noradrenaline and related amines are transported across the axonal membranes from the extracellular space to the axoplasm of the adrenergic nerve ending (Uptake I) is inhibited by the drug (Iversen, 1973). Phenoxybenzamine also appears to block an "auto-inhibition" mechanism whereby released noradrenaline acts on presynaptic α-receptors to inhibit its own further release (Kirpekar and Puig, 1971; Enero, Langer, Rothlin and Stefano, 1972). In addition extraneuronal uptake and metabolism of noradrenaline (Uptake 2) is inhibited by the drug (Lightman and Iversen, 1969). There is no effect on β-receptors and the blocking effect on cholinergic receptors by the drug in higher doses than those used in these experiments were neglected (Goodman and Gilman, 1970).

(iv) Phentolamine (Regitine, Ciba)

A competitive postsynaptic α-adrenergic nerve block was produced by this drug with a dose of 4 mg i.v. The drug results in an effective α-blockade to the constrictor effects of noradrenaline 4 μg i.v. Phentolamine is related to

histamine and has a different mode of action at the α-adrenergic nerve terminal to that of phenoxybenzamine. As well as its α-adrenergic blocking action the drug has a weak β-adrenergic effect on the heart which is blocked by propranolol (Gould, 1969). This may be due to partial inhibition of Uptake I and consequent release of noradrenaline (Nayler and Carson, 1972). The drug also has a direct vasodilator action which was considered to be small and has been neglected (Taylor, Sutherland, MacKenzie, Staunton and Donald, 1965).

(v) Guanethidine Sulphate (Ismelin, Ciba)

Guanethidine 12.5 mg/kg i.v. as a single dose was given to produce presynaptic sympathetic nerve block. The effectiveness of the block was tested during the experiments by the absence of a baroreflex constrictor response in the hindlimb bed following a fall in arterial pressure (Chapter 7). The effects on hindlimb flow from electrical stimulation of the lumbar sympathetic chain has been used previously in this laboratory to test the adequacy of the block (Chalmers, Korner and White, 1967b). Guanethidine is bound specifically at adrenergic nerve endings and produces with a single dose considerable depletion of catecholamines in all tissues of the rabbit

except the brain and adrenal medulla (Cass, Kuntzman and Brodie, 1960; Cass and Spriggs, 1961; Abercrombie and Davies, 1963; Boura and Green, 1965). The drug is taken up into the nerve endings by the same transport mechanism as for noradrenaline (Uptake I, Iversen, 1973). The initial sympathomimetic effects of the drug due to release of noradrenaline, disappear as the nerve endings become depleted of transmitter. Depletion of noradrenaline at the endings is not due to direct replacement of catecholamines by guanethidine but may be due to the failure of guanethidine to destroy intraneuronal monoamine oxidase which continues to metabolise the remaining noradrenaline (Laverty, 1973). Following an injection of guanethidine in the rabbit, there is a transient vasodilatory action (Chapter 7), the reason for which is unknown. It is unrelated to β-adrenergic stimulation since the effect is not blocked in the presence of propranolol. After presynaptic block, constrictor responses to exogenous catecholamines are increased. This phenomenon occurs rapidly and may be due to direct sensitization of smooth muscle by the lowering of the background catecholamine concentration or by blockade of catecholamines to non-specific receptors such as Uptake 2

(Vane, 1962; Trendelenburg, 1963). Guanethidine has small transient effects at cholinergic receptor sites which have been neglected in these studies (Boura and Green, 1965).

NEUROMUSCULAR BLOCK

Decamethonium iodide (Koch-Light Laboratories Ltd., Colnbrook, England)

This drug was used to produce muscle relaxation in those experiments where ventilation was maintained at a constant level. A dose of 1 mg/kg i.v. initially was followed by 0.5 mg/kg i.v. every 15 minutes and was sufficient to prevent movement without interference with autonomic ganglionic transmission (Korner, Uther and White, 1969).

ADMINISTRATION OF HYPOXIC GAS MIXTURES

Gas mixtures containing 6-8% oxygen in nitrogen were prepared before experiments from cylinders of oxygen and nitrogen (Commonwealth Industrial Gases (Australia) Ltd.). The oxygen concentration was determined using a Beckman model E2 paramagnetic oxygen analyser. The gas mixtures were administered to the rabbits from a light rubber meteorological balloon through a one-way respiratory flap valve connected to a tracheo tomy tube.

VARIABLES RECORDED DURING EXPERIMENTS

(i) Blood pressure and heart period

Ear artery and right atrial pressures were measured using Statham P23 Db and P23 Ac transducers, respectively. The signals were recorded in ink on a Grass model 7 polygraph recorder. Mean pressures were obtained by electronic damping. Zero pressure was taken at a plane 5 cm above the floor of the box in which the rabbit sat and was at about the same level as the top of the animal's sternum. The calibration and linearity of the measuring system were checked periodically against mercury and water manometers.

The heart period (pulse interval) was obtained from the arterial pressure pulse using a circuit triggered by the upstroke of the pressure pulse. The signal was then passed into the Grass recorder. This system was calibrated against an interval oscillator. The pulse interval recording system responded to step changes in heart period input with a time delay of 1 period and thus reflected faithfully the beat to beat fluctuations in heart period.

(ii) Measurement of Cardiac Output and Hindlimb Blood Flow

Blood flow was measured by the Doppler frequency shift method using a previously implanted cuff transducer and Doppler ultrasonic flowmeter as described by Franklin and colleagues (Franklin, Watson, Pierson and Van Citters, 1966; Van Citters and Franklin, 1969; Vatner, Franklin and Van Citters, 1970; made by Pierson Laboratories, San Diego, California). The device measures blood velocity but was used to measure changes in volume flow within animals in these experiments. Under the conditions of chronic implantation used, the transducer becomes firmly attached to the vessel and the cross-sectional area of the vessel enclosed within the transducer becomes fixed (Vatner, Franklin, and Van Citters, 1970).

Cardiac output and hindlimb flow were measured using transducers 5 mm i.d. placed around the aortic root and 4 mm i.d. placed around the lower abdominal aorta respectively. The emitter and sensing crystals of the transducer were mounted at a constant angle of 45 degrees to the direction of blood flow. On the day of the experiment the colour coded transducer wires from the animal's back were connected to the emitter and demodulator

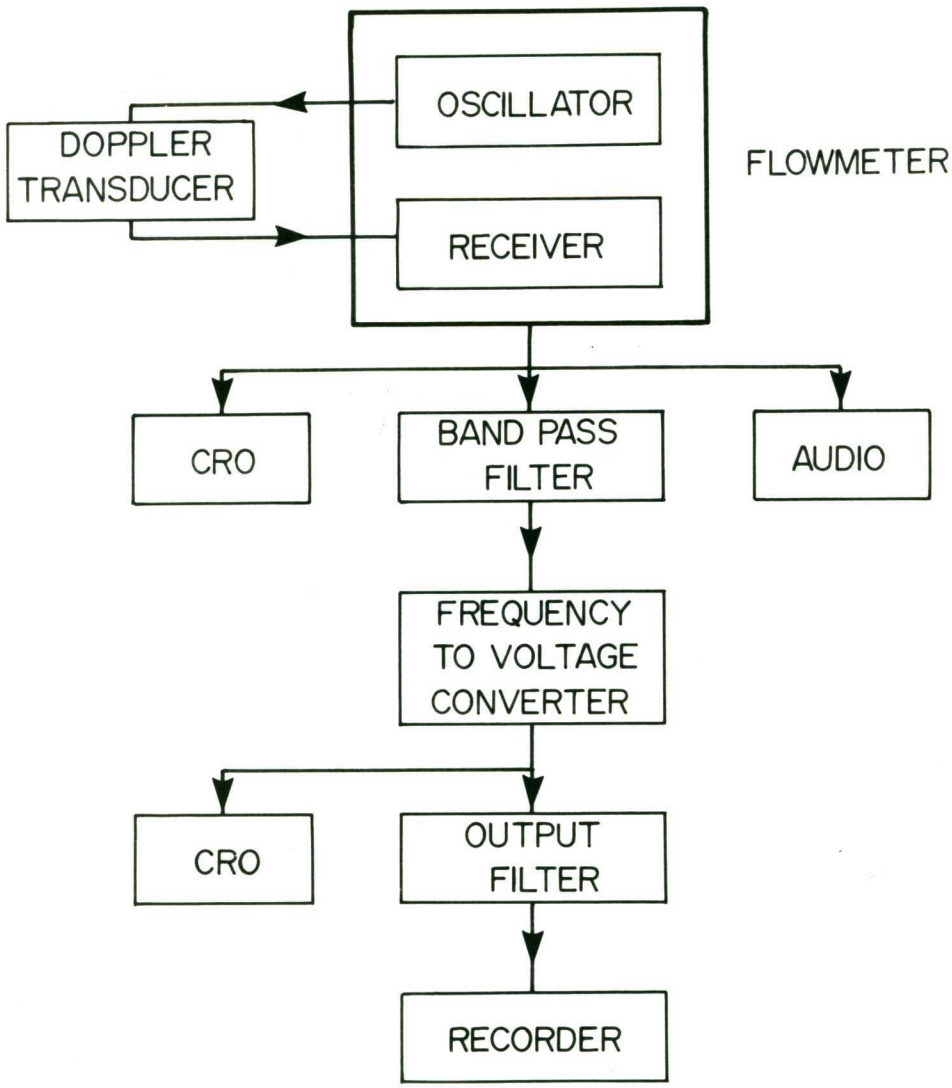

FIGURE 5

Flow diagram of Doppler flowmeter assembly.

terminals of the flowmeter. 9MHz ultrasound was transmitted into the moving blood and the frequency shift (incident frequency minus reflected frequency) was continuously monitored over an audio-amplifier (Fig. 5). Since each Doppler flow transducer is resonant at a slightly different frequency the exciter and demodulator frequencies were tuned to match the resonant frequency of the transducer by adjusting the frequency lugs of both oscillators to give an optimal signal both audibly and visually. The gain control on the demodulator was adjusted to give the maximum signal without distortion from electrical noise.

The Doppler flow (frequency) signal was then passed through a band pass filter (Krohn-Hite model 3500 filter) set to pass signals between 20 Hz and 20 kHz into a frequency-to-voltage converter (zero crossing detector, Pierson Laboratories, San Diego, California). Since signals from the aortic root have frequency components up to 20 kHz, the 15 kHz input filter of the converter had previously been removed. In this device the frequency of the signal was converted to an analogue voltage proportional to the frequency shift by means of a comparator with a hysteresis band centred on zero volts. The threshold of the

zero crossing detector was varied by controlling the amplitude of the signal fed to the comparator. This control was adjusted prior to each experiment to the centre of the sensitivity range where the analogue voltage waveform was independent of signal amplitude variations. For the measurement of cardiac output this position was determined by the position of zero end-diastolic flow. Since there are a number of sources of noise in Doppler systems (Wells, 1969) the absence of a range of sensitivity through which no amplitude changes occured indicated the signal to noise ratio of the signal was not high enough for a reliable estimate of flow velocity and the results were not used.

The analogue voltage was recorded on a Grass polygraph and mean flow was computed from phasic flow using an integrating circuit with a 2 second time constant.

Prior to each experiment the output frequency of the flowmeter was calibrated against an external frequency source (Heathkit sine-square wave generator, model IG-18) passed through the same frequency to voltage converter and recording system.

Absolute blood velocity was obtained from the Doppler equation

$$F = 2.f_e.V.\cos a/c$$

where F is the absolute frequency shift between the emitted signal frequency f_e and the reflected frequency f_r, V is the velocity of the scattering medium (blood), a is the angle between the acoustical axes and blood velocity and c is the velocity of sound in blood.

For a typical application with f_e = 9 MHz, cos a = 0.7 (45^o), c = 1.5 x 10^5 cm/sec, and range of F, 0 to 20 kHz, velocity of blood may range from 0 to 200 cm/sec.

Zero flow was established by removing the input to the frequency-to-voltage converter since the frequency shift and thus flowmeter output is zero when blood is stationary (Van Citters and Franklin, 1969; Vatner, Franklin and Van Citters, 1970).

The frequency response of the flowmeter recording system has been previously determined in this laboratory as - 3 dB at 60 Hz with a rise time (10-90%) of 10msec

and was found to be linear up to 20 kHz (McRitchie, 1973).

CALIBRATION OF THE DOPPLER FLOWMETER

Previous workers have demonstrated a linear relationship between the output of the Doppler flowmeter and volume flow both in vitro and in vivo (Van Citters and Franklin, 1969; Vatner, Franklin and Van Citters, 1970). In this laboratory during determinations of cardiac output in the rabbit a similar relationship has been found (Fig. 6) where comparisons were made between Doppler, thermodilution and electromagnetic flowmeter methods (McRitchie, 1973; White, McRitchie and Porges, 1974).

Thus for the series of cardiac output measurements described in Chapter 6, the linearity of the Doppler flowmeter system was regarded as confirmed. The instrument was calibrated at a single level of flow using the thermodilution method (Korner, 1965a). 7 to 23 days (mean 14 days) after implantation of the flowmeter under sodium pentobarbitone anaesthesia (approximately 30 mg/kg i.v.), an aortic thermistor catheter was placed in the upper abdominal aorta through the femoral artery while a right atrial catheter was inserted through a branch of the right external jugular vein. Ten comparisons of Doppler and thermodilution cardiac

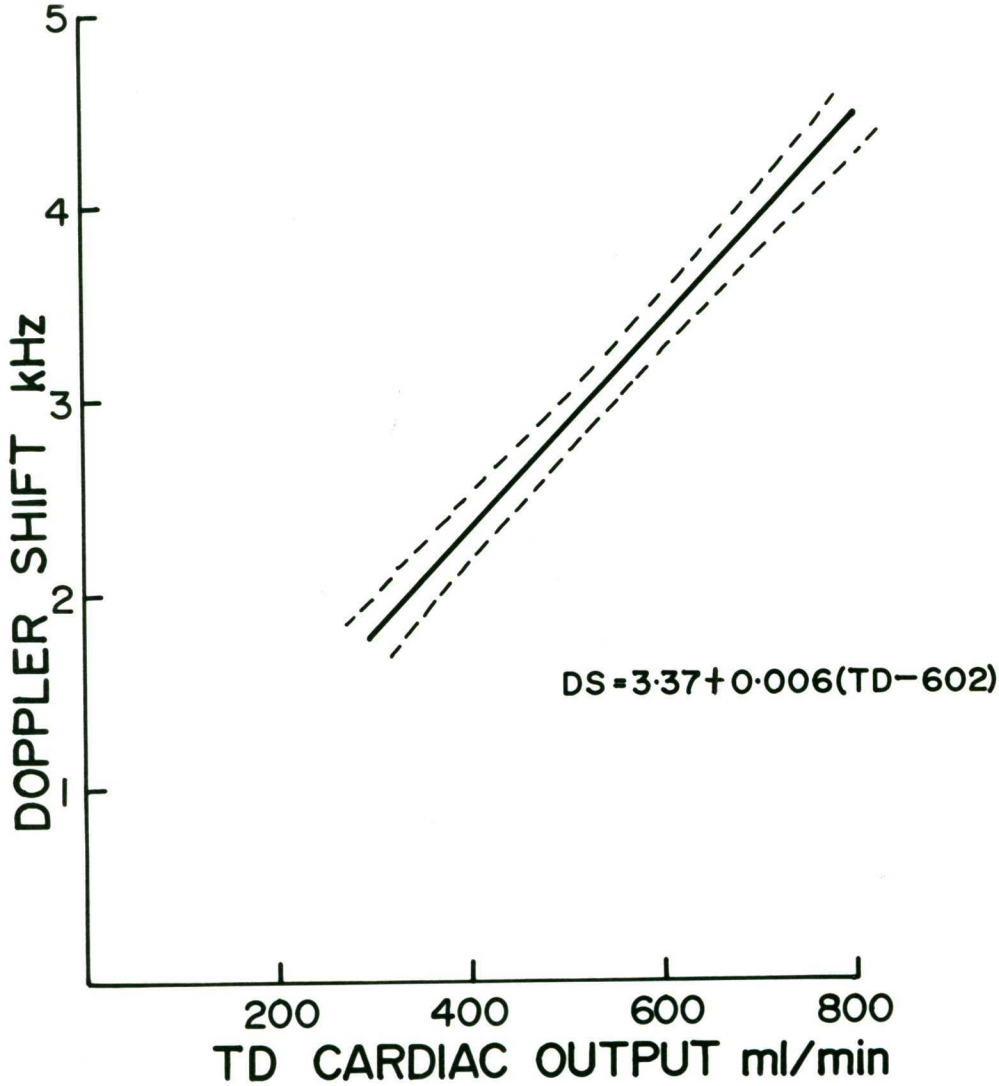

FIGURE 6

Graph illustration calculated regression line (solid line) for in vivo comparison of thermodilution cardiac output (TD) and Doppler shift (DS) together with the 95% confidence limits (dashed line). The comparison was made 7 days after the implantation of the transducer (From McRitchie, 1973).

output were made at intervals of 3 minutes. The catheters were then removed and the animals allowed to recover from the anaesthetic. The average calibration was 132 ± 3 ml/min per kHz. There were no differences between the cardiac outputs of the two groups of animals subsequently subjected to a sham procedure or rendered hypertensive (129 ± 3.5, 134 ± 4.2 ml/min per kHz respectively) although there were small differences between individual transducers within each group.

A series of in vitro volume flow calibration studies of the lower abdominal aortic Doppler flowmeter was carried out in sham-operated and hypertensive animals 10 to 35 days after implantation. The main purpose of these studies was to examine the degree of variation between the calibration curves of individual transducers especially with regard to the effect of differences in intravascular pressure on the individual calibration curves. At the conclusion of an experiment, the transducer and lower abdominal aorta were removed intact and placed in 10% formalin in 0.9% NaCl. In most cases calibration was carried out on the following day. There were no significant differences between the calibration curves performed immediately after experiments and on subsequent occasions.

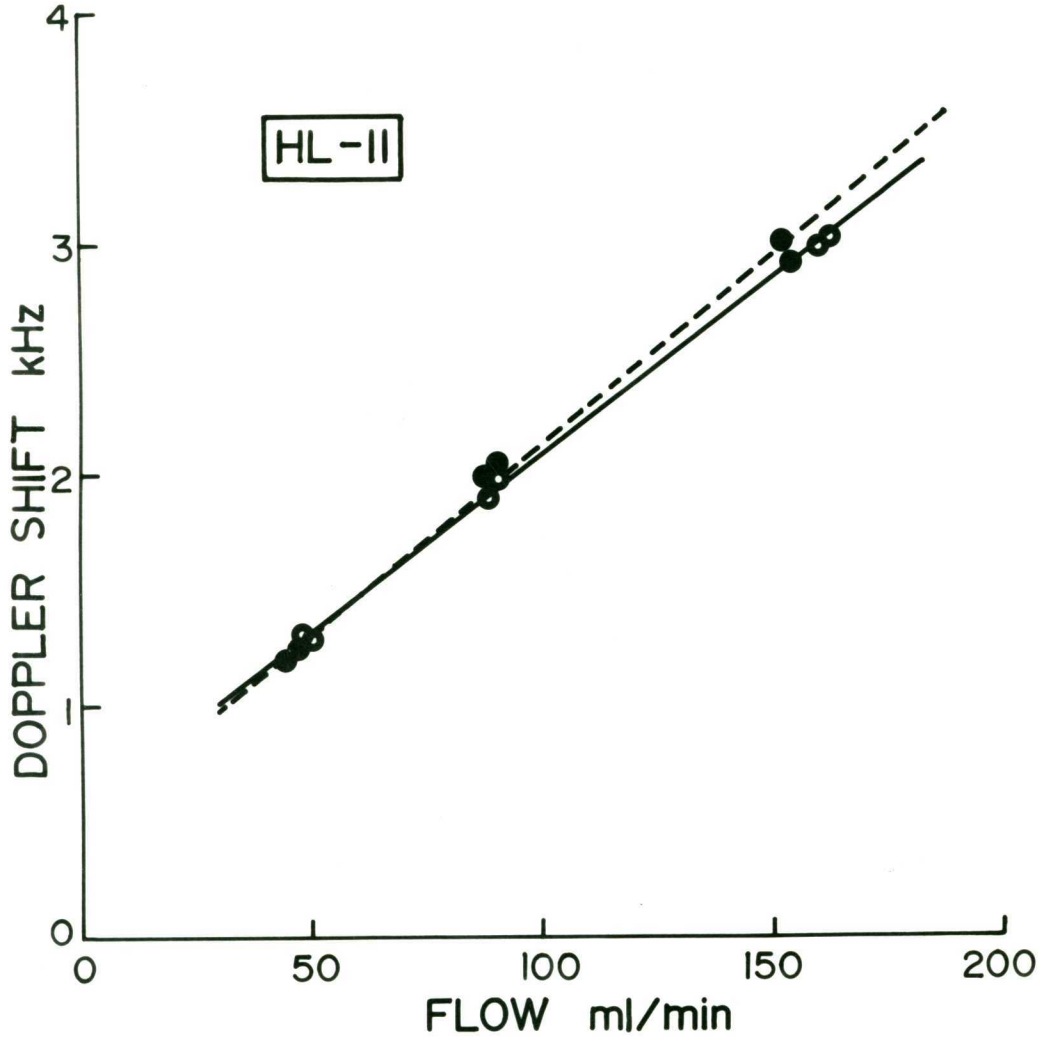

FIGURE 7

Graph illustrating the calculated regression lines for the calibration of a single hindlimb transducer (HL-11) at a high (open circles, solid line) and a low (closed circles, dashed line) pressure.

(see continuation)

FIGURE 7 continued...

High pressure	Low pressure
$\bar{X}^*$ = 95.7 ml/min	$\bar{X}$ = 100.8 ml/min
$\bar{Y}^*$ = 2.05 kHz	$\bar{Y}$ = 2.08 kHz
Sy^2 = 3.075	Sy^2 = 2.948
Sx^2 = 11627.33	Sx^2 = 12504.83
Sxy = 188.3	Sxy = 191.9

Analysis of Variance Tables

	D/F	SS	EMS	D/F	SS	EMS
Regression	1	3.049		1	2.944	
error	4	0.016	0.004	4	0.004	0.001
Total	5	3.075		5	2.948	

Calculated Regression Lines

Y = 0.50 + 0.0162X	Y = 0.46 + 0.0153X
S_a = 0.06	S_a = 0.03
S_b = 0.0006	S_b = 0.0003

*X, Y are the flow (ml/min) and Doppler shift (kHz) respectively.

Volumetric calibration was carried out at two different pressures (approximating the normotensive and hypertensive mean arterial pressures) using a pump perfusion circuit, measuring cylinder and stopwatch. Linear regression lines were constructed for the calibration curves of each transducer at each pressure level (Fig. 7) and the slopes and intercepts of the lines were compared as illustrated in Table 1a using the method of Snedecor and Cochran (1969, pp 432-436).

Under these conditions the output for a particular Doppler transducer was linearly related to volume flow from 40 ml/min up to 150 ml/min and remained constant in the pressure range between 50-150 mmHg examined. There were small differences between the output of individual transducers in both the normotensive and hypertensive groups of animals for a given flow (Table 1b), probably due to small differences in vascular calibre within the transducer shell plus differences in the angle of orientation between the emitting and sensing crystals on the one hand and the blood stream on the other. For a given Doppler shift the average volume flow difference was $\pm$ 5%. There remains the possibility of systematic differences between the mean aortic flow estimate in transducers implanted in hypertensive animals and those implanted in sham-operated animals since

TABLE 1A

Comparison of Regression lines at two pressure levels for a single transducer.

	Sx^2 *	Sxy	Sy^2	b	df	Error SS	EMS
Low pressure	12504.83	959.4167	73.7083	0.0767	4	0.0983	0.0246
High pressure	11627.00	941.5000	76.8750	0.0810	4	0.6367	0.1592
					8	0.7350	0.0919
Combined	24131.83	1900.9200	150.583	0.0788	9	0.8431	0.0937
			Difference between slopes		1	0.1081	0.1081
Overall	24212.25	1903.5000	150.6667	0.0786	10	1.0188	0.1019
			Difference between intercepts		1	0.2378	0.2378

Comparison of slopes F = 0.1081/0.0919 = 1.18 (df 1,8) N.S.

Comparison of intercepts F = 0.2378/0.0937 = 2.54 (df 1,9) N.S.

* Sx^2, Sy^2, Sxy sums of squares and products

X_i = flow (mls/min)

Y_i = Doppler shift mm (5 mm = 1 kHz)

TABLE 1B

Comparison of average regression lines of sham transducers

	Sx^2 *	Sxy	Sy^2	b	df	Error SS	EMS
HL 36	13925.06	1061.47	111.7647	0.0762	15	30.8516	2.06
HL 18	8285.33	759.67	70.3333	0.0917	4	0.6809	0.17
HL 10	35164.00	3413.50	350.2375	0.0971	18	18.8765	1.05
HL 30	9745.88	688.59	51.0588	0.0707	15	2.4071	0.16
					52	52.8161	1.016
Combined	67120.27	5923.23	583.3943	0.0883	55	60.6819	1.103
			Difference between slopes		3	7.8658	2.62
Overall	94404.58	8585.33	873.8333	0.0909	58	93.0667	1.60
			Difference between intercepts		3	32.3848	10.79

Comparison of slopes F = 2.62/1.02 = 2.6 (df 3,52) NS.

Comparison of intercepts F = 10.79/1.10 = 9.8 (df 3,55) Sig. P < 0.01

* Notation as in Table 1A

there were small differences between individual transducers and since there were no calibrations carried out prior to the induction of hypertension. However the systematic differences are probably small since in each transducer there was no difference between the calibration curves at the high and low pressure levels, and there were no significant differences between the transducers (normotensive and hypertensive) when they were all grouped together.

Noise

Unavoidable noise in the Doppler flowmeter assembly evident at low signal strength was probably due to the use of an artificial perfusate mixture (milk) which made the flowmeter difficult to tune. This noise accounted for the positive intercept of the calibration regression lines. At zero flow there was an unstable baseline offset from zero Doppler shift. However at very low rates of flow (0.8 ml/min) the signal was stable and the Doppler shift was close to the value of the intercept predicted from the calculated regression line. This effect occurred with the hindlimb transducers only when the signal to noise ratio was poor and was not detected when using the transducers to determine cardiac output. Since end-diastolic flow was set to zero Doppler shift in the latter case, the calibration curves pass through zero (Fig.6).

Backflow

The Doppler flowmeter does not detect changes in direction of flow and the small diastolic backflow component at the aortic root is measured as an additional component of forward flow. Electromagnetic flowmeter studies in 4 rabbits in this laboratory indicated this to be an average of 4.2 $\pm$ 0.4% (S.E. of the mean) of the stroke volume in forty measurements (McRitchie, 1973) and this correction has been neglected in the present study.

Backflow has been observed in the lower abdominal aorta of the anaesthetized rabbit (Shipley, Gregg and Schroeder, 1943; McDonald, 1960; Spencer and Denison, 1973). In the unanaesthetized resting rabbit McRitchie (1973) found no backflow using electromagnetic flow transducers. However with severe vasoconstriction when zero flow levels were approached following nasopharyngeal stimulation, backflow was observed in 5 out of 16 tests in the animals studied. The backflow under these conditions never exceeded 15% of total blood flow. Since in the present series of experiments during reflex vasoconstriction or during noradrenaline-induced vasoconstriction the fall in hindlimb blood flow was not as marked (minimum flow of approximately 30 mls/min), corrections due to backflow in

the lower abdominal aortic transducers were also neglected.

(iii) Vascular Resistance

Total vascular resistance and regional hindlimb resistance were measured and recorded continuously in arbitrary units as mean arterial pressure/Doppler shift (kHz) using an electrical divider circuit. The effects of changes in right atrial pressure and peripheral venous pressure, respectively, were neglected in these estimations of vascular resistance.

(iv) Respiratory Variables

Tidal volume and respiratory rate were measured using a differential manometer attached to the inflow side of a one way flap valve. The device was designed and constructed in this department by Mr. R. Hilder and consisted of a gauze wire mesh fixed across a chamber through which air was passed. The pressure difference across the mesh was measured by the deflection of a rubber diaphragm within a second chamber. The deflection, which was proportional to air flow, was read from a meter and a trigger measured the respiratory rate. The air flow meter was calibrated using a Starling respiratory pump which had previously been standardised with volumes of air

measured in a gas meter (Alex, Wright and Co.(Westminster) Ltd.).

(v) Blood Gases

Arterial PO_2, PCO_2 and pH were estimated using an Instrumentation Laboratory model IL 213 blood gas analyser and pH meter. 1.5 ml blood samples were collected from the ear artery cannula anaerobically in greased syringes containing heparin in the dead space. The analyses were carried out within 2-3 minutes of collection at 40°C which is the normal body temperature of these rabbits. Blood gas tensions could be determined with a reproducibility of ± 1 mmHg and pH with a reproducibility of ± 0.01 units.

EXPERIMENTAL PROCEDURE

All experiments were carried out in unanaesthetized rabbits while they were sitting quietly in their boxes. After preliminary procedures the animal was allowed to settle for 30 minutes before observations commenced. In the case of neurological procedures which required a general anaesthetic prior to the experiment, observations commenced 3 hours after cessation of the anaesthetic. During the course of experiments, the animals remained quiet and suffered no obvious distress. In some cases where the

animal became agitated and would not settle, the experiment was abandoned.

PART II

STATISTICS AND ANALYSIS OF RESULTS

Tests of significance

These were performed using Student's t test or Fisher's F ratio. The Analysis of Variance (Snedecor and Cochran, 1969, pp 258-338) was used for assessing changes in haemodynamic variables over different time or treatment periods (Table 2a). In some cases, further partitions of the sums of squares into individual degrees of freedom was carried out (Table 2b) by constructing sets of linear functions which were mutually orthogonal (Cochran and Cox, 1953, pp 55-72).

CONSTRUCTION OF BAROREFLEX STIMULUS-RESPONSE CURVES

In studies of the baroreceptor-heart rate reflex, a range of small rises and falls in mean arterial pressure was induced in pairs by injections of vasoconstrictor or vasodilator drugs in the human studies (Chapter 3) and by inflations of previously implanted aortic or inferior vena caval balloons in the rabbits (Fig. 9, Chapters 4 and 5). For each pair of changes in the blood pressure (i.e. rise

TABLE 2A

Analysis of changes in blood pressure during development of hypertension.

ANIMALS	I*	II	III	IV	
	74	81	80	84	319
	93	91	90	93	367
	77	77	80	84	318
SHAM	72	78	72	77	299
	87	87	89	92	355
	84	87	88	91	350
	81	99	95	95	370
TOTAL	568	600	594	616	2378
AVERAGE	81	86	85	88	
	85	79	91	95	350
	78	99	112	117	406
HYPERTENSIVE	87	95	97	109	388
	87	111	110	142	450
	78	92	89	109	368
	60	94	111	108	373
	106	116	126	135	483
TOTAL	581	686	736	815	2818
AVERAGE	83	98	105	116	
	1149	1286	1330	1431	5196

* I - control reading
II, III, IV - 10,21 and 33 days after sham operation or renal wrapping

ANALYSIS OF VARIANCE TABLE

	df	SS	EMS	F
Between animals	13	8040.9286	618.53	
Between groups)	1)	3457.1429	3457.14	83.0
Animals in groups)	12)	4583.7857	381.98	9.1
Between times	3	2932.4286	977.4762	23.4
Times x Groups	3	1352.1428	450.7143	10.8
Times x animals within groups (error)	36	1503.9286	41.7758	
TOTAL	55	13829.4286		

TABLE 2B

Analysis of Changes in Blood Pressure during Development of Hypertension
Partition of Degrees of Freedom for Times

1. Between Times

I	II	III	IV	PARTITION	DIVISOR	SS	F
3	-1	-1	-1	3 x 1149-(1286 + 1330 + 1431)	14 x 12	2142.8571	51.0
0	-2	+1	+1	2 x 1286+(1330 + 1431)	14 x 6	425.2500	10.2
0	0	-1	+1	- 1330 + 1431	14 x 2	364.321	8.7
					TOTAL	2932.4281	

2. Times x Groups

I	II	III	IV	SHAM PARTITION	HYPERTENSIVE PARTITION	DIVISOR	SS	F
3	-1	-1	-1	-106 av.effect 5.04 mmHg	-(-494) 23.5 mmHg	14 x 12	896.0952	21.5
0	-2	+1	+1	10 av.effect 0.7 mmHg	-(179) 12.8 mmHg	14 x 6	340.0119	8.1
0	0	-1	+1	22 av.effect 3.14 mmHg	-(79) 11.3 mmHg	14 x 2	116.0357	2.8
						TOTAL	1352.1428	

and fall) the mean arterial pressure (MAP), pulse pressure (PP), right atrial pressure (RAP) and the heart period (HP) were averaged over 10-15 seconds before the change in blood pressure and during the 'steady-state' plateau phase of the reflex heart period response (approximately 20-30 seconds after the change in blood pressure).

MAP and HP before and during each change in blood pressure were expressed as a percent of the mean of the resting values just preceding each pair of changes in blood pressure. The gain (G) of the heart period response was calculated as

$$G = \Delta HP / \Delta MAP \qquad (1)$$

where ΔHP and ΔMAP are the percent differences in heart period and blood pressure respectively, before and during each blood pressure change.

Stimulus-response curves relating the reflex changes in HP to the changes in MAP were calculated in percentage units by expressing the changes in heart period and mean arterial pressure as percentages of the mean of the resting values, HP_r and BP_r, immediately preceding each pressure change. Thus each pair of pressure changes was related to a common resting value, and fluctuations in this resting value were subsequently taken into account when

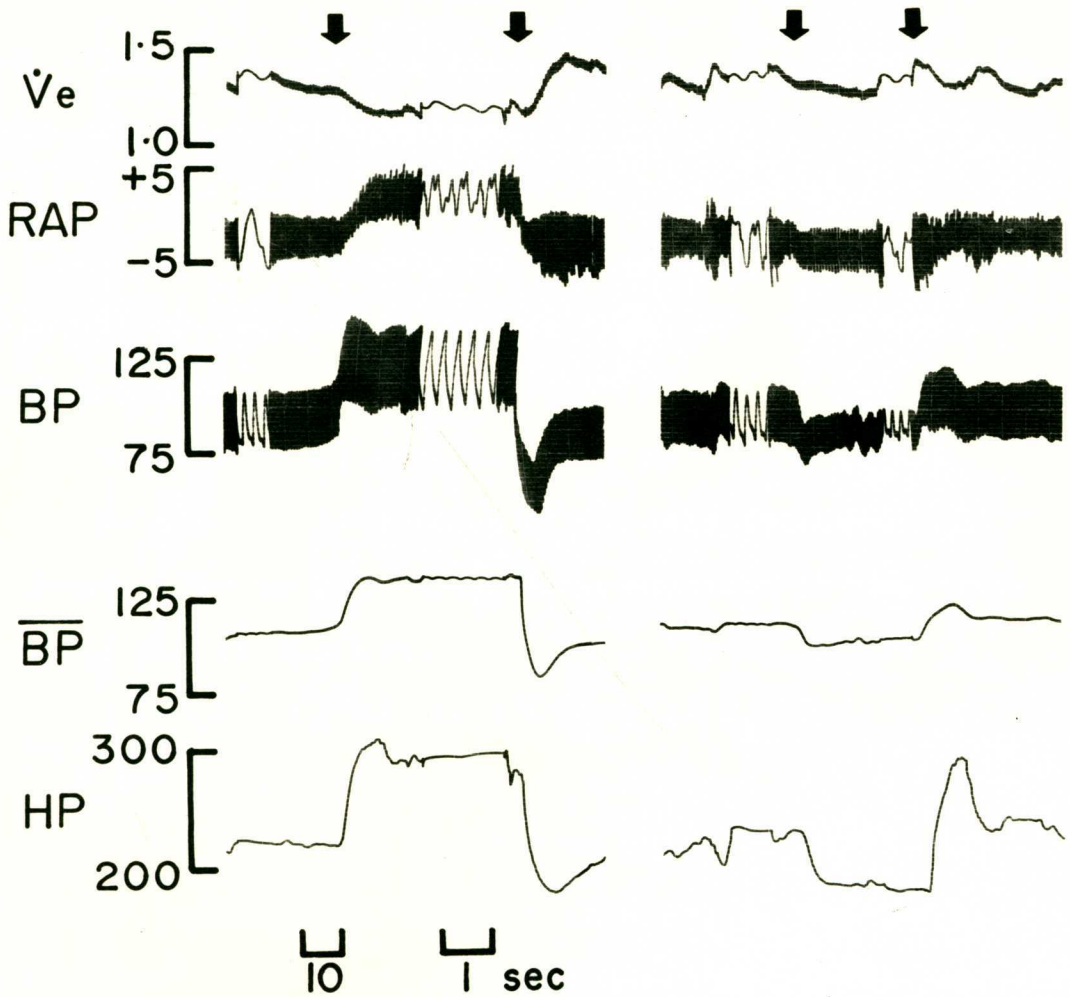

FIGURE 9

Records of respiratory minute volume ($\dot{V}e$, litres/min), right atrial pressure (RAP, mmHg), phasic ear artery blood pressure (BP, mmHg), mean ear artery blood pressure ($\overline{BP}$, mmHg), and heart period (HP, msec) during aortic balloon inflation (LEFT) and during inferior vena caval balloon inflation (RIGHT). Balloons were inflated between the arrows.

the standard errors for different portions of the stimulus-response curve were derived.

The relationship between heart period and mean arterial pressure was S-shaped (Fig. 10). Multiple regression equations were derived separately for all the responses following increases and decreases in MAP respectively for each group of animals (Snedecor and Cochran, 1969 pp 381-418). Each equation in the human studies was of the form

$$G = a + b_1 \log \Delta MAP + b_2 BP_r + b_3 \Delta PP \qquad (2)$$

and in the rabbit experiments

$$G = a + b_1 \log \Delta MAP + b_2 \Delta RAP + b_3 \Delta PP \qquad (3)$$

In these equations a is the intercept, b_1, b_2 and b_3 are the partial regression coefficients, ΔRAP (mmHg) and ΔPP (% of resting) are the changes in right atrial pressure and pulse pressure respectively during each pressure change, BP_r is the resting blood pressure as defined above and G and MAP are as defined previously.

With these equations, curves were constructed by calculating G from each equation using values of ΔMAP within the range of observations, and using constant values of ΔRAP, ΔPP and BP_r. The curves were then converted into absolute HP and MAP units by using as 100% scaling factors,

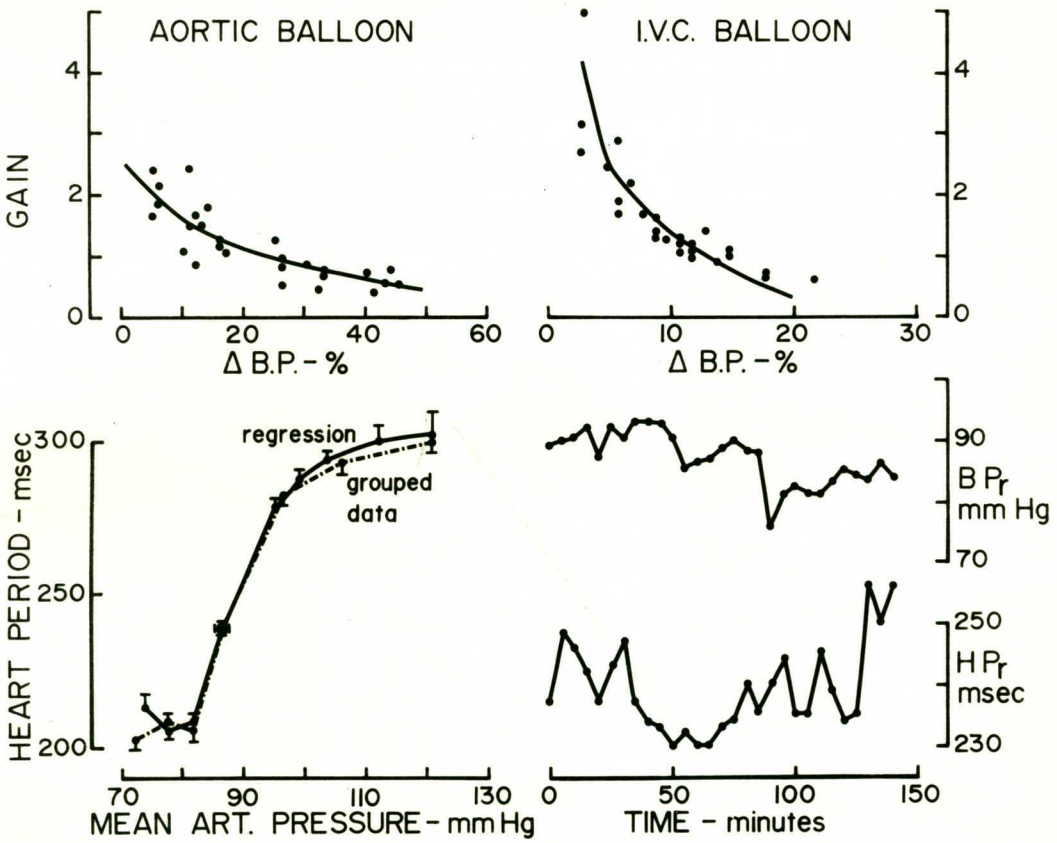

FIGURE 10

Results obtained from one sham-operated rabbit during pairs of balloon inflations performed every 5 minutes.

TOP: Relationship between gain of heart period response and percentage deviations in mean arterial pressure from the resting value during aortic balloon inflation, and during inferior vena caval balloon inflation. The line in each graph is the calculated regression line.

BOTTOM: On the left the relationship between mean ear artery pressure and heart period calculated by regression, and also by grouping the blood pressure values into a number of intervals above and below resting; the SE of the mean values are also shown. The resting blood pressure and heart period are shown as a larger point $\pm$ 1 SE on the left. In the graph on the right are shown the fluctuations in resting values (BP_r, HP_r) with time, during the course of the experiment.

the mean of all the resting HP and MAP values contributing to the equations. The role of factors such as ΔRAP, ΔPP and BP_r in the reflex response was determined by the significance of the respective partial regression coefficients which was a measure of the linear independence of the particular variable.

The standard error of the heart period (SE_{HP}) corresponding to a particular value of ΔMAP was

$$SE_{HP} = \left[SE^2_{\overline{HP}_r} + (\overline{HP}_r / 100)^2 . \Delta MAP^2 . SE_G^2 \right]^{0.5} \qquad (4)$$

where $\overline{HP}_r$ is the mean resting heart period (msec), $SE_{\overline{HP}_r}$ is the standard error of $\overline{HP}_r$ derived as appropriate from the sums of squares between animals, the sums of squares within animals or the pooled sums of squares for the whole group (see below) and SE_G is the standard error of G for the particular value of ΔMAP derived by standard formulae (Snedecor and Cochran, 1969, equation 9, page 171).

Computer programmes in Fortran IV language were written to perform the above calculations which were carried out using an IBM 7040 computer (see Appendix).

Stimulus-response curves obtained by fitting the regression functions to the data were in close agreement with curves obtained after grouping the arterial values (ΔMAP) into three or four intervals above and below the resting value, determining the heart period for each interval and then converting the mean percent differences into absolute units as before. The advantages of using the regression equations for curve fitting instead of grouping the data were that the standard errors of the stimulus-response curves parameters (see below) could be conveniently calculated by standard statistical methods.

CALCULATION OF STIMULUS RESPONSE CURVE PARAMETERS

Three parameters were calculated: the pressure-dependent heart period range (HPR, msec), the median blood pressure (BP_{50}, mmHg) and the average gain ($\bar{G}$, msec/mmHg).

HPR was calculated as the mean plateau value once the upper saturation pressure had been reached minus the mean plateau value below threshold pressure. Its standard error was determined using the regression equation for each set of pressure changes (i.e. rises or falls). Thus;

$$SE_{HPR} = (SE^2_{HP_R} + SE^2_{HP_F})^{0.5} \qquad (5)$$

where the values in parenthesis are values of SE_{HP} calculated in accordance with Eq. 4 for a rise (R) or fall (F) in MAP. The values of SE_{G_R} and SE_{G_F} used to determine SE_{HP_R} and SE_{HP_F} were taken at the mean values of ΔMAP which were near the beginning of the heart period plateau at upper saturation and at threshold pressure respectively.

BP_{50} was the estimated blood pressure at half HPR. The heart period, obtained from the two regression equations , was expressed as a fraction of HPR, and the probit of this value was plotted against the mean arterial blood pressure. The relationship between the heart period probit and the mean arterial pressure was approximately linear (Fig. 11) suggesting that, as a reasonable first approximation, the stimulus-response curve could be described as a normal Gaussian distribution function (Finney, 1952, pp183-198; Aichison and Brown, 1957). The probit regression line was determined, and BP_{50} was estimated at a probit of 5.0. The standard error of BP_{50} was calculated as $SD/(N)^{0.5}$, where SD is the reciprocal of the regression coefficient of the probit regression line (Finney, 1952) and $N = n_R + n_F + n_P$, is the number of vasoconstrictor, vasodilator and paired resting measurements.

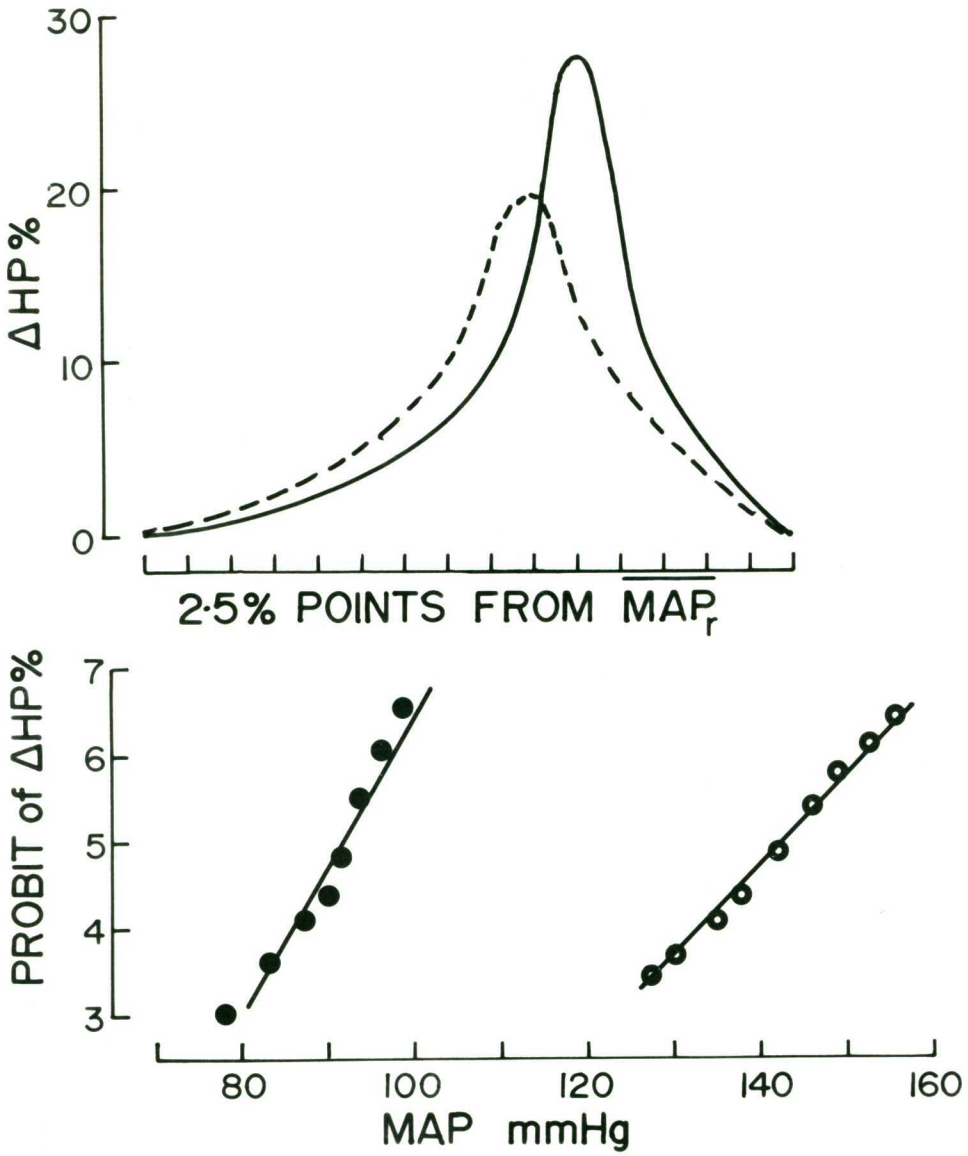

FIGURE 11

TOP: Curves showing relationship between mean arterial pressure (mmHg) and change in heart period (ΔHP%) between mid-points of successive blood pressure intervals, obtained from the stimulus response curves for the younger normotensive (group 1) and hypertensive (group 2) subjects in Figure 14.

BOTTOM: Relationship between mean arterial pressure (mmHg) and probit of heart period (expressed as the probit of pressure-dependent HPR).

$\bar{G}$ was assessed as the integrated mean gain between + 1 and -1 standard deviations (SD) from the BP_{50} of the distribution function and was calculated as $\bar{G}$ = 0.683 HPR/SD; 0.683 is the fraction of the area under the normal distribution curve between + 1 and - 1 SD (Snedecor and Cochran, 1969, Table A3), and $SE_{\bar{G}}$ is approximately $\bar{G}$. $\left[(SE_{G_R}/G_R)^2 + (SE_{G_F}/G_F)^2\right]^{0.5}$, where the expressions in brackets are obtained from the constrictor and dilator regression equations and G_R and G_F are the gains at the mean arterial blood pressures + 1 and - 1 SD, respectively from the mean (BP_{50}) of the distribution function.

Effect of Variation Between Animals

Several sources of variation determined the mean-stimulus response curve of a particular group of animals. To assess these different variations, 3 sets of average MAP-HP curves were calculated in each group, derived from 'between animal' data, 'within animal' data and the pooled results. This was done using the Analysis of Covariance to partition the pooled sums of squares and crossproducts of G, log ΔMAP and other variables used in the multiple regression equations, into 'between animals' and 'within animal' components (Korner, Shaw, West and

Oliver, 1972; Snedecor and Cochran, 1969, pp 419-446). In addition, the sums of squares of HP_r and BP_r were also partitioned into 'between' and 'within' animal components for calculating SE_{HP} and SE_{BP}. The differences between MAP-HP curves calculated from the three sets of equations were minimal but the standard error of the mean (S.E.M.) of the HP values were significantly greater using the 'between animal' data than the curves obtained from the 'within animal' data. This suggests that there were systematic differences in HP response to a given pressure change between the different animals of the group but that these were distributed normally about the mean response.

For calculating the standard error of the difference in the values of $\bar{G}$ and of HPR between hypertensive and normotensive groups as in Chapters 3 and 4, it is appropriate to use 'within animal' S.E.M. for these parameters in each group. Each animal of the group has contributed a number of HP responses during the rises and falls in MAP from its own resting MAP. Hence the S.E.M. of each parameter calculated from the 'within animal' data provides a measure of the average variability of individual HP responses in each group.

The position of each MAP-HP curve along the MAP axis is determined by the error limits of BP_{50} which are virtually the same as the error limits of the average resting MAP. In the average MAP-HP curves these are not determined by the small fluctuations in resting MAP occurring spontaneously in each animal during the experiment. The fiducial limits for fixing the mean MAP (or BP_{50}) of the group depend on the range of variation in these measurements between individual members of the group. Hence for assessing the significance of the difference in BP_{50} between hypertensive and normotensive groups, the standard error of the difference must be calculated using the 'between animals' S.E.M. of the parameter for each group.

BAROREFLEX CURVES IN THE RABBIT HINDLIMB BED

Curves relating changes in hindlimb vascular resistance (HVR) to falls in MAP were constructed similarly to the MAP-HP curves described in the previous section. In this case variable inflations of the vena caval balloon resulted in a range of constrictor responses which formed part of a reversed S-shaped curve (Chapter 7). The dilator responses due to rises in MAP were not examined. An estimate of maximal vasodilation attributable to the baroreceptor system was made from the HVR after autonomic block.

This enabled the curve parameters to be calculated using a similar technique to that used for the MAP-HP curves.

CHAPTER 3

BARORECEPTOR-HEART RATE REFLEX IN NORMAL MAN AND IN HUMAN ESSENTIAL HYPERTENSION

INTRODUCTION

One of the principal mechanisms involved in the moment to moment control of the blood pressure are the baroreceptor reflexes. Alteration of baroreflex function has been suggested as a possible mechanism for the abnormal regulation of blood pressure in hypertension (McCubbin, Green and Page, 1956; Kezdi and Wennemark, 1958; Merrill and Schupak, 1964). A reduction in baroreflex sensitivity (gain) in hypertensive subjects has been recently demonstrated by Sleight and colleagues (Bristow, Honour, Pickering, Sleight and Smyth, 1969; Bristow, Honour, Pickering and Sleight, 1969; Gribbin, Pickering, Sleight and Peto, 1971) following the development of a quantitative method for assessment of baroreflex sensitivity.

The principle of their method involved the use of drugs to induce a small ramp change in blood pressure by vasoconstriction or vasodilation but which had no direct effect on the heart. The analysis then depended on the associated

change in heart rate being entirely reflex in origin. The systolic (or mean) arterial pressure of each pressure pulse in the ramp was related to the heart period (pulse interval) of the succeding beat by a linear plot, the slope of which was a measure of the gain of the reflex (Smyth, Sleight and Pickering, 1969; Pickering, Gribbin and Sleight, 1972). The heart period response was measured during the time of rapid blood pressure change. The method does not evaluate the 'steady-state' properties of the baroreceptor reflex which may be more appropriate in conditions such as hypertension where the disturbance is prolonged.

In this study the 'steady-state' characteristics of the baroreceptor-heart rate reflex were examined in normotensive and hypertensive subjects. A range of injections of the vasoconstrictor drug phenylephrine and the vasodilator drug glyceryl trinitrate were used to cause small 'steady-state' changes in intravascular pressures about the resting value of arterial pressure. The reflex heart rate responses to changes in arterial pressure were then expressed in quantitative terms by the construction of average stimulus-response curves in three groups of subjects classified according to their mean arterial pressure. Each group was

further subdivided into 2 subgroups according to age.

METHODS

Subjects

23 normotensive subjects (21 male, 2 female) and 24 subjects with essential hypertension (14 male, 10 female) were studied. 14 of the group with hypertension had not received any previous treatment for hypertension while in the remaining 10, all anti-hypertensive therapy had been discontinued for 2-3 weeks before the study. The diastolic blood pressure in the normotensive subjects was below 90 mmHg on two occasions one week apart and at the time of the study. They were clinically healthy as assessed by history, physical examination, chest X-ray, ECG and standard haematological and biochemical tests. Secondary causes of hypertension were excluded in the hypertensive subjects by serum electrolytes and urinary catecholamine estimations, microurine examination, intravenous pyelography and in 13 subjects by renal arteriography. The diastolic blood pressure was greater than 95 mmHg on three examinations made at intervals of one week and at the time of the study. In all patients retinal vascular changes were only mild and most had a normal ECG although some had minor voltage abnormalities. There was no clinical evidence of cardiac

failure. All subjects were placed on a diet containing approximately 100 mEq Na^+/day and 80 mEq K^+/day for two weeks before the study.

Preparation for study

Following explanation of the protocol and after having given consent to the procedure which had been approved by the Clinical Investigation Committee of the Royal Prince Alfred Hospital, the subjects were admitted to hospital on the morning of the study or on the previous day. An antibiotic (penicillin or erythromycin) was given 30 minutes before the study but the subjects were not sedated. Using local lignocaine anaesthesia (Xylocaine 1%, Astra) a Cournand 18T needle was inserted percutaneously into the brachial artery and a 5 or 6F cardiac catheter was placed via a cutdown in the antecubital fossa into the right atrium.

Arterial pressure was measured using a Sanborn transducer, and heart period was obtained from the RR interval of the ECG. These variables were recorded on a photographic Sanborn recorder. In later experiments, the blood pressure was recorded using a Statham P23 Db transducer and the heart period (pulse interval) was obtained from the arterial pressure pulse (Fig 12). Recordings were made in ink on a

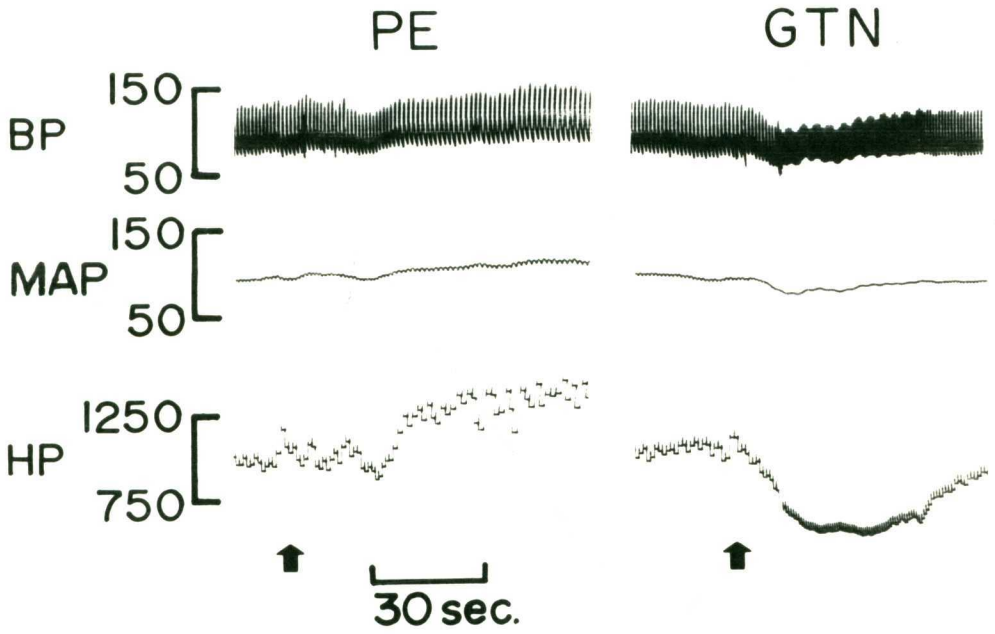

FIGURE 12

Record showing effect of injecting phenylephrine (PE) and glyceryl trinitrate (GTN) on arterial blood pressure (BP, mmHg), mean arterial pressure (MAP, mmHg) and heart period (HP, msec). Drug injections were made at the arrows.

Grass polygraph.

Experimental Protocol

After a 30 minute rest period with the subject supine, pairs of injections of small amounts of phenylephrine (Neosynephrine, Winthrop; dose 20-300 µg) and of glyceryl trinitrate (5% w/v solution prepared by Burroughs Wellcome (Australia) Ltd., dose 20-300 µg) were made into the right atrium over a period of 15-20 seconds to elicit small rises and falls in mean arterial pressure (Fig 12). Each drug dose was diluted in 5% dextrose in water and injected in a total volume of 5ml and was flushed by 5ml of 5% dextrose in water. In each subject the effects produced by 3 or 4 different rises and 3 or 4 different falls in mean arterial pressure were studied in an attempt to produce small (2-5%), moderate (6-15%), and large (>15%) blood pressure changes. Five minute intervals were allowed between the alternate injections of phenylephrine and glyceryl trinitrate, each of which was given in a random dosage schedule. After drug injections the blood pressure rose or fell from the resting value over a period of 10-20 seconds and reached a plateau which was maintained for 15-30 seconds (Fig 12). The heart period also reached a plateau at this time. Mean arterial pressure, pulse

pressure and heart period were measured just before drug injection and during the plateau phase.

In four other subjects (3 normotensives, 1 hypertensive) 14-16 pairs of phenylephrine and glyceryl trinitrate injections were given over a period of approximately 3 hours to derive full stimulus-response curves in individual subjects.

Stimulus-Response Curves

The method of curve fitting was similar to that described in Chapter 2.

RESULTS

Properties of Mean Arterial Pressure-Heart Period Curves in Man

The 'steady-state' relationship between mean arterial pressure (MAP) and heart period (HP) was sigmoid and approximately symmetrical as shown in the curves from individual subjects and from the average response of a given group (Fig. 13).

Curvilinearity in the MAP-HP relationship was demonstrated from individual observations in the normotensive

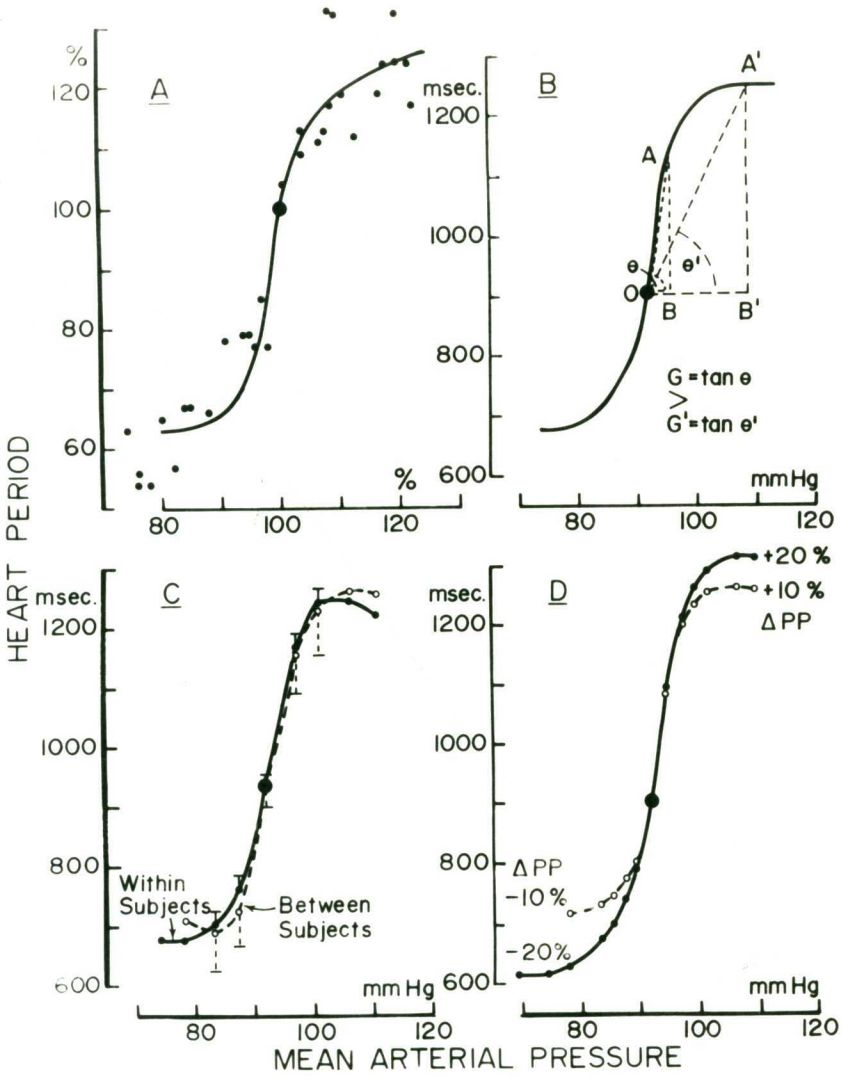

FIGURE 13

Relationship between mean arterial pressure and heart period. A : Results in an individual subject (JW), showing the observed points and the stimulus-response curve derived from the regression equations. B : Schematic illustration showing that the gain of the sigmoid MAP-HP curve is greater for small deviations in MAP in a given direction, than for large deviations, G = AB/OB = tan Θ>G' = A'B'/OB' = tan Θ'. C : MAP-HP curve calculated from univariate regression equations derived from 'between subjects'(broken lines) and 'within subjects' (solid lines) data in younger Group 1 subjects. The bar at some of the mean HP values is the SE of the mean HP. D : MAP-HP curve, calculated from 'within subject' data of subgroups of Group 1, showing the effects of different changes in pulse pressure (ΔPP, % of resting) on the curve.

and hypertensive subjects by examination of the changes in gain (G) for different percentage values of ΔMAP from resting MAP. For sigmoid curves, G will be greater for smaller values of ΔMAP in a given direction than for larger ΔMAP (Fig. 13) and this was observed in both normotensives and hypertensives (Table 3).

Administration of phenylephrine and glyceryl trinitrate altered not only MAP but also pulse pressure (PP) (Fig. 12). The multiple regression equations were used to evaluate the role of variation in ΔPP on the MAP-HP curve. ΔPP exerted significant effects on the HP response at a given MAP in equations derived "within subjects" and from the pooled data (Table 4). The effect was particularly on the heart period range (HPR) (Fig.13) in agreement with the findings in animals (Chapt. 4; Korner, Shaw, West and Oliver, 1972).

Common values of ΔPP% within the range of observations of each group were substituted into the multiple regression equation of each group and the MAP-HP curve reconstructed (Fig.13). ΔPP was expressed as a percentage of each group's resting value rather than as an absolute change since this tends to offset differences in afferent neural input

TABLE 3

Gain of the heart period response in relation to the change in mean arterial pressure evoked by phenylephrine and glyceryl trinitrate in normotensive and hypertensive subjects.

	NORMOTENSIVES (MAP 80 - 110 mmHg)			HYPERTENSIVES (MAP 117 - 163 mmHg)		
ΔMAP	n	Mean G	SE_G	n	Mean G	SE_G
			PHENYLEPHRINE			
0 - 5%	11	4.60	0.96	16	3.92	0.58
6 - 10%	33	3.51	0.41	22	2.03	0.42
11 - 15%	22	2.16	0.31	25	1.87	0.21
>16%	21	1.77	0.17	22	1.46	0.19
			GLYCERYL TRINITRATE			
0 - 5%	20	4.09	0.55	16	3.39	0.65
6 - 10%	33	2.07	0.24	30	1.65	0.29
11 - 15%	14	1.64	0.22	22	1.04	0.21
>16%	13	0.98	0.13	12	0.69	0.13

n = number of observations

ΔMAP = mean arterial pressure percentage difference

TABLE 4

Partial regression coefficients ± SE, in multiple regression functions[+] of younger Group 1 subjects.

	b_1	b_2	b_3
PHENYLEPHRINE			
Between subjects	-5.94	-0.109	+0.004
(13)*	±2.1	±0.04	±0.03
Within subjects	-6.89	-0.008	+0.039
(37)	±1.2	±0.034	±0.013
Pooled data	-6.32	-0.088	+0.018
(50)	±1.9	±0.021	±0.013
GLYCERYL TRINITRATE			
Between subjects	-5.46	-0.027	-0.006
(13)	±1.7	±0.028	±0.038
Within subjects	-3.39	+0.041	-0.069
(33)	±1.43	±0.050	±0.021
Pooled data	-4.86	-0.026	-0.036
(46)	±1.0	±0.017	±0.019

+G = a + b_1 log ΔMAP + b_2 MAPr + b_3 ΔPP; ΔMAP is percentage deviation of mean arterial pressure from resting; MAPr = resting mean arterial pressure (mmHg); ΔPP = percentage change in pulse pressure.

* Degrees of freedom.

from the baroreceptors due to differences in the MAP. In experimental hypertension it has been shown that the increased arterial wall stiffness contributed to a reduction in fibre discharge which was related to the absolute ΔPP (Peterson, 1966; Kezdi, 1967; Aars, 1968).

Absolute resting MAP (MAP_r) exerted a significant effect on G in the phenylephrine equations derived "between subjects" (Table 4). For example in the younger normotensives, in those with higher MAP_r, G was smaller for a given rise in MAP suggesting that the resting MAP value was close to the upper saturation pressure of their stimulus response curve than in subjects in whom MAP_r was lower. The effect due to MAP_r was not significant in the equations derived "within subjects" and thus the smaller fluctuations in MAP_r which occured in each subject during the study were neglected.

The MAP-HP curves derived from the "between subjects" regression equation were closely similar (Fig. 13) to those derived from the "within subjects" data (Korner, Shaw, West and Oliver, 1972). Since the residual error mean square was significantly greater in "between subjects" groups ($P < 0.001$), systematic differences in HP response between subjects of a given group were present but were distributed

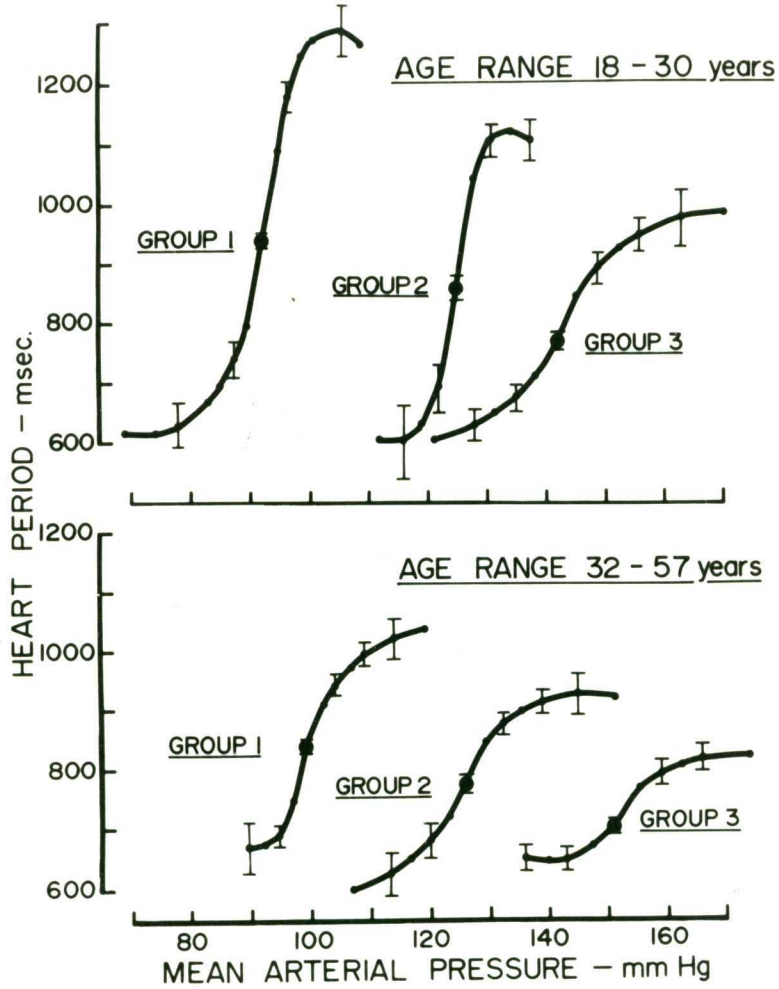

FIGURE 14

MAP-HP curves ('within subjects') in young and older subgroups. Group 1, resting MAP range 80 to 110 mmHg; Group 2, 117 to 136 mmHg; Group 3, 143 to 163 mmHg. Each curve has been adjusted to a similar ΔPP value (expressed as a percentage of each subject's resting PP) of +15% after phenylephrine, and -20% after glyceryl trinitrate. The between subject SEM of the different resting values are given in Table 3.

normally about the group mean.

Effect of Hypertension and of Age on MAP-HP Curve

The subjects were classified into 3 groups according to the average of each person's resting MAP at the time of the study. Group 1 were normotensive, with an MAP range 79 to 109 mmHg. Group 2 had moderate hypertension with an MAP range 117 to 136 mmHg. Group 3 had more severe hypertension, with MAP between 143 and 163 mmHg. Each group was further subdivided into two subgroups according to age: (1) subjects of 30 years or less (range 18 to 30 years, mean 25 years); (2) subjects over 31 years (range 32 to 57 years, mean 42 years). The number of subjects in each group are shown in Table 5. The MAP-HP curves in Fig. 14 were derived from the "within subjects" equations of each group; the standard error of each mean HP value thus represents the average variation in response occuring within subjects of the particular group.

The resting HP values differed also between the groups (Table 5), being progressively lower with each group, so that in each age range, Group 3 subjects had a significantly lower resting HP value than Group 1 subjects (P between subjects < 0.05).

TABLE 5

Mean resting values and curve parameters in the different groups

	GROUP 1		GROUP 2		GROUP 3	
AGE RANGE	<30	>30	<30	>30	<30	>30
MEAN AGE	23.6 ± 4.1[1]	43.9 ± 4.5	26.4 ± 2.7	39.1 ± 7.8	25.2 ± 5.2	47.0 ± 9.0
n	14	9	5	11	4	4
MEAN ART. PRESSURE mmHg	92 ± 2.5[2]	99 ± 3.2	125 ± 3.1	126 ± 1.7	142 ± 0.5	151 ± 8.0
PULSE PRESSURE mmHg	53 ± 1.8[2]	57 ± 3.2	68 ± 3.5	73 ± 3.6	70 ± 4.6	90 ± 7.4
HEART PERIOD msec	904 ± 39[2]	841 ± 39	860 ± 50	775 ± 51	768 ± 44	705 ± 36
BP_{50} mmHg	92 ± 0.6[3]	101 ± 0.7	125 ± 0.6	124 ± 1.0	143 ± 1.8	154 ± 1.1
HPR msec	670 ± 45[3]	371 ± 32	520 ± 52	322 ± 30	397 ± 43	173 ± 31
$\bar{G}$ msec/mmHg	41 ± 5.3[3]	21 ± 2.8	49 ± 9.3	13 ± 2.4	13 ± 2.6	11 ± 3.2
N	105	78	36	81	31	30

n is the number of subjects.

1 SD

2 SE between subjects.

3 SE within subjects; curves adjusted to ΔPP + 15% (phenylephrine); -20% (glyceryl trinitrate)

N is the number of observations used to derive each curve; i.e. phenylephrine + glyceryl trinitrate + pairs of resting values.

The resting PP differed between the groups and was higher in Group 2 and 3 subjects than those of Group 1.

There was a reduction in the heart period range (HPR) and an alteration in average gain ($\overline{G}$) in MAP-HP curves of the hypertensive subjects compared with the normotensives of similar age range (Fig. 14 , Table 5). The percentage reduction in HPR was approximately the same in each age range and was related to the degree of hypertension. Thus the HPR of the curves of Group 2 and Group 3 subjects averaged 80% and 55% respectively of the HPR of the curves in Group 1 subjects. The reduction in HPR in each age range was due to a progressive lowering of the level of the upper HP plateau with increasing severity of hypertension. There was no significant difference in the level of the lower plateau between the 3 groups.

There was a significant lowering of $\overline{G}$ in each age range of hypertensive Group 3 compared to the normotensive Group 1. In the hypertensive subjects there were no significant differences between the MAP-HP curves related to sex.

The older subjects of each group had stimulus-response

curves with an HPR of 40 to 60% of the corresponding younger subjects (Fig. 14 , Table 5). This reduction in HPR was mainly due to lowering of the upper plateau level in the older age group at each MAP level. Likewise $\overline{G}$ was significantly reduced in the older Group I and 2 subjects, compared with the corresponding younger subgroups ($P < 0.001$, Table 5). In group 3 subjects however the differences were smaller and not statistically significant.

DISCUSSION

The present analysis depends on the HP response to injections of phenylephrine and glyceryl trinitrate being reflex in nature. Previous studies in man and animals showing virtual abolition of the response after cardiac autonomic block with atropine and propranolol support this condition (Robinson, Epstein, Beiser, and Braunwald, 1966; Korner, and Shaw, 1971). Animal studies suggest the arterial baroreceptors are the major determinants of the response with contributions from the cardiopulmonary baroreceptors (Korner, and Shaw, 1971; Korner, Shaw, West and Oliver, 1972).

The stimulus-response curves are altered both by the effects of hypertension and by age. The reduction in HPR with increasing degrees of hypertension was due principally

to a fall in the upper plateau while the lower plateau was relatively unaffected. The increase in HP due to a rise in arterial pressure, as reflected in the upper half of the sigmoid response curve, depends on the extent of vagal excitation and sympathetic inhibition. The mechanism involved in the fall in the HP plateau was not studied and may be due to an abnormality of either cardiac effector mechanism. It seems more likely to result from impairment of vagal excitation in hypertension than from impaired sympathetic inhibition in view of (1) the findings that the lower resting HP of essential hypertension was due almost entirely to reduction of vagal tone (Korner, Shaw, Uther, West, McRitchie and Richards, 1973) and (2) the demonstration of a more marked fall in gain of the reflex using the ramp method (Bristow, Honour, Pickering and Sleight, 1969) but which is more sensitive to the effects of decrease in vagal tone than to the sympathetic component of the effector response (see below).

In the absence of alterations in excitability of the remaining cardiac effector motoneurones, attenuation of the baroreflex-dependent HPR should be associated with a fall in gain (Korner, Shaw, West and Oliver, 1972) as has occurred

in each age range in Group 3 compared to Group 1. The absence of a change in gain in the younger subjects of Group 2 despite a reduction in HPR suggests an increase in excitability of the motoneurones receiving baroreceptor projections.

The effects of age were to reduce both HPR and $\overline{G}$ at each level of arterial pressure. The attenuation of HPR in the older subjects involved mainly a lowering of the upper plateau at each level of blood pressure.

The effects of age and hypertension were relatively independent in agreement with the findings of Gribbin, Pickering, Sleight and Peto (1971) using the ramp method.

The degree of reduction in baroreflex gain in hypertension is much less pronounced with the 'steady-state' method than with the ramp method. With the latter method the baroreflex gain of hypertensive subjects averaged about 20% that of the normotensives (Bristow, Honour, Pickering, Sleight and Smyth, 1969; Gribbin, Pickering, Sleight and Peto, 1971) while with the 'steady-state' method in these studies, $\overline{G}$ for Groups 2 and 3 taken together was about

60% that of the average normotensive value. Two factors contribute to this effect.

Firstly, most comparisons between normotensive and hypertensive subjects using the ramp method have been made during rises in pressure from the resting value. There is a marked difference in the vagal (approximately one second) and cardiac sympathetic (approximately 10-20 seconds) time constants. Thus the reflex heart period response at the beginning of the ramp rise in pressure must be due entirely to changes in vagal efferent activity, with the sympathetic effectors responding increasingly over the next several beats (Wang and Borison, 1947; Warner and Cox, 1962; Katona, Barnett and Jackson, 1967; Thames and Kontos, 1970; Scher and Young, 1970). An impairment of the vagal component of the reflex would be most evident during rapid blood pressure changes and would account for the greater loss of reflex sensitivity in hypertension by the ramp method than by the 'steady-state' method.

Secondly, the ramp method shows unidirectional rate sensitivity in that the baroreflex gain is greater during rises than during falls in arterial pressure from the resting value (Clynes, 1969; Scher and Young, 1963; Pickering

Gribbin and Sleight, 1972). This is in contrast with the virtually equal responses in HP during the 'steady-state' pressure changes in the present study (cf. Table 3).

CONCLUSION

Alteration of baroreceptor function in man with essential hypertension is not only the result of adaptation to the raised arterial pressure but also due to impairment of the reflex. The site of impaired reflex function has not been localised. Baroreceptor disfunction due to receptor degeneration from increased pressure and the effects of age has been suggested as a possible site (Angell-James, 1973). However predominance of a reduced vagal response in the reflex, suggests that other mechanisms may be involved.

As a result of baroreflex impairment in hypertensive subjects, reflex adjustments in heart rate are probably both less rapid and less extensive than in normal subjects during moment to moment fluctuations in arterial pressure, but the importance as far as overall circulatory control is concerned, cannot be assessed.

CHAPTER 4

BARORECEPTOR-HEART RATE REFLEX IN UNANAESTHETIZED RABBITS WITH RENAL HYPERTENSION

INTRODUCTION

In the previous chapter it was shown that the 'steady-state' properties of the baroreceptor-heart rate reflex in essential hypertension in man differ from those of normotensive subjects of the same age. In essential hypertension the sigmoid mean arterial pressure-heart period curves are reset about a higher mean arterial pressure with a reduction in average gain of the reflex and in the pressure dependent heart period range. It is not known whether these changes in baroreflex function in human essential hypertension are the result of a basic disorder of the autonomic control system or whether they are merely a secondary consequence of chronic elevation of blood pressure. In this chapter, the effects of a relatively brief period of renal hypertension on the baroreceptor-heart rate reflex were examined in the rabbit.

METHODS

At an initial operation inflatable balloons were placed around the abdominal aorta and the inferior vena cava. In one group of rabbits renal hypertension was induced by

means of cellophane wrapping of the kidneys, while in another group a sham procedure took place. Operations were carried out as described in Chapter 2. Experiments were performed 4 to 6 weeks later in 21 hypertensive rabbits and in 28 sham-operated normotensive rabbits. At this time degeneration of baroreceptors in the arterial wall was probably minimal (Angell-James, 1973) and the arterial baroreceptors should have fully adapted to the new blood pressure level (Aars, 1968b).

After a 30 minute rest period, pairs of graded rises and falls (2 to 50 mmHg) in mean arterial pressure (MAP) were produced at 5 minute intervals by means of inflations of the aortic and vena caval balloons respectively. The steady state heart period (HP) response, change in pulse pressure (PP) and right atrial pressure (RAP) after a 30 second inflation were measured. In each rabbit 5-6 pairs (i.e. rise and fall in pressure) of balloon inflations were made.

Analysis of Results

For each group of rabbits MAP-HP curves and curve parameters were derived as described in Chapter 2. As outlined inChapter 2, standard errors were calculated

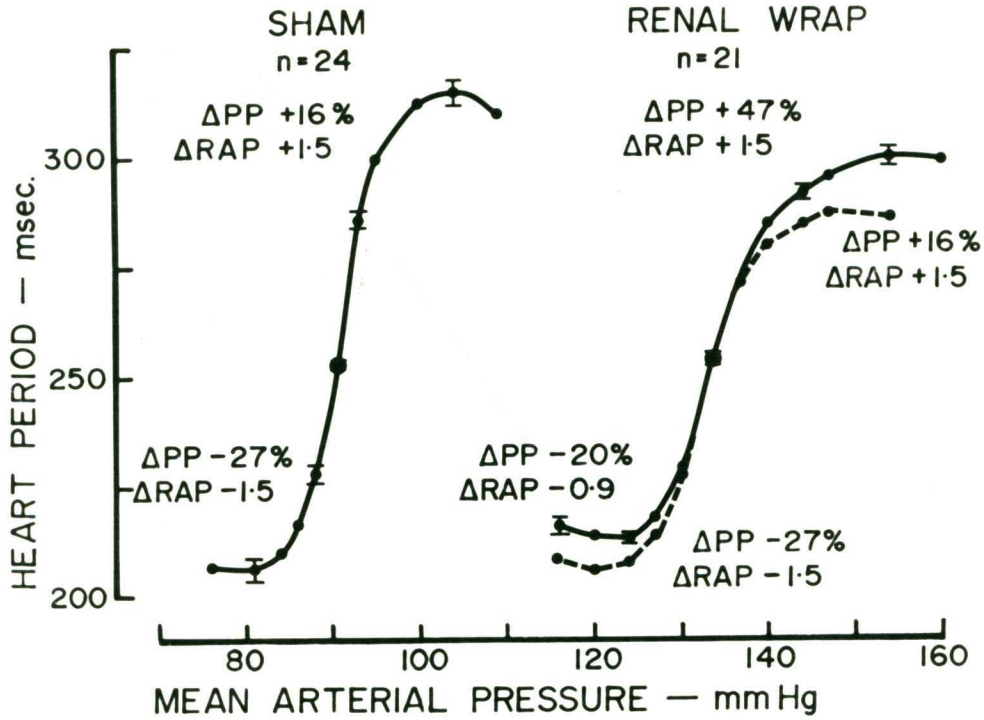

FIGURE 15

Average MAP-HP curves calculated from 'within animal' data of 21 renal wrap hypertensive rabbits and 28 sham-operated normotensive animals. The solid lines are the unadjusted results in each group. The interrupted line is the curve of the hypertensive group adjusted to the same changes in pulse pressure and right atrial pressure as in sham-operated rabbits. The large point inthe centre of each curve is the mean resting MAP and HP value, whilst the smaller points are the calculated HP values at different values of MAP within the range of observation of each group. Each bar is $\pm$ 1 s.e.m. of the mean HP.

TABLE 6

Resting values in renal wrap hypertensive and sham-operated normotensive rabbits.

	HYPERTENSIVE		NORMOTENSIVE	
n	21		28	
HEART PERIOD msec (s.e.m.)[1]	254	(13.5)	258	(3.2)
MEAN ARTERIAL PRESSURE mmHg (s.e.m.)	134	(5.7)	91	(4.0)
PULSE PRESSURE mmHg (s.e.m.)	36.6	(3.7)	31.5	(2.3)
RIGHT ATRIAL PRESSURE mmHg (s.e.m.)	-0.1	(1.3)	0.5	(0.8)

[1] All s.e.m. values are between animals.

TABLE 7

Parameters of MAP - HP curves in renal hypertensive and normotensive rabbits.

	RENAL HYPERTENSIVE		NORMOTENSIVE
	UNADJUSTED*	ADJUSTED**	
Number of animals	21		28
N^{+}	339		474
BP_{50} mmHg (s.e.m.)[1]	135 (5.7)	133 (5.9)	91 (4.2)
HPR msec (s.e.m.)[2]	85.5(2.5)	79.5 (2.8)	107.5 (3.3)
$\bar{G}$ msec/mmHg (s.e.m.)[2]	4.6(0.3)	4.9 (0.4)	9.7 (0.6)

+N is the number of aortic balloon inflations + number of IVC balloon inflations + number of paired resting values.

* For ΔPP and ΔRAP values see Table 9.

** Adjusted to the same ΔPP and ΔRAP values as observed in normotensive animals, Table 9.

1 s.e.m. calculated from between animal data.

2 s.e.m. calculated from within animal data.

appropriately for 'between' or 'within' animals variation.

RESULTS

Resting Values in Hypertensive and Sham-Operated Normotensive Rabbits

The resting values obtained in this series of rabbits after 4-6 weeks of hypertension compared to the values in the sham-operated normotensive group are shown in Table 6. The mean arterial pressure (MAP) was 44 $\pm$ 7 mmHg (SE diff between animals, $P < 0.001$) higher in the hypertensive group. There were no significant differences between the two groups in the resting values for heart period (HP), right atrial pressure (RAP) or pulse pressure (PP).

MAP-HP Curves in Renal Hypertension

The MAP-HP curve derived from rabbits with renal hypertension was shifted to the right along the pressure axis from the curve obtained in sham-operated normotensive animals (Fig. 15, Table 6). In each group the resting MAP was close to the BP_{50} value (Tables 6 and 7). The HPR of hypertensives was about 80% of that of the normotensive animals ($P < 0.001$) while the average gain $\bar{G}$ was 50% of the value obtained in the sham-operated group (Table 7 , $P < 0.001$).

TABLE 8

Changes in pulse pressure and right atrial pressure during aortic and IVC balloon inflation.

	AORTIC BALLOON				IVC BALLOON			
	HYPERTENSIVE		NORMOTENSIVE		HYPERTENSIVE		NORMOTENSIVE	
Δ PP % of resting (s.e.m.)[1]	+47	(1.8)	+16	(1.0)	-20	(1.1)	-27	(1.1)
Δ RAP mmHg (s.e.m.)	+1.5	(0.1)	+1.5	(0.1)	-0.9	(0.1)	-1.5	(0.1)

[1] s.e.m. between animals.

TABLE 9

Regression equations $G = a + b_1 \log \Delta MAP + b_2 \Delta RAP + b_3 \Delta PP$ calculated from 'within animal' data obtained during aortic and IVC balloon inflation in renal hypertensive and normotensive rabbits.

	D/F	a	b_1	b_2	b_3
RENAL HYPERTENSIVE					
Aortic Balloon[1]	93	3.492 (0.44)	-2.59 (0.21)	+0.138 (0.042)	+0.012 (0.003)
IVC Balloon	93	4.993 (0.96)	-4.23 (0.62)	-0.069 (0.090)	-0.037 (0.012)
NORMOTENSIVE					
Aortic Balloon	128	5.554 (0.82)	-3.72 (0.37)	+0.099 (0.06)	+0.0085 (0.006)
IVC Balloon	132	4.341 (1.35)	-2.68 (0.55)	+0.070 (0.18)	-0.0182 (0.009)

[1] Values in brackets are s.e.m.

In the multiple regression equations derived for aortic and inferior vena caval balloon inflations both ΔPP (% of resting) and ΔRAP (mmHg) exerted significant effects on the gain of the heart period response in both the normotensive and hypertensive animals (Fig. 15 , Table 9) in agreement with previous findings (Korner, Shaw, West and Oliver, 1972). In each group a greater rise in ΔPP and ΔRAP from resting increased HP for a given rise in MAP during aortic balloon inflation, while a greater fall in ΔPP and ΔRAP after caval balloon inflation lowered HP for a given fall in MAP from the resting value. However during aortic balloon inflation, the rise in PP for a given ΔMAP was significantly greater in the hypertensive rabbits than in the sham-operated normotensive animals (Table 8). This greater change in PP in the hypertensive animals tended to minimise the differences in HPR between the two groups (Fig. 15 , Table 7). When the hypertensive MAP-HP curve was adjusted to the same values of ΔPP and ΔRAP as in the normotensive group (Fig. 15) its HPR was 74% of the value of the normotensive curve since the upper HP plateau in this adjusted curve of the hypertensive rabbits was more markedly below that of the sham-operated group ($P < 0.001$). Thus, a similar 'pressure-input' to the circulating baroreceptors evokes a less marked bradycardia in the

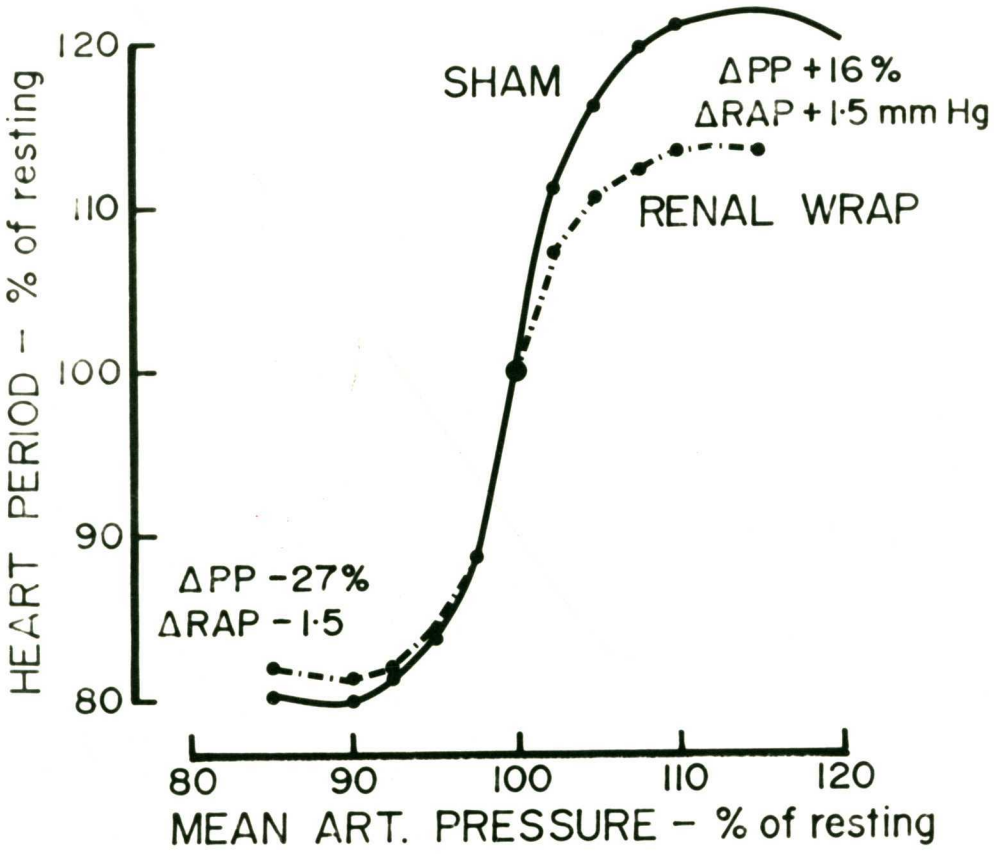

FIGURE 16

MAP-HP curves from 21 renal wrap hypertensive rabbits and 28 sham-operated normotensive animals. The scales along the MAP and HP axes are in percentage units of each group's mean resting MAP and HP.

hypertensive than in the normotensive rabbits. The lower HP plateau below threshold pressure was now identical in each group indicating that arterial pressure reduction produced tachycardia of identical magnitude in normotensive and hypertensive rabbits.

When the curves were expressed in percentage units of each group's resting MAP and HP, the difference in the value of $\overline{G}$ between the two groups was considerably reduced (Fig. 16). In the hypertensive rabbits $\overline{G}$ was 2.9 $\pm$ 0.23 ΔHP%/ΔMAP% compared with 3.63 $\pm$ 0.28 ΔHP%/ΔMAP% in the sham group (cf Table 10). That is, when the MAP-HP curves were expressed in percentage units, $\overline{G}$ for the hypertensive rabbits was 80% of the value in normotensive rabbits, compared with 50% of the value when using absolute HP and MAP units.

DISCUSSION

The changes in properties of the baroreceptor-heart rate reflex in the present group of hypertensive rabbits were closely similar to those observed in patients with essential hypertension (Chapter 3). In the two different types of hypertension the average elevation of blood pressure was the same, though the duration of the hypertension

TABLE 10

Parameters of MAP-HP curves in renal hypertensive and normotensive rabbits and in patients with essential hypertension and normotensive subjects.

	BP_{50} mmHg	HPR msec	$\frac{G}{msec}$/mmHg
MAN+			
Normotensive	95.5	553	33.2
Essential Hypertensive	132.4	351	20.2
%*	139	63	61
RABBIT			
Normotensive	91	107.5	9.7
Renal Hypertensive	132.7	79.5	4.9
%*	146	74	51

+ Data from Chapter 3.

Hypertensive and normotensive values in each group adjusted to common value of ΔPP

%* = 100 x Hypertensive/Normotensive value.

was much shorter in the animal studies. The duration seems to be relatively unimportant since in the two types of hypertension the percentage rise in BP_{50} and in threshold pressures were similar, as were the percentage reductions in $\bar{G}$ and in HPR compared with the appropriate normotensive parameters (Table 10). This suggests that the changes in baroreflex parameters depend on nonspecific factors associated with chronic elevation of blood pressure. They do not apparently represent a specific abnormality of autonomic function in essential hypertension.

The MAP-HP curve measures the overall characteristics of the baroreceptor-heart rate reflex. Since balloon inflation activates all the circulatory mechanoreceptors (including arterial, cardiac and pulmonary baroreceptor groups), each contributes to the reflex response. However under the conditions of the present study, the input from the arterial baroreceptor zones is probably of greater importance (Korner, Shaw, West and Oliver, 1972; Chapter 5). Changes in baroreflex parameters during hypertension may be due to changes in the properties of (i) the peripheral baroreceptor zones, (ii) of central neurones receiving baroreceptor projections, or (iii) of the peripheral autonomic effectors.

Studies on the relationship between arterial pressure and arterial baroreceptor fibre discharge suggest that most of the changes in overall reflex properties can be accounted for by changes in the properties of the peripheral arterial baroreceptor zones (McCubbin, Green and Page, 1956; Kezdi, 1962; Kreiger and Marseillan, 1966; Spickler and Kezdi, 1967; Aars, 1968a, Angell-James, 1973). A rise in threshold pressure of the baroreceptors may thus lead to the rise in threshold pressure of the MAP-HP curve. Such a rise in threshold may be due to adaptation of the receptors to a new pressure level, a property which is common to all types of mechano-receptors during prolonged changes in environmental conditions (Mountcastle and Baldessarini, 1968). Aars (1968a) has shown in studies of the relationship between arterial pressure and integrated whole aortic baroreceptor nerve activity in the rabbit, that adaptation to elevation in mean pressure occurs relatively rapidly over 1-2 hours. Angell-James (1973) has demonstrated in a single aortic nerve fibre analysis that the elevation in threshold in rabbits with renal hypertension involves every type of baroreceptor fibre, and that the distribution of fibre size is the same in hypertensive as in normotensive animals.

Furthermore she has shown that the gain of the baroreceptor (impulse frequency/mmHg ΔMAP) in hypertensive animals averages about half that observed in normotensive rabbits, suggesting that most of the changes in absolute overall baroreflex $\bar{G}$ can be accounted for by changes in the sensitivity of single receptor units. The process of adaptation of the arterial baroreceptor to a new pressure level may also be related to the fall in gain, since the peripheral baroreceptors when adapted do not sense absolute wall tension (or transmural pressure) but only changes in tension. Hence their sensitivity at any pressure level is probably a function of the prevailing resting tension. Accordingly, a pressure change of 10 mmHg in animals with a MAP of 100 mmHg might produce a similar alteration in fibre discharge as a pressure change of 15 mmHg in an animal with a MAP of 150 mmHg. This would account for the reduction in the difference in overall reflex $\bar{G}$ between the hypertensive and normotensive groups in the present study when the parameter is expressed in percentage rather than absolute units. However even then there are still significant though relatively small differences between the two groups. This is probably because the use of percentage units may not adequately account for the nonlinear changes in mechanical wall properties during hypertension which include an increase in initial aortic volume and a reduction

in distensibility (Peterson, 1966; Aars, 1968b; Wolinsky, 1970; Angell-James, 1973). It is of interest that the greater rise in PP occuring in the hypertensive animals during increases in peripheral resistance (e.g. aortic balloon inflation) tends to elevate baroreceptor firing at a given rise in MAP, thus compensating to some degree for the reduction in receptor sensitivity.

The reduction in HPR is probably related to an impairment of the function of the arterial baroreceptors. This is suggested by the findings of Spickler, and Kezdi (1967) of a reduction in range of integrated whole baroreceptor aortic nerve activity. It is not known whether this depends on an actual reduction in the number of units because of degenerative changes in some of the receptors, or whether it is due to an overall impairment of receptor function. The study of Angell-James suggests that overt baroreceptor degeneration requires elevation of blood pressure that is of longer duration and/or more severe than in the present study. However she found minor histological changes in the vessel wall and in the receptors, which could well have contributed to the reduction in HPR.

While the changes in baroreflex BP_{50} and in $\bar{G}$ appear thus to be largely an accompaniment of the adaptation process of the receptors to chronic elevation of the blood pressure, the changes in HPR suggest a real impairment of function of the reflex. If at least some of it is due to degenerative changes in receptors it seems likely that with chronic elevation of blood pressure a component of neurogenic 'receptor denervation' hypertension becomes superadded to the primarily renal hypertension.

CONCLUSION

The present findings in moderate experimental renal hypertension of 4 weeks duration indicate that the changes in properties of the baroreceptor-heart rate reflex under steady-state conditions are similar to those observed in man with uncomplicated essential hypertension. The changes thus appear to be a nonspecific accompaniment of the development of the hypertensive state.

CHAPTER 5

INTERACTIONS BETWEEN BARORECEPTORS AND OTHER INPUTS IN THE CARDIAC SYMPATHETIC MOTONEURONE POOL

This chapter illustrates how the properties of the MAP-HP curve can be changed by a physiological disturbance. The method of analysis is discussed for a particular example (arterial hypoxia) and the possible application of the technique to the study of differences in autonomic excitability in renal hypertension is briefly discussed.

A generalised disturbance involving the cardiovascular system usually results in changes in the activity of all of the cardiovascular receptor groups including arterial, cardiac and pulmonary baroreceptors. However little is known about the mechanism by which these various inputs modify the baroreceptor response. Since all the major cardio-respiratory inputs converge onto the autonomic motoneurone pool of the cardiac effectors (Korner, 1971) the heart rate response to a generalised disturbance will depend on the extent to which each input activates specific motoneurones in the pool and interacts with other inputs (e.g. by convergence). Using this model, the autonomic responses produced by a disturbance and identified by

different types of shift in the MAP-HP curves may be partitioned into components which are dependent and independent of baroreceptor influences (cf Fig. 18).

Severe arterial hypoxia was used to displace the normal MAP-HP curve from its resting position. This permitted analysis of some of the interactions between the different peripheral afferents involved in the response.

METHODS

Implantation operations, neural preparations and drug dosage schedules were carried out as described in Chapter 2. Normotensive animals with previously implanted aortic and vena caval balloons were used. Neural sham-operated and pontine animals were prepared on the day of the experiment. In some of these animals, section of the carotid sinus and aortic nerves was performed under anaesthesia immediately after craniotomy and in others, bilateral vagotomy was performed half an hour before the start of the experiment. The experiments commenced 3 hours after the craniotomy operation.

The experimental protocol consisted of the construction

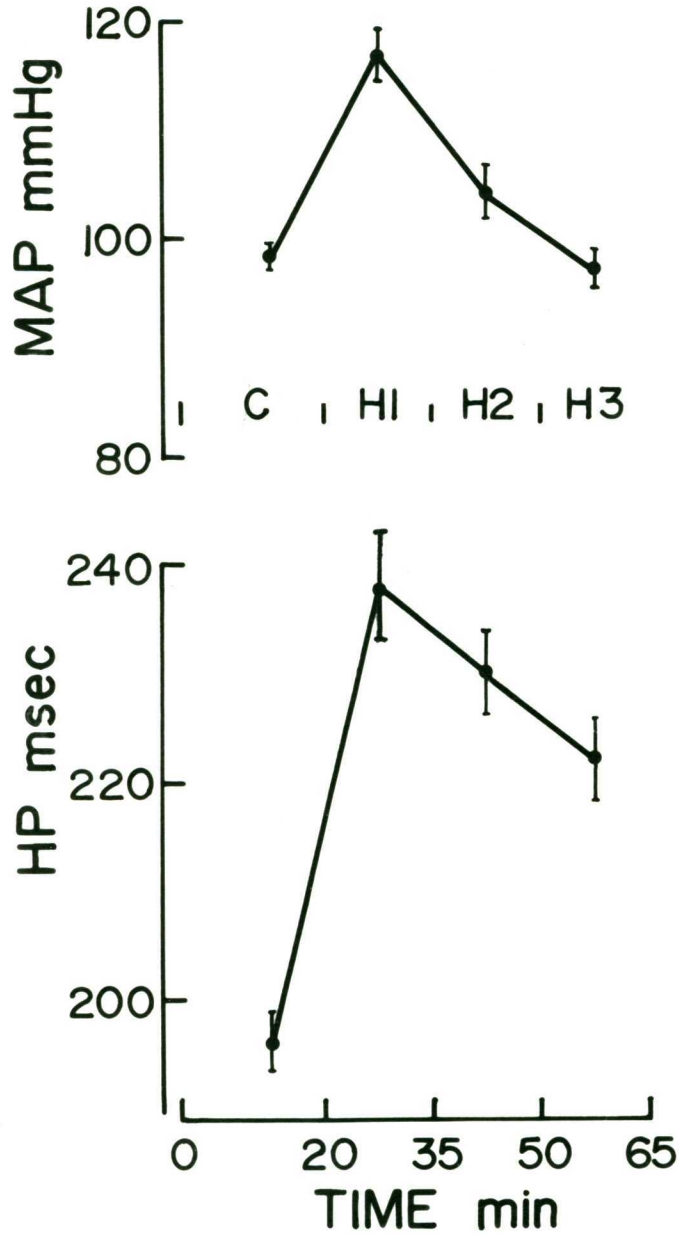

FIGURE 17

Average results of resting mean arterial pressure and heart period obtained in 5 atropinized sham-operated rabbits at a constant ventilation of 2 litres/min. Aortic and caval balloons were inflated to test baroreceptor function at the various times of the experiment. The pooled data from the effects of balloon inflation obtained during the control period (C), early (H_1), middle (H_2) and late (H_3) periods of hypoxia, and during recovery (R) was used to calculate the stimulus-response curves for the sham-operated group of rabbits in Fig. 20. Each bar is $\pm$ 1 S.E. of the mean value at a single time interval during periods C to H_3.

of baroreceptor stimulus response curves by means of paired balloon inflations exactly as described in the previous chapter. During the control period 5 pairs of balloon inflations were performed while the animal was breathing air. This was followed by 48 minutes of hypoxia (art. PO_2 mean 30 mmHg; range 28-35 mmHg) in which 10 pairs of balloon inflations were made starting 2 minutes from the induction of hypoxia. Lastly 3 pairs of balloon inflations were made during a 15 minute recovery period following hypoxia (Fig. 17). Arterial blood samples were taken at the start of the control period and 12 and 37 minutes from the start of hypoxia. Each animal was subjected to only one run on hypoxia and tolerated the level of hypoxia without apparent distress (Korner, Uther and White, 1969; Uther, Hunyor, Shaw and Korner, 1970).

The experiments were performed under controlled ventilation and all the rabbits were atropinized half an hour before the start of the experiment. They were then connected to a respiratory pump and ventilated at the average resting respiratory minute volume and rate of spontaneously breathing normal rabbits (i.e. 1 litre/min, at a rate of 60/min). Muscular relaxation was induced with decamethonium iodide. No operative intervention was undertaken while the animals

were under the influence of decamethonium, and they were placed on sponge rubber, wrapped in cotton wool, and Dow Silicone Medical Fluid No. 360 was instilled into their eyes, to minimise somatic stimuli (Korner, Uther and White, 1969). The experiment began 15-20 minutes after decamethonium had been given. In the first part of the study the animals were ventilated with room air and the effects were examined of changing ventilation from 1 litre/min to 2 litre/min by increasing the pump stroke volume while keeping the rate constant. At each level of ventilation 5 pairs of balloon inflations were carried out. During the second part of the experiment ventilation was maintained constant at 2 litre/min during control, hypoxia and recovery periods. The ventilation of 2 litre/min was chosen to imitate the normal respiratory minute volume during hypoxia of the spontaneously breathing rabbit. The length of the control, hypoxia and recovery periods was the same in all experiments. The baroreceptor-sympathetic heart rate effects were studied in 5 sham-operated and 5 pontine rabbits, in 4 sham-operated animals with section of carotid sinus and aortic nerves, and in 5 sham-operated and 6 pontine rabbits following vagotomy.

Stimulus-Response Curves

Stimulus-response curves were calculated from the response of 4 to 5 animals in each set of experiments with several measurements made in each animal. In the hypoxia experiments each animal contributed results to the various calculated curves from the following number of balloon inflations; 5 pairs during the control period (C in Figs. 17 and 20); 3 pairs during early hypoxia at 2, 7 and 12 minutes (HI); 3 pairs during the middle part of hypoxia (H2); 4 pairs during the latter part of hypoxia (H3) and 3 pairs during recovery (R). In the hyperventilation study, results from 4-5 pairs of balloon inflations per animal were used to calculate the curves at each level of ventilation.

The mean arterial pressure-heart period response curves and their parameters at each time interval were derived as described in previous Chapters.

RESULTS

Significance of Changes in Stimulus - Response Curves

When a disturbance acts on the cardiac autonomic motoneurone pool independently of the baroreceptor input only the mean level of the heart period will be altered and the

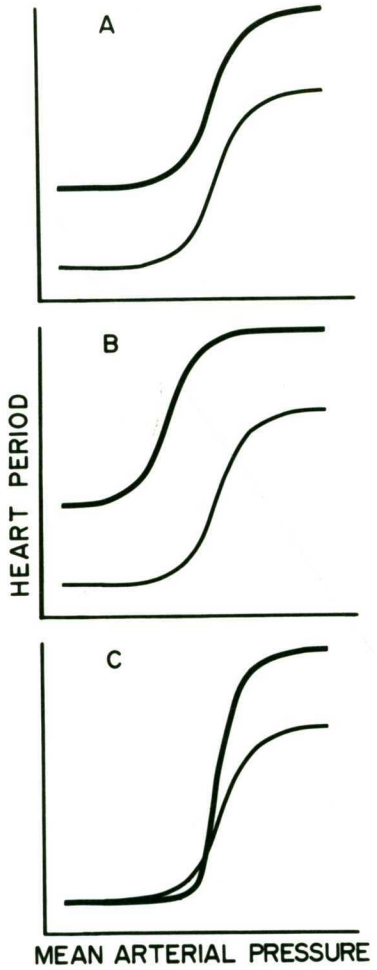

FIGURE 18

Schematic illustration of different mechanisms involved in altering of baroreceptor stimulus-response curves from their control position during a disturbance (heavy curve). A : Reflex cardiac motoneurone activation is entirely independent of the input from circulatory baroreceptors. Only the mean level of the heart period has altered, but none of the pressure-dependent parameters of the curve. B : The disturbance has evoked (i) pressure-independent effects, with equal shifts in both plateau levels; (ii) effects on pressure-sensitive motoneurones, with resulting fall in threshold and BP_{50} but no change in $\bar{G}$ or HPR. C : Reflex effect is entirely 'baroreflex dependent' with changes in every parameter of the curve. The shift in upper plateau is entirely due to the rise in HPR.

stimulus-response curve will be displaced vertically with no change in any of the pressure-dependent parameters (Fig.18). Conversely if the inputs activated by the disturbance converge with the input from the baroreceptors onto common motoneurones, the pressure-dependent parameters will become altered from their control values. Alterations in parameters thus reflect interaction between the various input components activated and the baroreceptor system.

Sympathetic Responses During Controlled Ventilation

(i) Effects of Hypoxia

To eliminate the reflex effects of hyperventilation induced by the hypoxia, respiration was maintained constant at 2 litres/min during control and hypoxia periods. The period of hypoxia resulted in a transient rise in arterial pressure (MAP) and right atrial (RAP) pressure, and a persistent elevation in heart period (Fig. 17). In pontine rabbits the arterial pressure also increased while RAP remained unchanged and heart period fell significantly below control (Fig. 20). Changes in arterial blood composition were the same as described previously from this laboratory (Korner, Uther and White, 1969; Uther, Hunyor, Shaw and Korner, 1970).

TABLE 11

Curve parameters in atropinized rabbits ventilated at constant (2 litres/min) controlled ventilation.

	C	H_1	H_3
SHAM + ATROPINE[1] (5)[2]			
BP_{50} mmHg[3]	100 + 1.0[4]	114 ± 1.1	97 ± 0.9
HPR msec	48 ± 3.8	42 ± 7.0	65 ± 6.3
$\bar{G}$ msec/mmHg	1.8 ± 0.2	2.0 ± 0.7	4.0 ± 0.6
SHAM + ATROPINE + VAGOMOTY (5)			
BP_{50} mmHg	99 ± 1.0	125 ± 1.9	96 ± 1.2
HPR msec	36 ± 5.0	40 ± 7.0	43 ± 5.6
$\bar{G}$ msec/mmHg	1.4 ± 0.2	1.2 ± 0.4	1.6 ± 0.4
PONTINE + ATROPINE (5)			
BP_{50} mmHg	84 ± 0.6	102 ± 1.8	86 ± 1.6
HPR msec	28 ± 5.1	42 ± 5.8	32 ± 3.6
$\bar{G}$ msec/mmHg	1.8 ± 0.3	1.2 ± 0.3	1.0 ± 0.2

Resting mean arterial pressure and heart period plotted in Fig. 17.

1 Results are for control period breathing air (c); early period of hypoxia (H_1), later period of hypoxia (H_3).

2 Number of animals.

3 BP_{50} = median blood pressure; HPR = heart period range; $\bar{G}$ = average gain.

4 Mean and SE from pooled data; curve calculated from number of aortic balloon inflations + number of caval balloon inflations + number of pairs of resting values (see Chapter 2).

During hypoxia the curves shifted from their control positions. In the sham-operated rabbits, the shifts in upper and lower plateaux were approximately equal during each period (i.e. were independent of baroreflex influences) and were maximal during the early part of hypoxia (Fig. 20). The parameters of the stimulus-response curves altered during hypoxia with transient rises in threshold pressure and BP_{50} and a maximum rise in gain of 100% in the latter part of hypoxia (Table 11, Fig. 20). At each time interval during hypoxia the rise in resting heart period from control was greater than the rise in plateau levels and was thus in part dependent on baroreflex pressure sensitive changes. This effect is illustrated in Figure 19 where the average rise in resting heart period above control during H_1 was 42 mec. The baroreflex-independent component of this rise, determined by the average rise in plateau levels was 36 msec, which left 6 msec as the baroreflex-dependent component. The increased threshold of this early hypoxia curve has reduced the value of the baroreflex-dependent component since for a similar rise in blood pressure during the control period the baroreflex-dependent rise in heart period would have been greater (21 msec).

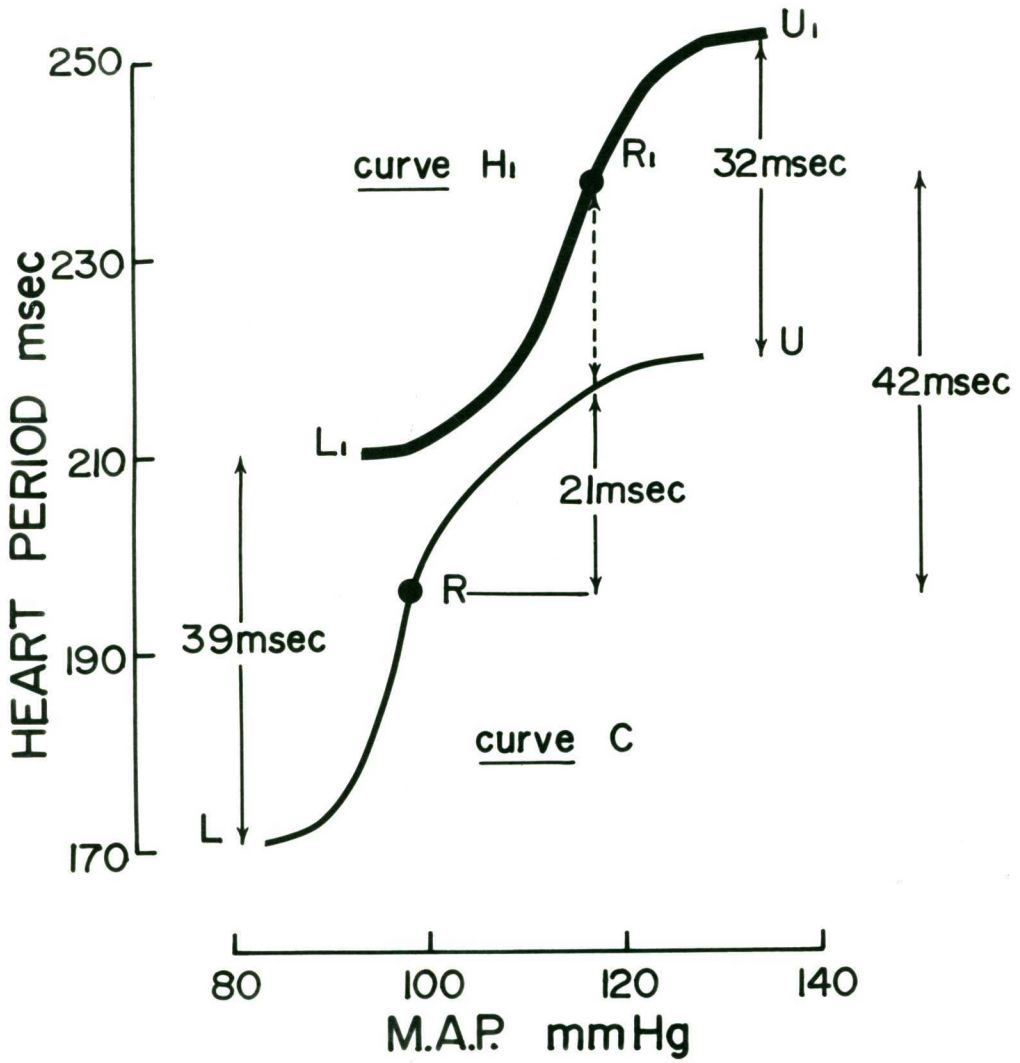

FIGURE 19

Diagram illustrating calculation of baroreflex-independent and baroreflex-dependent components of reflex response of heart period in atropinized, sham-operated rabbits with ventilation controlled at 2 litres/min. C is control curve, H_1 is curve early in hypoxia. R and R' are the resting heart period values; L and L' the lower plateau levels; U and U' the upper plateau levels of each curve; X is the point on the control curve at the same blood pressure as during hypoxia. The change in resting heart period equals R' - R = 42 msec; the baroreflex - independent component equals 0.5 ((L' - L) + (U' - U)) = 0.5 (39 + 32) = 36 msec; the baroreflex-dependent component equals 42 - 36 = 6 msec, which is less than the heart period change in curve C between R and X of 21 msec which the same rise in blood pressure would have evoked during the control period.

(ii) Role of Chemoreceptors, Arterial and Cardiac plus Pulmonary Baroreceptors

In sham-operated animals with section of the carotid sinus and aortic nerves which effectively eliminates all neural baroreceptor input in the rabbit (Chalmers, Korner and White, 1967a), balloon inflation did not elicit any reflex changes in heart period during any of the observation periods. The minimal shift from control in the mean heart period level during the early part of hypoxia indicates that the corresponding changes in the intact animal were reflex in nature (Fig. 20, curves C and H_1). During the middle and latter periods of hypoxia, the heart period increased by 16 $\pm$ 4 msec ($P < 0.05$) above control. This rise was probably partly due to cardiac depression since it was associated with a marked fall in mean arterial pressure (James and Nadeau, 1963).

The effects of vagotomy in atropinized animals during hypoxia were a more marked initial rise in BP_{50} and threshold than in animals given atropine alone, while the shift in plateau levels, HPR and $\bar{G}$ remained approximately constant throughout hypoxia and did not change during the middle and latter periods as in animals given atropine alone (Fig. 20, Table 11). In both groups caval balloon inflation was

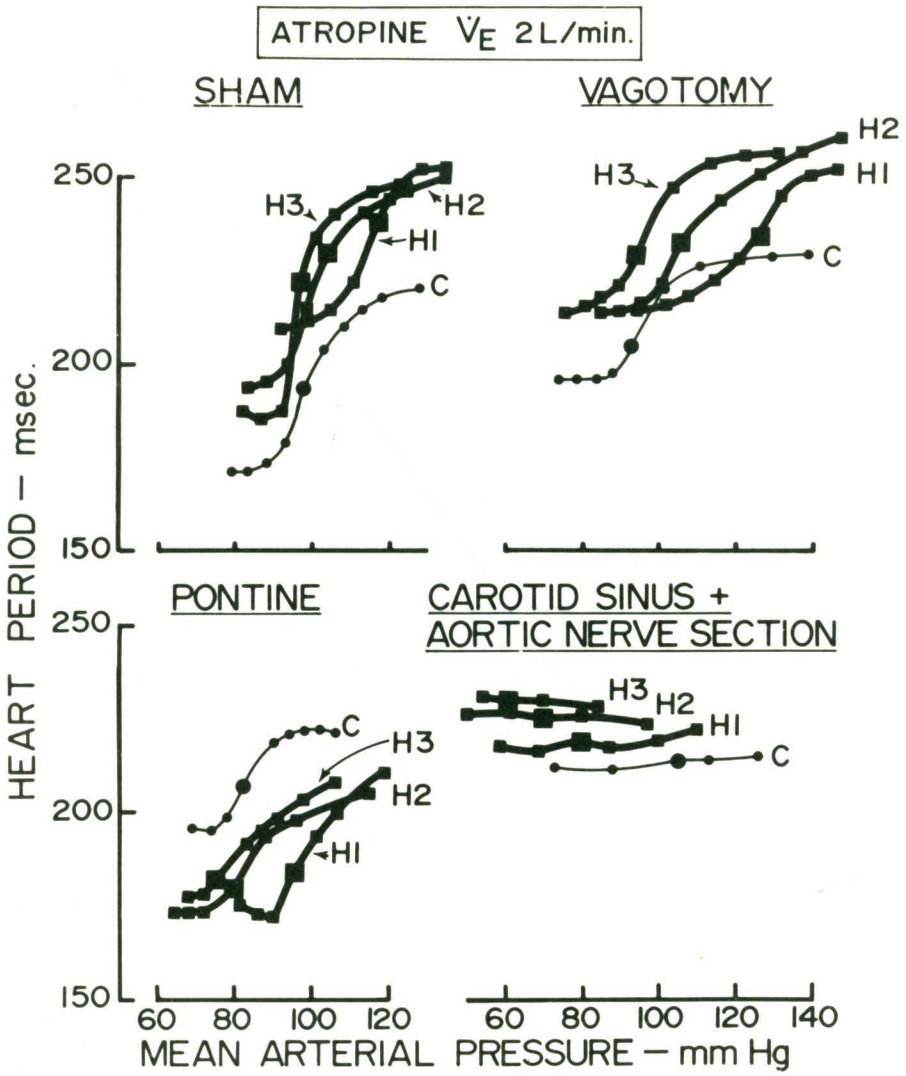

FIGURE 20

Mean arterial pressure-heart period curves obtained during control and hypoxic periods in the following groups of rabbits: 5 sham-operated; 5 vagotomized (intact CNS); 5 pontine; 4 section of carotid sinus and aortic nerves (intact CNS). All animals were given atropine and ventilation was controlled at 2 litres/minute throughout the experiment.

associated with a more marked reduction in RAP during hypoxia (Vagotomy + atropine; -1.1 ± 0.1 mmHg, air; 2.4 ± 0.2 mmHg, hypoxia. Atropine alone; 1.0 ± 0.15 mmHg, air; 2.5 ± 0.2 mmHg, hypoxia).

In sham-operated rabbits the baroreflex-independent rise in plateau level was thus mediated through the carotid sinus and aortic nerves (i.e. chemoreceptors) and not through vagal afferents. The rise in BP_{50} and threshold were similarly mediated by arterial baroreceptor-chemoreceptor interaction through the carotid sinus and aortic nerves. The effects of vagotomy arose from the removal of the cardiopulmonary baroreceptor influence since ventilation was constant. These receptors resulted in facilitation of the baroreflex-dependent response early in hypoxia since they reduced the rise in BP_{50} and the threshold while later they contributed to the rise in $\bar{G}$ and HPR (see Discussion).

There were only small differences in displacement of the plateau levels and in changes in curve parameters during hypoxia between pontine rabbits given atropine alone and pontine rabbits subjected to vagotomy plus atropine.

TABLE 12

Curve parameters in vagotomized atropinized rabbits ventilated with room air.

	VENTILATION 1 litre/min			VENTILATION 2 litres/min		
	BP_{50}	HPR	$\bar{G}$	BP_{50}	HPR	$\bar{G}$
'SHAM-OPERATED'	104 ± 0.6	35 ± 7.0	2.9 ± 0.8	99 ± 1.2	36 ± 5.0	1.4 ± 0.2
PONTINE	82 ± 0.9	44 ± 6.6	2.4 ± 0.4	87 ± 0.6	41 ± 6.3	2.7 ± 0.5

For notation see Table 11.

(iii) Effects of Hyperventilation with Room Air

Increasing respiratory minute volume from 1 to 2 litres/min in sham-operated rabbits ventilated with air produced cardioacceleration with systematic lowering in plateau levels and minimal changes in curve parameters suggesting independence from baroreceptor influences (Fig. 21, Table 13 c.f. 'intact columns at $\dot{V}_E$ 1 and 2 litres/min.). In pontine animals the lowering in plateau levels was about half that of the sham-operated group.

In rabbits with section of the carotid sinus and aortic nerves hyperventilation produced the same lowering of heart period as in sham-operated animals in which these nerves were intact (Fig. 21). The effects of hyperventilation on the cardiac sympathetic were thus independent of the arterial baroreceptors.

In vagotomized and atropinized animals the shift in plateau levels due to hyperventilation was much reduced and the small residual lowering of the plateau level was not statistically significant ($P = 0.15$). The systematic shift in the curves during hyperventilation is thus mediated through vagal afferents, probably arising from lung inflation

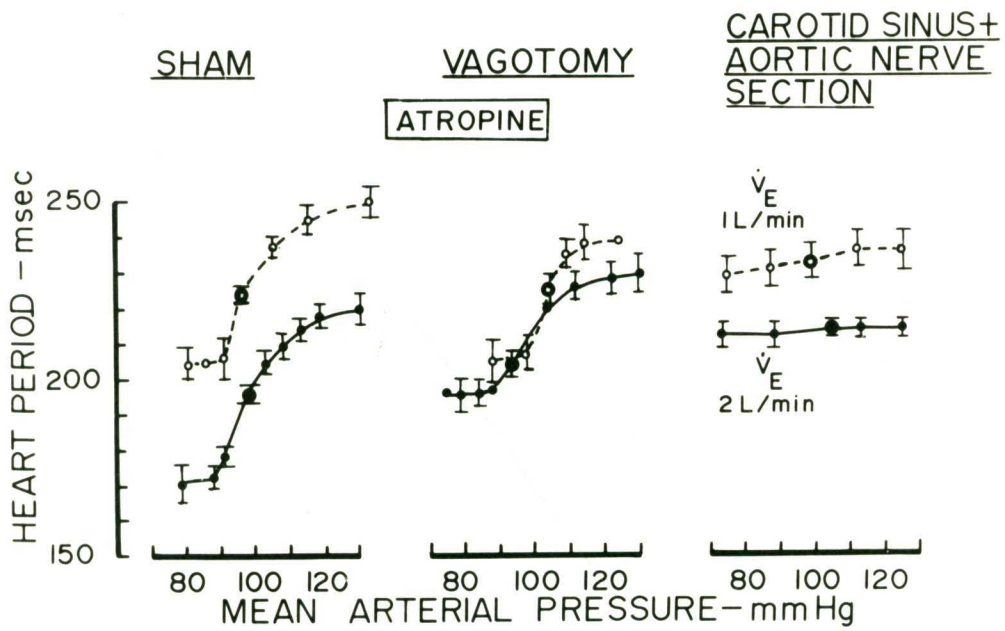

FIGURE 21

Effect of changing ventilation with room air from 1 litre/min to 2 litres/min in 5 rabbits with all afferents intact, 5 vagotomized rabbits, and 4 animals with section of carotid sinus and aortic nerves. All groups were given atropine.

receptors (Daly and Hazzledine, 1963; Crocker, Johnson, Korner, Uther and White, 1968).

Suprapontine and Pontine Influences on the Baroreflex-Dependent Component of the Cardiac Sympathetic

(i) Arterial Baroreceptors

The role of higher centres on bulbar sympathetic neurones from this input was assessed by a comparison of the stimulus response curves, at the same respiratory minute volume, from sham-operated and pontine preparations following atropine and vagotomy and ventilated with air (Table 12). BP_{50} and threshold were higher in the sham-operated group than in the pontine animals, both at a ventilation of 1 litre/min and at 2 litres/min ($P < 0.001$) (see Discussion).

(ii) Cardiopulmonary Baroreceptors

In order to estimate the effects arising from vagal afferents on ventilation (i.e. cardiopulmonary influences), the curve parameters in sham-operated animals were averaged over both levels of ventilation and compared in vagotomized plus atropinized animals with those animals given atropine alone (Table 12). There was no significant difference between the two groups. In a similar comparison in pontine

TABLE 13

Curve parameters in atropinized and in atropinized + vagotomized rabbits ventilated with room air.

		VENTILATION 1 litre/min		VENTILATION 2 litres/min		AVERAGE EFFECT	
		Intact Afferents	Vagotomy	Intact Afferents	Vagotomy	Intact Afferents	Vagotomy
SHAM OPERATED	$(BP_{50}$	98 ± 1.7	104 ± 0.6	100 ± 1.2	99 ± 1.0	99 ± 1.8	101.5 ± 1.1
	(HPR	45 ± 5.0	35 ± 7.0	48 ± 3.8	36 ± 5.0	46.5 ± 6.3	35.5 ± 8.7
	$(\bar{G}$	1.2 ± 0.3	2.9 ± 0.8	1.8 ± 0.2	1.4 ± 0.2	1.5 ± 0.3	2.2 ± 0.8
PONTINE	$(BP_{50}$	87 ± 1.4	82 ± 0.9	84 ± 0.7	87 ± 0.6	85.5 + 1.6	84.5 ± 1.1
	(HPR	39 ± 5.0	44 ± 6.6	28 ± 5.0	41 ± 6.3	33.5 ± 7.0	42.5 ± 8.9
	$(\bar{G}$	1.2 ± 0.3	2.4 ± 0.4	1.8 ± 0.3	2.7 ± 0.5	1.5 ± 0.4	2.6 ± 0.6

For notation see Table 11.

rabbits there was also no significant difference in curve parameters. Thus at arterial P_{O_2} of 100 mmHg, the input from cardiopulmonary baroreceptors had no significant effect on the stimulus-response curve which acted independently of ventilation.

There were however effects dependent on ventilation. In sham-operated animals at a ventilation of 1 litre/min, BP_{50} was significantly higher in vagotomized plus atropinized animals than in rabbits with intact afferents given atropine alone (i.e. cardiopulmonary afferents reduce BP_{50} and threshold). In pontine animals BP_{50} was significantly lower in vagotomized plus atropinized than in those given atropine alone (i.e. cardiopulmonary afferents here have the effect of raising threshold and BP_{50}). Since these parameter differences were absent at 2 litres/min, a significant interaction between the lung inflation input and that from cardiac plus pulmonary baroreceptors is likely.

DISCUSSION

Functional Organisation of the Sympathetic Motoneurone Pool

The arterial baroreceptors, chemoreceptors and lung inflation receptors each activate distinctive parts of the

cardiac sympathetic motoneurone pool. During a given set of conditions (e.g. at P_{O_2} either 100 or 30 mmHg) changes in activity in one of these specific pool sites may take place independently of the others and account for the major part of the reflex changes in resting heart rate. There are also interactions between the various cardiorespiratory inputs which can be explained by convergence onto common motoneurones and which may modify the response to a particular input during a disturbance such as hypoxia. In the present study, only the cardiopulmonary baroreceptors had no effects which were independent of other inputs, but interacted particularly with the arterial baroreceptors and chemoreceptors. The pathways from the various peripheral receptors to the baroreflex-dependent and independent groups of motoneurones are shown in Fig. 22.

Arterial Baroreceptors

This is the necessary input to the pressure sensitive part of the sympathetic notoneurone pool since without it there are no reflex changes in heart period during balloon inflation at either arterial P_{O_2} of 100 or 30 mmHg. At arterial P_{O_2} of 100 mmHg suprapontine mechanisms have an inhibitory effect on the group of motoneurones receiving

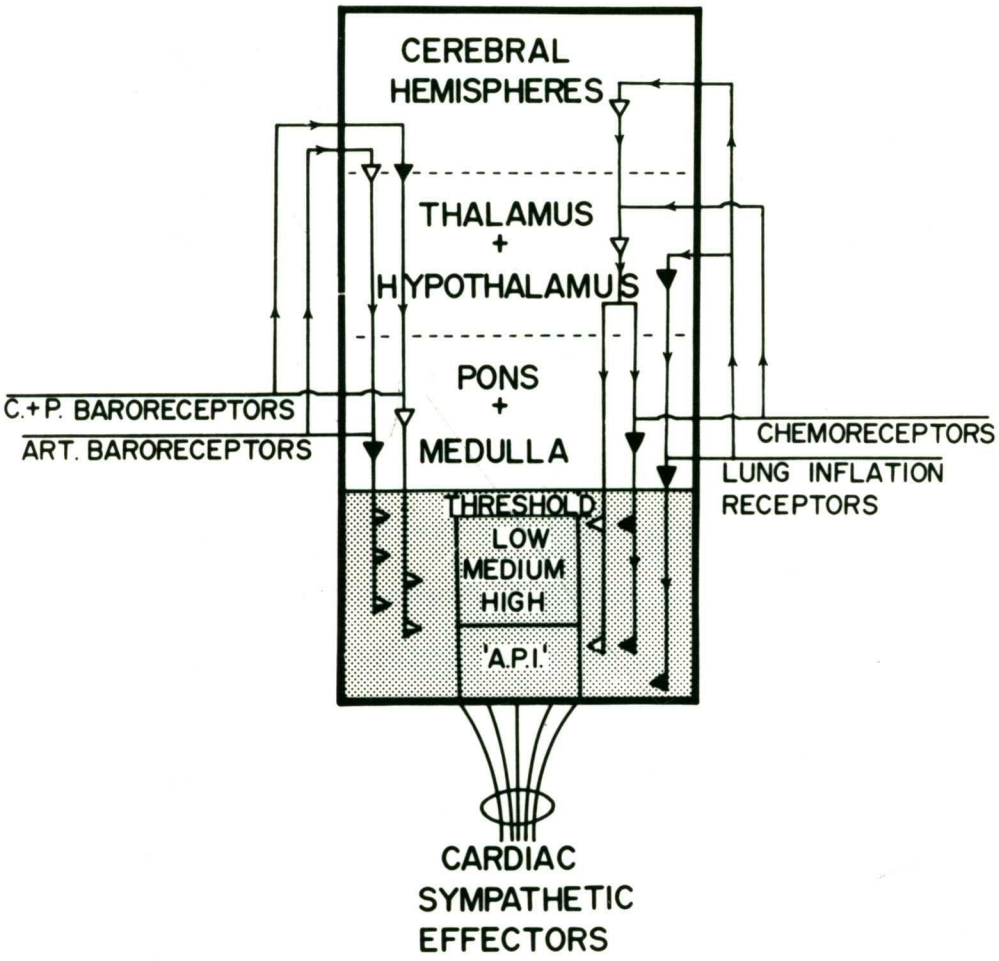

FIGURE 22

Schematic illustration of pathways from arterial baroreceptors, cardio-pulmonary baroreceptors, chemoreceptors and lung inflation receptors to cardiac sympathetic motoneurones. Motoneurone pool has (i) a pressure-sensitive component with units of low, medium and high threshold to baroreceptor stimuli; (ii) an arterial pressure-insensitive (API) component, which does not receive arterial baroreceptor projections. Pathways shown are based on differences in responses of sham-operated, thalamic and pontine atropinized groups. Solid triangle = excitatory (facilitatory) effect; open triangle = inhibitory effect; half solid-half open triangle shows that baroreceptor inputs can evoke either excitation or inhibition of pressure-sensitive motoneurones, depending on the direction of the intravascular pressure changes. The pathways and various interactions are discussed in the text.

arterial baroreceptor projections since BP_{50} and threshold is lower in pontine animals both with respiration controlled or spontaneous (Korner, Shaw, West and Oliver, 1972).

Cardiopulmonary Baroreceptors

The effects of this input on the curve parameters at arterial P_{O_2} 100 mmHg were not exactly determined. However during severe hypoxia this input produced definite effects which occurred in association with a greater reduction in RAP during caval balloon inflation. These changes were probably related to a rise in intrathoracic blood volume due to the bradycardia and vasoconstriction of hypoxia.

The large rise in threshold and BP_{50} found in vagotomized plus atropinized animals during early hypoxia was reduced in the presence of the cardiopulmonary input. This suggests an interaction between the projections from this group of receptors and those from the arterial baroreceptors by means of convergence on to high threshold pressure sensitive motoneurones. This interaction together with the cardiopulmonary - chemoreceptor interaction (see below) also contributes to the rise in $\bar{G}$.

Suprapontine centres also play a role since there is a fall in $\bar{G}$ in pontine animals rather than a rise during the course of hypoxia.

Chemoreceptors

Because of the rise in plateau during hypoxia, projections from this input in sham-operated animals inhibit sympathetic arterial pressure in dependent motoneurones (Fig. 22, API). Conversely there is excitation of these neurones in pontine animals. Since there is a rise in HPR and in $\bar{G}$ later in hypoxia in animals with intact afferents compared to vagotomized animals, it seems that some of the arterial pressure independent motoneurones (Fig. 22, API) at first inhibited by the chemoreceptors may now be excited by right atrial pressure reductions. Thus there is convergence on API motoneurones of projections from the chemoreceptors and the cardiopulmonary baroreceptors. The reason why this effect is unmasked later in hypoxia may be related to adaptation in the chemoreceptors (Eyzaguirre and Lewin, 1961).

The rise in threshold and BP_{50} in sham-operated and pontine rabbits during the early part of hypoxia may be explained by convergence of the chemoreceptors probably on

to low threshold pressure sensitive motoneurones (Fig. 22).

Normally the pressure sensitive sympathetic motoneurone pool responds with increased or decreased activity during small arterial pressure changes. Strong inhibition in sham-operated animals by the high level of chemoreceptor activity during the early part of hypoxia makes these neurones relatively refractory to any further pressure change and converts the low threshold population into one with a high threshold. Thus during chemoreceptor stimulation there are only small effects from changes in arterial pressure.

Lung Inflation Receptors

The systematic shift in the stimulus-response curve with increased minute ventilation in both sham-operated and pontine animals suggests an excitation of arterial pressure-independent motoneurones by this input.

Korner, Uther and White (1969) suggested there was an interaction with the chemoreceptors through a pathway from the lung inflation receptor to the cerebral hemispheres which suppresses the sympatho-inhibitory effects of chemoreceptor stimulation during mild hypoxia. With more

severe hypoxia (30 mmHg) in the sham-operated animal the diencephalic chemoreceptor inhibitory effects dominate, and result in the bradycardia which is observed (Korner, 1971; Korner, Uther and White, 1969).

CONCLUSION

This model of the sympathetic cardiac autonomic motoneurone pool and its various afferent inputs indicates that during severe arterial hypoxia, the sympathetic reflex changes in heart period are the result of activation of autonomic motoneurones not receiving arterial baroreceptor projections and those which do receive projections from the baroreceptor system.

The baroreceptor-heart period reflex under resting conditions depends almost entirely on the input from the arterial baroreceptors whereas during the disturbance other inputs become important determinants of the response to changes in arterial pressure stimuli. During hypoxia the major part of the initial rise in heart period is contributed by the arterial pressure insensitive pool of motoneurones whose main inputs are projections from the chemoreceptors

and lung inflation receptors. The baroreceptor system with the various interactions accounts for about 14% of the initial increase in heart period in the sham-operated animal.

The displacement of the MAP-HP curve from its own control position during a disturbance could be used to assess the relative excitability of two preparations with different resting values of MAP such as hypertensive and normotensive rabbits. In order to perform such an analysis in the future, it would be necessary to examine curve displacement at several levels of hypoxia and to determine the relative effects of the hypoxic stimulus on the changes in the different parameters of the MAP-HP curves.

CHAPTER 6

THE DEVELOPMENT OF RENAL HYPERTENSION IN THE RABBIT

It has been proposed that in the development of renal hypertension the rise in total peripheral resistance is preceded by a phase of increased cardiac output (Ledingham and Cohen, 1963; Guyton and Coleman, 1967). The resultant perfusion of the tissues in excess of their metabolic requirements is believed to provide the stimulus for gradual elevation of vascular resistance through local autoregulatory mechanisms (Ledingham and Cohen, 1963; Guyton and Coleman, 1967; Freis, 1960; Conway, 1966). According to this view, as the resistance becomes raised, cardiac output should return towards normal. During the development of experimental renal hypertension a rise in cardiac output has been observed in the rat and dog (Ledingham and Cohen, 1964; Ledingham and Pelling, 1967; Ferrario, Page and McCubbin, 1970) but such a rise was not present in the series of Olmsted and Page (1965). In the studies of Ledingham and Pelling (1967) and of Ferrario, Page and McCubbin (1970) the rise in cardiac output was small but also sustained during the period of observation. There have been no previous studies of the role of autonomic and nonautonomic

factors in the flow and resistance changes during the evolution of experimental renal hypertension.

In the studies described in this chapter, the haemodynamic changes during the development of renal hypertension were examined in unanaesthetized rabbits. The contribution of autonomic and nonautonomic factors to the various changes in circulatory parameters were assessed by an examination of the haemodynamics before and after complete autonomic block at different times in the course of the development of the hypertension. In addition, the vascular resistance changes due to noradrenaline infusions under 'open loop' conditions were studied so that the opposing interaction of circulatory reflexes and the direct effects of noradrenaline on the vascular smooth muscle was avoided.

METHOD

A Doppler ultrasonic flow transducer was fixed in position around the aortic root at an initial operation 15-37 days (mean 23 days) before the first experiment. Seven to 23 days (mean 14 days) after this operation each animal was anaesthetized and a 'spot' calibration of the Doppler flowmeter was performed using the thermodilution method (Korner, 1965a) . The third operation was carried out

6-20 days (mean 10 days) after the first experiment (that is, an average of 33 days after the implantation of the aortic Doppler transducer). Hypertension was induced in one group of rabbits by renal wrapping while another group underwent a sham procedure. All three operations were carried out as described in Chapter 2.

The main series of experiments were performed in 14 rabbits each of which was studied on 4 occasions. The first (control) experiment was carried out an average of 23 days after implantation of the aortic root transducer (Fig. 26). Ten days later on the average, 7 animals were subjected to bilateral renal cellophane wrapping and 7 to a sham operation. The next experiment was performed on the average 10 days later (range 8-14 days in each group). Two further experiments were performed in each animal on the average 21 days after the operation (range 18-23 days) and 33 days after the operation (range 29-42 days). In the first (control) experiment haemodynamic observations were made with all autonomic effectors functioning normally and after total autonomic block with guanethidine, propranolol, atropine and phenoxybenzamine. During the phase of presynaptic block, vascular responsiveness to pressor stimuli was studied. The first and third experiments after renal

wrapping were carried out similarly, but in the second experiment, only haemodynamic measurements with intact effectors were made.

On the day of an experiment the animal was prepared as described in Chapter 2 and rested in the rabbit box one hour prior to the commencement of a 30 minute recording of arterial pressure, cardiac output, total peripheral resistance and heart rate. Measurements were usually stable and the control readings were expressed as the mean of 6 measurements taken at 5 minute intervals. Animals were then subjected to sympathetic presynaptic block with guanethidine sulphate. This resulted in a marked reduction in arterial pressure and total peripheral resistance and a rise in cardiac output and heart rate (Fig. 23). These variables returned gradually toward resting levels over the next 30-40 minutes without further treatment of the animal which remained in good condition. An hour after injection of guanethidine the animals were treated with atropine and propranolol which blocked the vagal efferents, the cardiac sympathetic effects and the β-adrenergic dilator effects on muscle due to adrenaline release from the adrenal gland. After a further rest period of one hour (that is, two hours

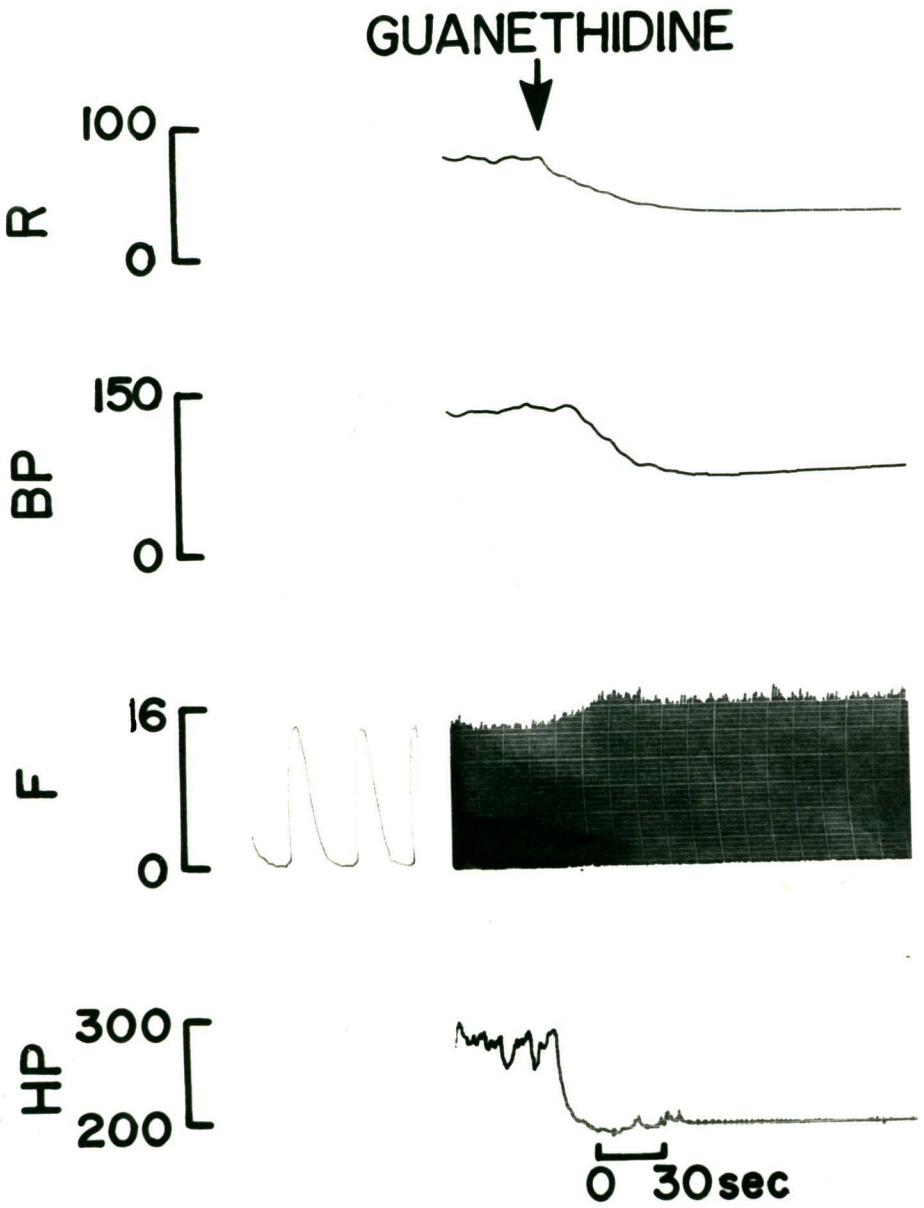

FIGURE 23

Graph illustrating the initial transient effects of guanethidine (12.5 mg/kg) in a hypertensive rabbit, on total peripheral resistance (R, units), mean arterial pressure (BP, mmHg), phasic cardiac output (F, kHz) and heart period (HP, msec). A record of the waveform of the Doppler flow signal precedes the resting cardiac output.

after the injection of guanethidine), when the haemodynamic variables were in a steady state, a dose-response curve was constructed relating the dose of injected noradrenaline (µg I.V.) to changes in total peripheral resistance (Fig. 24, 25). Doses administered to all animals were 0.5, 1, 2, 4, 8, 16 and on some occasions 32 µg. A rest period of 15-20 minutes was then allowed and the animals were given phenoxybenzamine to complete the block (Fig. 24). Thirty minutes later continuous circulatory measurements were made for a further 30 minutes and the effects of total autonomic blockade expressed as the mean of five sets of measurements obtained at five minute intervals. The animals were then returned to their cages after removal of catheters. Drugs were given in the doses outlined in Chapter 2.

In an additional 8 rabbits (6 renal wrap and 2 sham) full measurements were obtained during the first preoperative (control) experiment and during the first postoperative experiment which was performed earlier (6-8 days) after operation than in the main series.

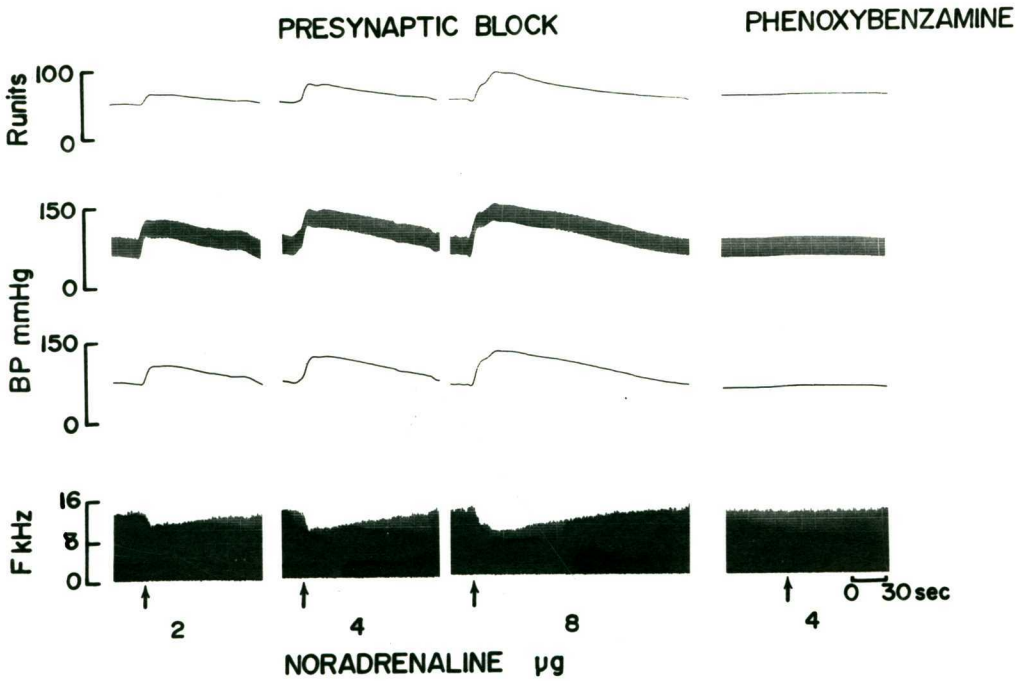

FIGURE 24

(LEFT) Effect of increasing doses of noradrenaline (µg, i.v.) on total peripheral resistance (R, units), phasic and mean arterial pressure (BP, mmHg) and phasic cardiac output (F, kHz) in the open loop preparation after presynaptic block with guanethidine in the presence of atropine and propranolol. The change in resistance with each dose of noradrenaline was determined planimetrically. Following α-block with phenoxybenzamine (RIGHT) there was no pressor response to a challenging dose of noradrenaline (4 µg) indicating total autonomic block.

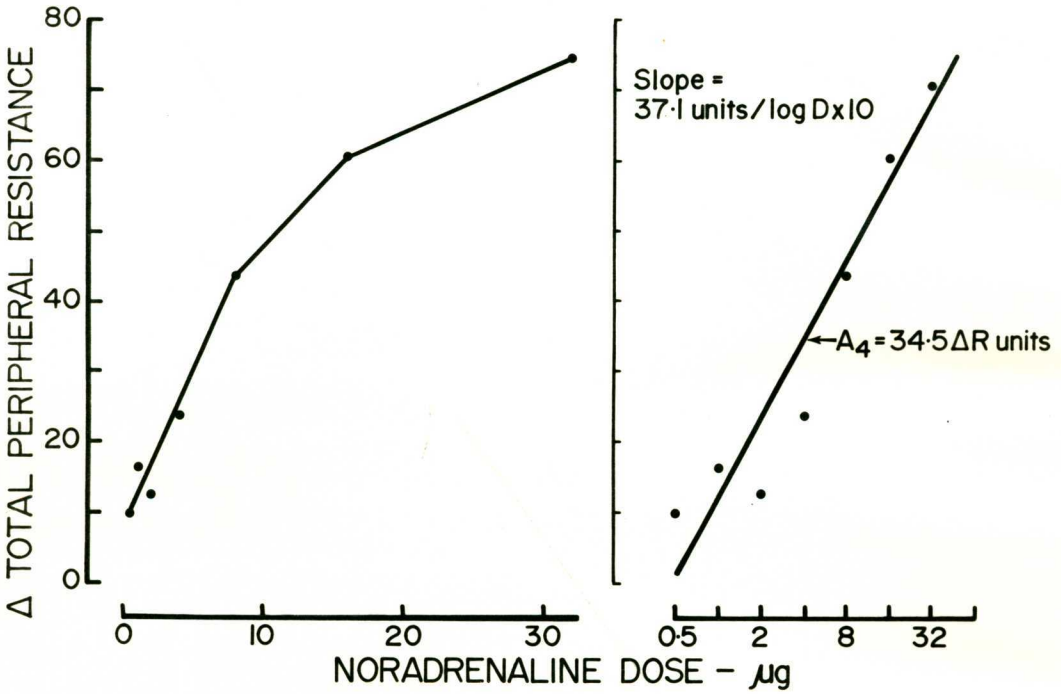

FIGURE 25

(LEFT) Relationship between the dose of noradrenaline (µg) and the change in total peripheral resistance (units) for a single experiment.

(RIGHT) With the use of a logarithmic scale for noradrenaline dosage, the relationship approximates a straight line. The slope of the line and the change in resistance for 4 µg of noradrenaline (A_4) could then be calculated using standard regression techniques.

ANALYSIS OF RESULTS

The changes in haemodynamic variables during the different treatment and time periods were analysed as described in Chapter 2 by the Analysis of Variance.

The dose-response curves were obtained by linear regression relating the logarithm of the dose of noradrenaline to the change in total peripheral resistance. At each time period the mean response slope and the mean response due to 4 μg of noradrenaline were calculated.

RESULTS

Control Experiments

Before renal wrap the cardiovascular variables of the hypertensive group of animals were closely similar to those of the sham-operated group (Fig. 26; Table 14).

Effects of 4 to 6 weeks of Renal Wrapping

In animals with intact autonomic effectors, after an average of 33 days of renal wrapping there was an increase of 40% ($P < 0.001$) in the level of mean arterial blood pressure (MAP) and 62% ($P < 0.001$) in the level of total peripheral resistance (TPR) (Fig. 26). There were no changes in the cardiac output or heart rate. The MAP and TPR

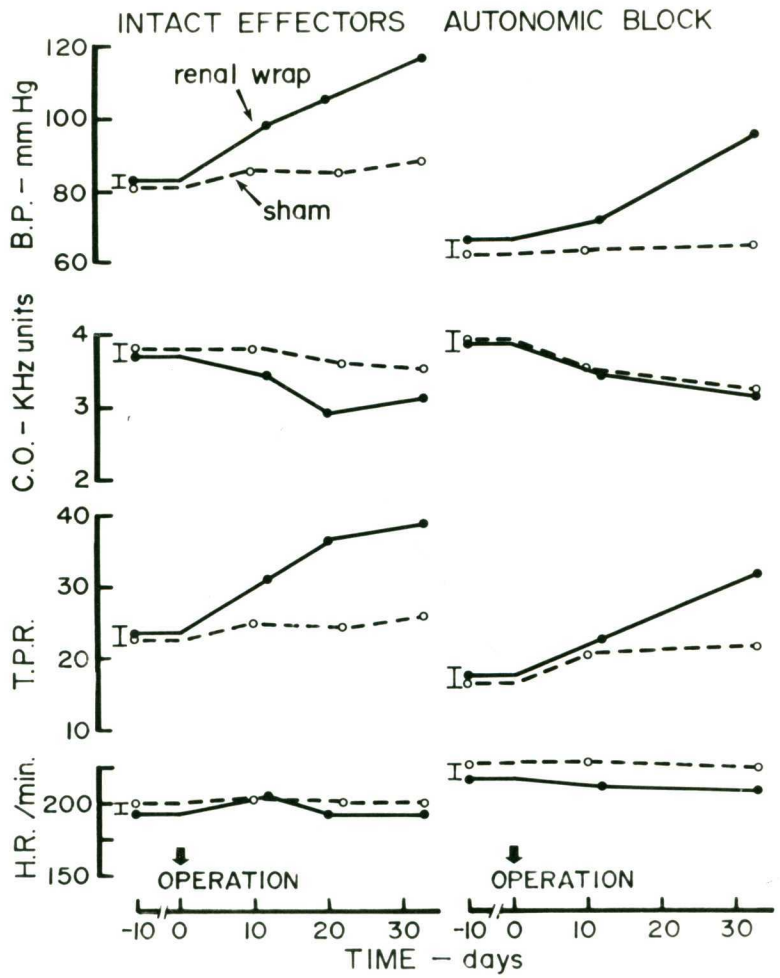

FIGURE 26

Changes in mean arterial pressure (B.P.), cardiac output (C.O.), total peripheral resistance (T.P.R.), and in heart rate (H.R.) during the development of renal hypertension. A renal wrap or sham procedure (OPERATION) was carried out after an initial control experiment. Measurements were made in 7 sham operated and in 7 hypertensive animals with all autonomic effectors intact (on the left) and after total autonomic block (on the right). The symbol on the left is $\pm$ 1 SE of the mean of a single time interval calculated by Analysis of Variance as illustrated in Table 2.

rose at a constant rate during this time period as determined from measurements of haemodynamics at 10, 21 and 33 days following the renal operation. These changes were significantly greater than in the sham-operated animals where there were small insignificant rises in MAP and TPR, a slight decrease in cardiac output and no change in heart rate (Fig. 26).

When the renal wrapped animals were subjected to complete autonomic blockade there was an increase of 45% ($P < 0.001$) in MAP and 79% ($P < 0.001$) in TPR attributable to nonautonomic factors compared to their control values (Fig. 26, Table 14). In the sham-operated animals there were small insignificant rises in the nonautonomic components of MAP and TPR. The nonautonomic TPR contributed 94% of the rise in TPR in the hypertensive animal, after 33 days of renal wrapping.

Early changes after renal wrapping

The absence of a rise in cardiac output at 10 days was not in agreement with the results obtained in either the rat or dog at this time (Ledingham and Pelling, 1967; Ferrario, Page and McCubbin, 1970). One difference in experimental procedure was that these workers produced

TABLE 14

Changes in circulatory variables during development of renal hypertension.

	INTACT					NON AUTONOMIC COMPONENT				
	I *	II	III	IV		I	II	III	IV	
SHAM										
BP (mmHg) T	81.1	85.7	84.9	88.0	(2.5)†	62.0	63.4		64.4	(3.2)
CO (kHz)	3.8	3.8	3.7	3.5	(0.2)	3.9	3.5		3.2	(0.2)
TPR (units)	23	25	24	26	(2)	16	21		21	(2)
HR (beats/min)	200	204	205	200	(5)	227	227		222	(7)
HYPERTENSIVE										
BP (mmHg)	83.0	98.0	105.0	116.4	(2.5)	65.5	70.8		94.7	(3.2)
CO (kHz)	3.7	3.4	3.1	3.1	(0.2)	3.9	3.4		3.1	(0.2)
TPR (units)	24	31	37	39	(2.0)	18	23		32	(2)
HR (beats/min)	192	205	183	191	(5)	216	210		207	(7)

* I - control reading, II, III, IV - 10, 21 and 33 days after sham operation or renal wrapping. Measurements were made with all autonomic effectors intact and after total autonomic block (guanethidine, atropine, propranolol and phenoxybenzamine). At time III measurements were made with autonomic effectors intact only.

T BP - mean arterial pressure, CO - cardiac output, TPR - total peripheral resistance, HR - heart rate.

† Figures enclosed within brackets are ± 1 SE of the mean of a single time interval calculated by analysis of variance as illustrated in Table 2. There were 7 animals in each group at each time interval.

hypertension by unilateral renal artery constriction or renal wrap together with removal of the other kidney. However it seemed possible that a rise in cardiac output may have occurred at an earlier time period. No deliberate experiments were performed to test this point but some data was available from animals studied before wrapping or sham-operation and studied again less than 10 days following these procedures. These animals were not studied further due to transducer failures and wire breakages.

The results from these animals were combined with those from the first postoperative experiment of the main series of rabbits. Two groups of sham-operated and two groups of renal wrapped data were formed from studies performed either less than 10 days or greater than 10 days after renal wrapping or sham-operation. As with the main series of rabbits there was no difference between the control values of this augmented series of renal wrapped and sham-operated animals. A significantly higher cardiac output and MAP was found in the group where the experiment was performed 6-10 days after renal wrapping ($P < 0.01$; Fig, 27). After autonomic block the cardiac output and MAP were also significantly higher in this group ($P < 0.025$).

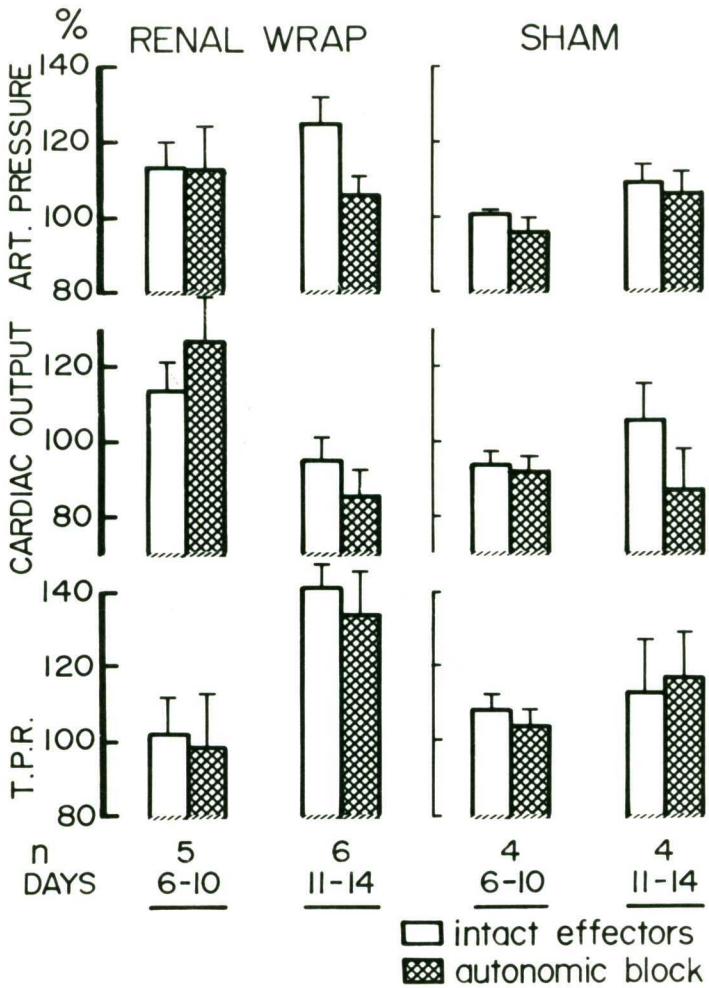

FIGURE 27

Changes (% of control values) in mean arterial pressure, cardiac output and total peripheral resistance after renal wrap or sham operation in a supplementary series of animals which had completed the first postoperative experiment (n is the number of animals in each group). The animals were grouped according to whether the experiment was performed 6-10 days (average 8 days) or 11-14 days (average 12 days) after the operation. Measurements were made in each group of animals with all autonomic effectors intact and after total autonomic block. Symbols are $\pm$ 1 SE of the mean.

There were no significant differences in the haemodynamics between the two sham groups either with effectors intact or after autonomic block.

Vascular Responsiveness to Noradrenaline

Curves relating the logarithm of the dose of noradrenaline to the change in TPR were constructed at several time periods during the development of hypertension. The experiments were carried out in the open-loop preparation after sympathetic block with guanethidine in order to remove inhibitory reflex effects. For the hypertensive animals there was some increase in the slope of the lines at both 10 and 33 days following renal wrap compared to the prewrap control value, but it was only at 10 days that the value was significant ($P < 0.01$) (Fig. 28, Table 15). There were small insignificant changes in the slope of the curves during the course of 33 days for the sham-operated animals. The effect of the increased responsiveness in the hypertensive animals was only marginal since the increment in TPR following a given dose of noradrenaline was only slightly greater (Table 15, Fig. 28).

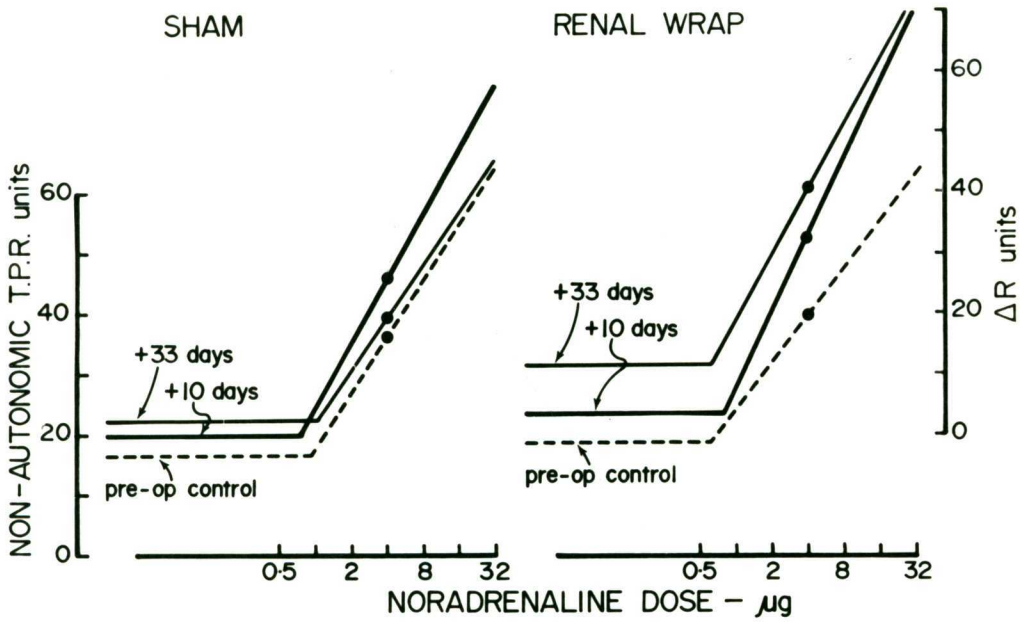

FIGURE 28

Comparison of the calculated regression lines relating the changes in total peripheral resistance (scale on right) to the logarithm of the dose of noradrenaline during the development of renal hypertension (on the right) and for sham operated animals (on the left) during the same time period. The changes after 4 µg of noradrenaline are indicated by the closed circles. The dose response curve at each time period is related to its respective nonautonomic total peripheral resistance value (scale on left). Also see Table 15.

TABLE 15

Parameters of dose response curves to noradrenaline during the development of renal hypertension.

	SHAM OPERATED		HYPERTENSIVE
1. Slope *			
control †	31.0 ± 3.7 T	(5) ∂	26.6 ± 2.6
10 days	35.5 ± 5.4	(8)	44.7 ± 5.5
33 days	28.9 ± 1.6	(7)	36.2 ± 5.6
2. A_4 response *			
control	19.8 ± 2.3		20.5 ± 1.4
10 days	25.3 ± 4.2		27.3 ± 3.8
33 days	17.2 ± 3.2		28.7 ± 4.0

* Slope of calculated regression line relating logarithm of dose of noradrenaline to change in total peripheral resistance (units). A_4 is the mean response to 4 µg of noradrenaline.

† Experiments performed during a control period and at 10 and 33 days following renal wrap or sham operations.

T ± 1 SE of the mean

∂ number of animals in each group.

DISCUSSION

The present method for measuring cardiac output (and hindlimb flow in Chapter 7) uses the Doppler principle. Enough time was allowed for fibrosis to take place between the cuff of the transducer and the vessel wall to keep the diameter of the vessel within a given transducer constant. Calibration studies indicated that in the hindlimb there was no difference in the calibration 4 to 6 weeks after renal wrapping compared to normotensive sham-operated animals. The theoretical possibility that the vessel diameter of the former was greater, leading to a fall in Doppler shift for a given flow could thus be excluded.

The haemodynamic findings in these experiments differ from those previously reported in other species in that there is an absence of a rise in cardiac output 10 days after renal wrapping at a time when previous investigators have demonstrated a rise in cardiac output (Ledingham and Pelling, 1967; Ferrario, Page and McCubbin, 1970). Similarly, after 4 to 5 weeks there was no residual increase in cardiac output as had been described previously. The present experimental findings do not preclude a raised cardiac output being present earlier than the time of the first observation period since cardiac output was greater

in the group of animals studied at less than 10 days after renal wrap compared to those studied more than 10 days after renal wrap. The conditions of the comparison were different however to the main series since there was no within group comparison between those animals studied less than 10 days and those studied after 10 days. Nevertheless the findings are supported by the studies of Ledingham and Cohen (1964) in the rat where cardiac output was raised prior to 10 days but was normal thereafter.

The absolute level of blood pressure developed in the hypertensive animals was less than that previously reported in the literature but the percentage change was similar to that in the studies of Ledingham and Pelling (1967). The use of antiadrenergic drugs before the onset and during the development of hypertension may have reduced the magnitude of the rise in blood pressure in the present experiments since in the studies of renal hypertension reported in Chapter 3, the increase in blood pressure was twice as great.

The increased blood pressure at 4 to 5 weeks after renal wrap is entirely accounted for by a rise in resting total peripheral resistance. Approximately 90% of the difference

in total peripheral resistance between hypertensive and normotensive animals is due to elevation of the nonautonomic component of TPR. These findings are similar to those found by Korner, Shaw, Uther, West, McRitchie and Richards (1973) in human essential hypertension where the nonautonomic component of TPR index contributed 60-80% of the rise. Since there have been no differences demonstrated in the length of resistance vessels or in viscosity between hypertensive and normotensive animals, the high nonautonomic total peripheral resistance is probably due to reduction in vascular calibre (Folkow and Neil, 1971). The autonomic and nonautonomic components of vascular resistance are discussed more fully in the next chapter.

The increase in vascular responsiveness of TPR to catecholamines in renal wrapped animals compared to normotensive animals was not significant. This contrasts with the hindlimb bed (Chapter 7, Fig. 35) where the increase in responsiveness to catecholamines in the hypertensive animals was much greater than in the present experiments and suggests the possibility of nonuniform changes in reactivity. However the rise in blood pressure in the present series was relatively less and may have contributed to the findings.

CONCLUSION

These experiments have shown that in the rabbit there is no change in cardiac output during the early stages of the development of hypertension. This difference from previous work cannot be related to systematic differences in calibration of the transducers between hypertensive and normotensive animals (see Discussion). Therefore species differences between the rabbit on the one hand and rat and dog on the other appear responsible. The stage in the development of hypertension less than 10 days after wrapping may be associated with a rise in cardiac output but this needs to be confirmed. In any case the rise in cardiac output appears to be more transient than previously suggested. The increased blood pressure in renal hypertension is due mainly to a rise in the nonautonomic component of TPR.

CHAPTER 7

VASCULAR RESPONSIVENESS IN THE HINDLIMB BED IN RENAL HYPERTENSION

In this chapter the vascular responses of the rabbit's hindlimb were studied with the object of assessing (i) the magnitude of the nonautonomic component in renal hypertension and the resistance of the maximally dilated hindlimb bed, (ii) the responsiveness of the 'open-loop' preparation to noradrenaline, angiotensin II and vasopressin and (iii) the responsiveness of the hindlimb bed of the unanaesthetised rabbit with renal hypertension to baroreflex stimuli.

Section (i) Nonautonomic Factors in Renal Hypertension

There have been few detailed studies of the haemodynamics in renal hypertension after complete autonomic block where the relative roles of the autonomic and nonautonomic components of vascular resistance have been analysed. An increased neural component of the blood pressure in renal hypertension has been suggested by studies which show a more marked decrease in arterial pressure than in normotension in response to ganglion blocking agents (Page and McCubbin, 1951; Doyle and Smirk, 1955; Laverty and Smirk; Phelan 1966; Smirk, 1970; Tarazi and Dustan, 1973). This conclusion

was also made in studies where neural effects had been assessed by tilting and with the Valsalva manoevre (Frohlich, Ulrych, Tarazi, Dustan and Page, 1967). In human essential hypertension (Korner, Shaw, Uther, West, McRitchie and Richards, 1973) and in the previous chapter in rabbit renal hypertension it was shown that nonautonomic effects played the major role in maintaining the raised blood pressure. In these studies presynaptic block was induced with guanethidine and the possible compensatory effects of unblocked circulatory adrenal medullary secretions and vagal effectors were appropriately blocked. Folkow and coworkers in studies of the hindlimb resistance at maximal vasodilation have suggested that structural adaptive changes in the vascular walls participate in the raised nonautonomic resistance of renal hypertensive rats (Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, 1973).

In the present experiments, the haemodynamic changes following autonomic block and maximal vasodilation in the hindlimb bed of renal hypertensive rabbits were examined.

METHOD

Two series of experiments (Fig. 29; series A and B) were performed in animals who had undergone either cellophane

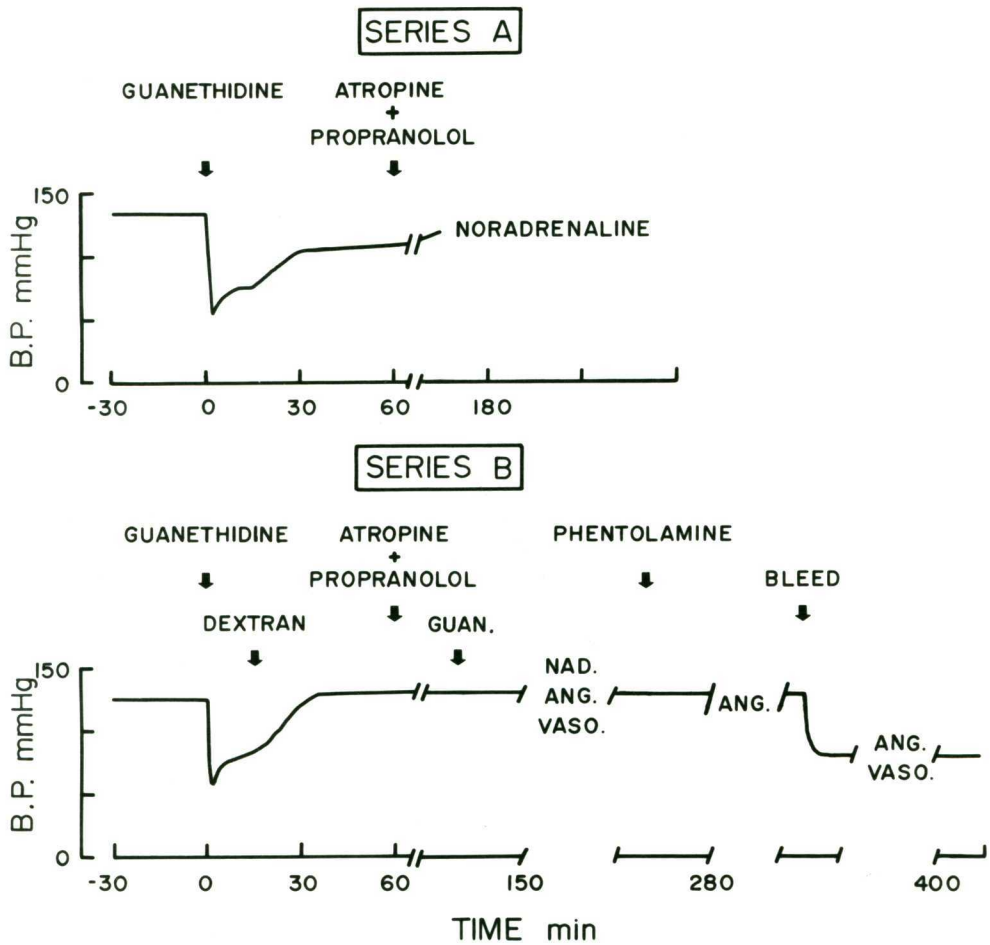

FIGURE 29

Protocol of the experiments carried out in series A (TOP) and in series B (BOTTOM). In series A the blood pressure was uncontrolled following presynaptic block. In series B, after guanethidine the blood pressure was restored to resting levels with dextran.

wrapping of their kidneys or a sham procedure 4 to 5 weeks earlier. The animals in series A had had in addition a silastic inflatable balloon fixed around the inferior vena cava for use in experiments described in Section (iii) (Fig.30). All operations and drug dosage schedules during experiments were carried out as described in Chapter 2 unless otherwise indicated.

In the experiments of series A, haemodynamic observations were made in 11 normotensive and 7 hypertensive animals with all autonomic effectors functioning normally and after presynaptic nerve block with guanethidine in the presence of atropine and propranolol (Fig. 29). Propranolol blocks the cardiac sympathetic and also prevents the β-adrenergic dilator effectors of circulating adrenal catecholamines on the muscle bed. Measurements of the circulatory variables after presynpatic nerve block were made after the administration of guanethidine when the blood pressure had been stable for at least 30 minutes. In this series the blood pressure was not brought back to resting levels and in both normotensive and hypertensive animals it was below resting control values at this time (Fig. 29). After making the various measurements in the guanethidine-atropine-propranolol treated animals they were given phentolamine to complete the block. This

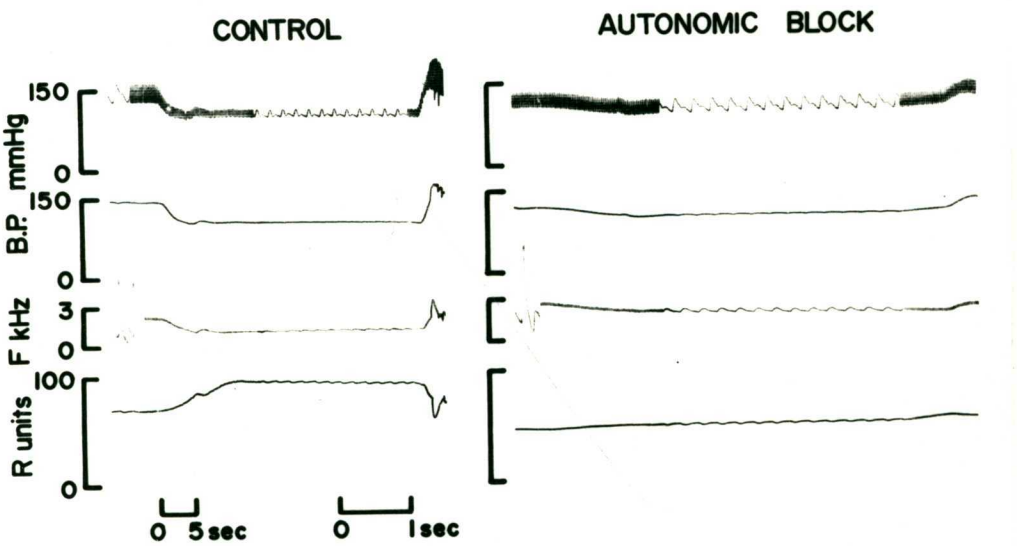

FIGURE 30

Changes in phasic and mean arterial blood pressure, hindlimb flow and hindlimb vascular resistance during vena caval balloon inflation. On the left all autonomic effectors are intact (CONTROL). Blood pressure changes elicit no rise in hindlimb vascular resistance after guanethidine, atropine and propranolol (AUTONOMIC BLOCK).

prevents the α-adrenergic constrictor effects of circulating adrenal catecholamines which are not blocked by guanethidine.

In series B, experiments were performed in 7 normotensive and 9 hypertensive rabbits. Haemodynamic observations were made as in series A before and after presynaptic block. In this series however the blood pressure in both the hypertensive and normotensive animals was restored to its respective resting level by infusions of dextran shortly after the administration of guanethidine (Fig. 29). After completion of autonomic block with phentolamine the hypertensive animals were bled to the blood pressure level of the normotensive animals. This enabled differences in hindlimb resistance at different arterial pressures to be examined.

Immediately after the administration of guanethidine, glyceryl trinitrate (GTN) was given to fully dilate the hindlimb bed. Two doses of GTN were used (2 µg and 8 µg) to demonstrate maximal vasodilation.

RESULTS

Series A

In the resting state hindlimb vascular resistance

TABLE 16

Changes in circulatory variables following autonomic block with guanethidine, atropine and propranolol.

	CONTROL		AUTONOMIC BLOCK		
	SHAM	HYPERTENSIVE	SHAM	HYPERTENSIVE	AFTER BLEEDING
1. Series A *					
BP (mmHg) †	93.2 ± 3.0 (11) ⊤	133.9 ± 5.9 (7)	73.4 ± 3.9	117.9 ± 5.8	
R (units)	33.7 ± 2.5	77.7 ± 6.4	22.3 ± 1.6	49.4 ± 3.6	
Flow (kHz)	2.9 ± 0.2	1.8 ± 0.1	3.6 ± 0.3	2.4 ± 0.1	
2. Series B *					
BP (mmHg)	83.7 ± 1.2 (9)	125.7 ± 3.5 (7)	79.0 ± 3.1	129.1 ± 3.3	80.3 ± 2.4
R (units)	24.7 ± 2.8	68.0 ± 10.0	20.0 ± 1.8	63.0 ± 11.0	45.0 ± 5.0
Flow (kHz)	3.4 ± 0.3	2.1 ± 0.3	3.5 ± 0.2	2.4 ± 0.4	2.0 ± 0.3

* Series A - blood pressure uncontrolled following autonomic block. Series B - blood restored to resting values following autonomic block.

† BP - mean arterial pressure, R - hindlimb vascular resistance, Flow - hindlimb blood flow.

⊤ Number of animals in each group.

∂ ± 1 SE of the mean.

(HVR) and mean arterial pressure (MAP) were significantly greater ($P < 0.001$ for both) in the hypertensive animals and the hindlimb flow was significantly lower ($P < 0.001$) (Fig. 31, Table 16).

After presynaptic block in both normotensive and hypertensive animals there was a significant reduction in MAP (approximately 20 mmHg in both, $P < 0.001$ for both) and in HVR ($P < 0.001$) compared to control conditions with all autonomic effectors intact. Hindlimb flow was increased ($P = 0.025$ normotensive, $P < 0.001$ hypertensive) above resting levels after presynaptic block. The blocked value of HVR in the hypertensives was significantly higher than the value in the normotensive animals ($P < 0.001$) and contributed 62% of the difference in resting HVR between normotensive and hypertensive animals.

There were insignificant effects on HVR following phentolamine which was used in only a few animals in this series. A more detailed study was carried out in series B.

Series B

The resting state circulatory variables in series B were similar to those in series A (Fig. 32). After

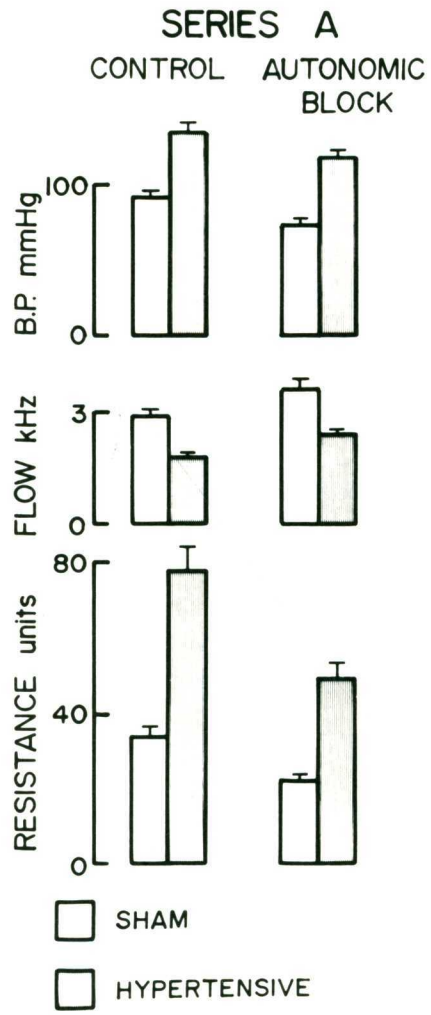

FIGURE 31

Values of mean arterial pressure, hindlimb flow and hindlimb vascular resistance in 11 sham operated and 7 hypertensive animals with all autonomic effectors intact (on the left), and after autonomic block with guanethidine, atropine and propranolol (on the right). Symbols are $\pm$ 1 SE of the mean. Also see Table 16.

presynaptic block with restoration of blood pressure to the initial value, the difference in HVR between renal hypertensive and normotensive rabbits was even greater than in series A (Fig. 32; Table 16). Under these circumstances (series B) the fall in HVR was the same in both normotensive and hypertensive animals after presynaptic block, whereas in series A where blood pressure was uncontrolled the fall in HVR was greater in the hypertensive animals.

Following postsynaptic block with phentolamine there was a small and insignificant fall in HVR (Fig. 33) in both the sham-operated and hypertensive animals. The effects of circulating catecholamines on HVR were considered to be small and have been neglected. HVR following presynaptic block has thus been termed the 'nonautonomic' HVR.

Bleeding reduced MAP of the hypertensive animals in series B to the same level as that of the normotensive animals (Fig. 32). The fall in blood pressure was accompanied by a reduction in nonautonomic HVR and by an insignificant fall in hindlimb flow. HVR was still higher in the hypertensive animals than in the normotensive ($P < 0.001$). Bleeding thus reproduces essentially similar results to series A.

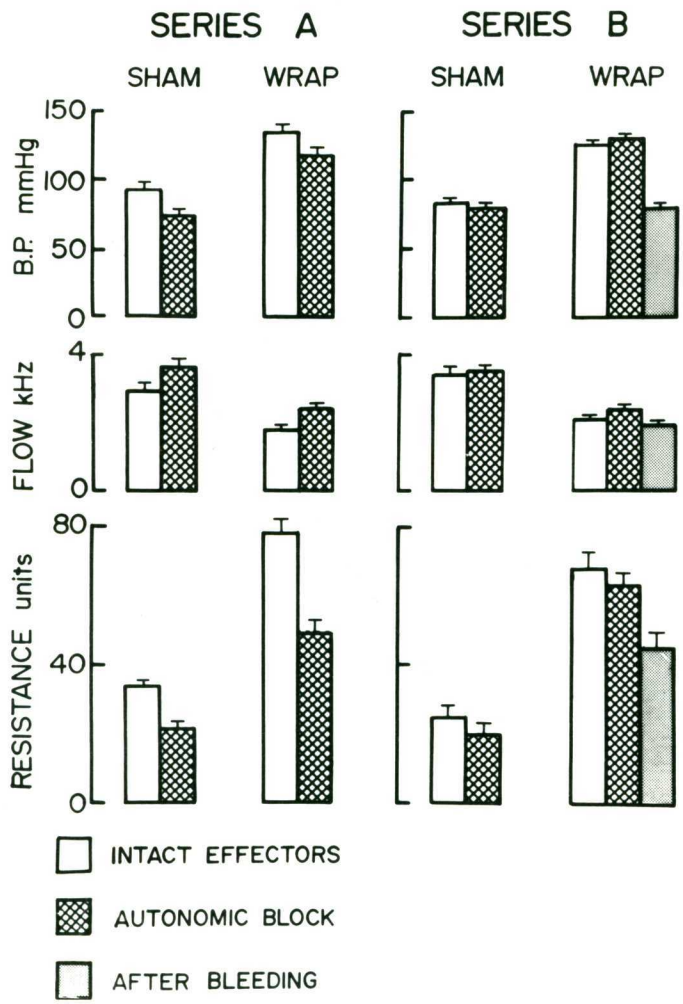

FIGURE 32

Series A: Values of mean arterial pressure, hindlimb blood flow and hindlimb vascular resistance before and after autonomic block with guanethidine, atropine and propranolol, in 11 sham operated and 7 hypertensive animals. Blood pressure was uncontrolled following autonomic block.

Series B: Blood pressure maintained at resting levels in 7 sham operated and 9 hypertensive animals following autonomic block. The blood pressure in the hypertensive animals was then reduced to normotensive levels by bleeding. Also see Table 16.

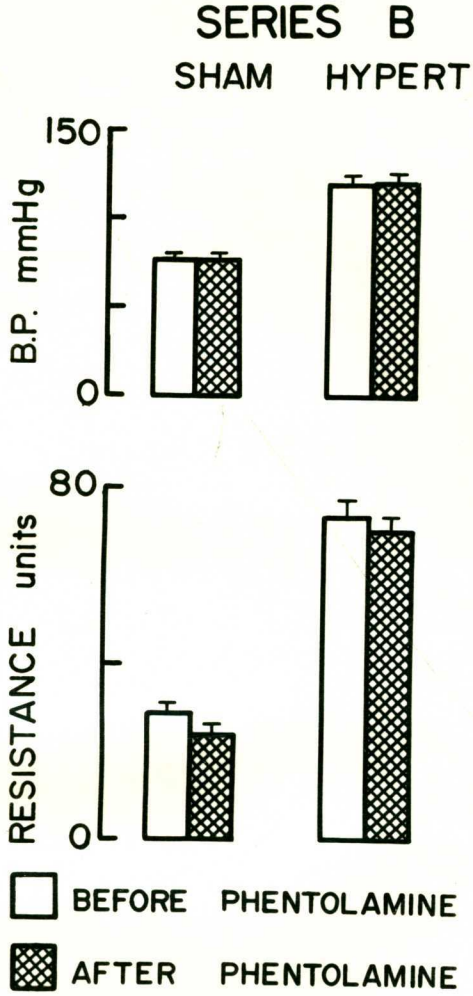

FIGURE 33

Effect of α-block with phentolamine on mean arterial pressure (BP, mmHg) and hindlimb vascular resistance (R, units) in 7 sham (LEFT) and 9 hypertensive (RIGHT) animals in series B. Symbols represent ± 1 S.E. of the mean.

During the transient vasodilatory response following guanethidine, a further small vasodilation could be induced with glyceryl trinitrate (GTN). The vasodilation with 8 µg of GTN was maximal and HVR in the hypertensive animals was greater than that in the normotensive animals in the ratio of 2.4 to 1 ($P < 0.001$) (Table 17).

Section(ii) Responses of Renal Hypertensive and Normotensive Animals to Pressor Stimuli

A number of studies have demonstrated increased vascular responsiveness in essential hypertension and in renal hypertension (Page and McCubbin, 1968; Smirk, 1970; Folkow, 1971; Doyle and Fraser, 1961). There have been few studies however where vascular sensitivity has been investigated in the 'open-loop' preparation without the influence of opposing baroreflex stimuli. In the previous chapter the total body vascular sensitivity in rabbit renal hypertension was not significantly different from control values although there was a rise during the development of hypertension. In the present section, vascular responsiveness to a variety of pressor stimuli were compared in hypertensive and normotensive animals following opening of the sympathetic effector feedback loop with guanethidine.

TABLE 17

Series B. Maximal Vasodilation of rabbit hindlimb with glyceryl trinitrate.

	SHAM OPERATED (7) *	HYPERTENSIVE (9)
R † (units)	5.8 ± 1.2 T	13.6 ± 2.6
BP (mmHg)	36.1 ± 4.3	41.8 ± 2.3
Flow (kHz)	4.4 ± 0.2	2.9 ± 0.3

* Number of animals in each group.

† R - hindlimb vascular resistance, BP - mean arterial pressure, Flow - hindlimb blood flow.

T ± 1 SE of the mean.

METHODS

After presynaptic block in the first group of normotensive and hypertensive rabbits (Series A) stimulus response curves relating the dose of noradrenaline to changes in HVR were constructed (Fig. 34).

The specificity of the altered vascular responsiveness in hypertension was examined in Series B when the experiments of Series A were repeated using angiotensin II and vasopressin as well as noradrenaline as the pressor agents (Fig. 29). The effects of α-block with phentolamine on vascular sensitivity in hypertension was examined with angiotensin II. Finally, the change in vascular sensitivity in hypertensive animals due to alteration in nonautonomic HVR was determined by constructing stimulus response curves using angiotensin and vasopressin, after the blood pressure in the hypertensive animals had been reduced to normotensive levels by bleeding.

RESULTS

Series A

The mean slope of the dose response curve using noradrenaline as the pressor agent was significantly greater in the hypertensive animals than in the normotensives in

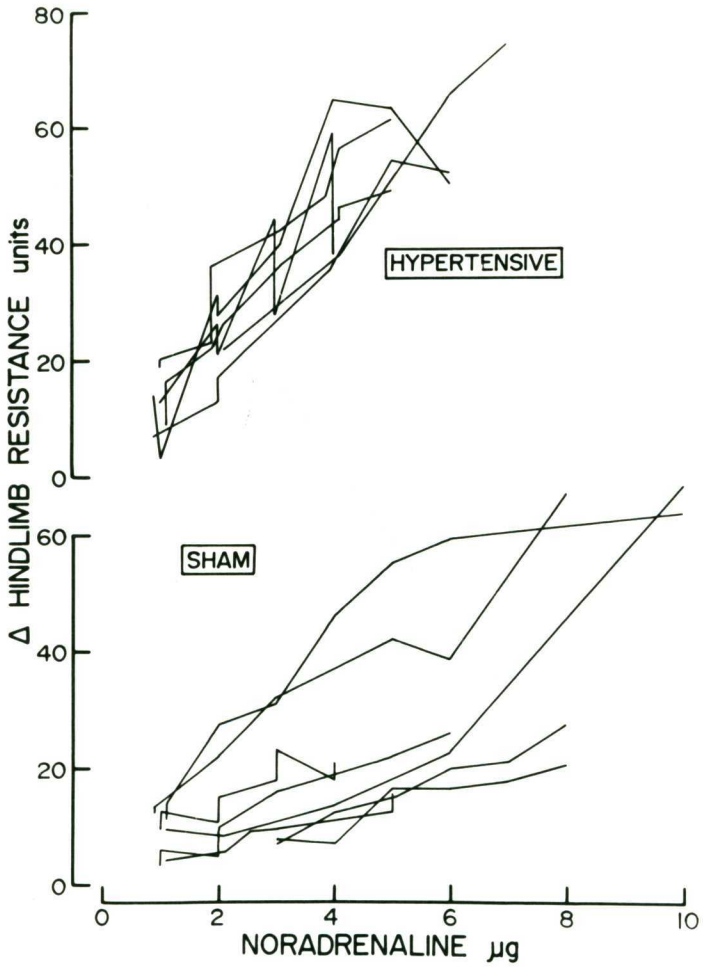

FIGURE 34

Curves relating the change in hindlimb vascular resistance to the dose of noradrenaline in 8 sham operated and 6 hypertensive animals of Series A. The change in resistance was integrated planimetrically for each dose of noradrenaline.

in the ratio of 1.9 to 1 ($P < 0.005$) (Fig. 35). The changes in HVR following a dose of 4 µg of noradrenaline was 98% greater in the hypertensive animals ($P < 0.05$) but the threshold dose of noradrenaline in both groups was close to 1 µg. After guanethidine the vascular muscle becomes hypersensitive to neurotransmitters and to exogenous catecholamines. The possibility exists that this might differentially affect the hypertensive group (see Discussion).

Series B

The slopes of the regression lines for the three pressor agents noradrenaline, angiotensin II and vasopressin were all greater in the hypertensive animals compared to the normotensive animals (1.7 to 1, 1.9 to 1 and 2.1 to 1 respectively) (Table 18). However due to the large variation between animals these results were not significantly greater in the hypertensive animals. The wide variation may be due to the single drug doses used in this series compared to duplicate ones in series A. These results suggest however that the increased vascular responsiveness to pressor drugs in renal hypertension is fairly non-specific.

After α-adrenergic block with phentolamine there was

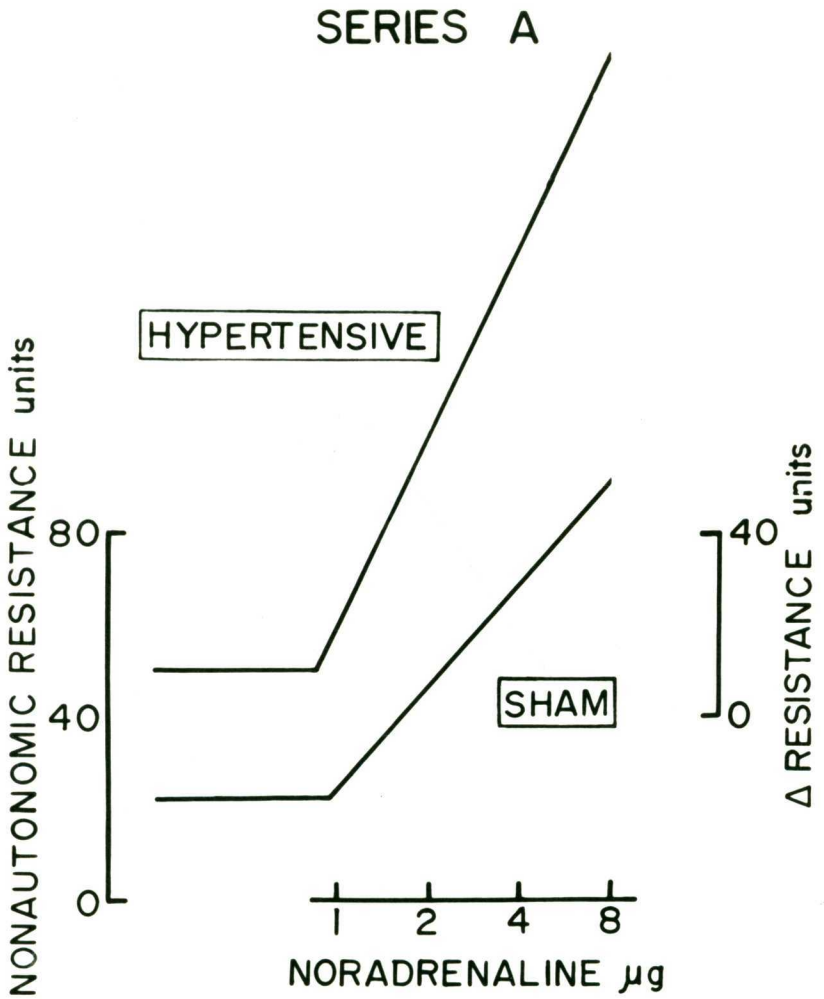

FIGURE 35

Calculated regression lines of data illustrated in Figure 34. The lines relate change in hindlimb vascular resistance (scale on right) to the dose of noradrenaline in sham operated and in hypertensive animals. The dose response curves are related to their respective nonautonomic hindlimb resistance values (scale on left).

no significant change in slope of the regression lines for angiotensin II compared to the pre-block values in either the sham or hypertensive rabbits (Table 19).

There was no significant difference in the dose response curves to angiotensin II and vasopressin of the hypertensive animals following bleeding (Table 20). Thus vascular responsiveness for angiotensin II and vasopressin was still greater in the hypertensive rabbits despite a reduction in nonautonomic HVR. The results suggest that the difference in responsiveness between hypertensive and normotensive animals is maintained over a range of values of nonautonomic resistance.

Section (iii) Responsiveness to Baroreflex Stimuli

The changes in vascular resistance induced by baroreceptor stimulation depend not only on the magnitude of sympathetic vasoconstrictor activity but also on the responsiveness of vessels to neural activity. In view of the increased sensitivity of the hindlimb vessels of presynaptically blocked hypertensive animals to noradrenaline shown in the previous section (Series A), it was of interest to compare the relative responsiveness with normotensive animals during reflex stimulation of the sympathetic constrictor nerves

TABLE 18

Parameters of dose response curves to noradrenaline in series A.

	SHAM OPERATED (8) †	HYPERTENSIVE (6)
1. Slope *	72 ± 11	137 ± 14
2. Intercept *	-69 ± 13	-127 ± 22

Parameters of dose response curves to various pressor agents in series B.

	SHAM OPERATED (6) †	HYPERTENSIVE (6)
1. Slope *		
Noradrenaline	65 ± 18	113 ± 21
Angiotensin II	61 ± 11	113 ± 24
Vasopressin	179 ± 42	359 ± 110
2. Intercept *		
Noradrenaline	-58 ± 19	-101 ± 18
Angiotensin II	-5 ± 9	4 ± 10
Vasopressin	-172 ± 53	-328 ± 112

* Slope and intercept of calculated regression line relating logarithm of dose of noradrenaline (µg), angiotensin II (µg) and vasopressin (units) to change in hindlimb vascular resistance (units).

† Number of animals in each group.

under conditions when the tissue catecholamine content had not been disturbed. This was examined in this section where the baroreflex constrictor properties of the hindlimb in normotensive and renal hypertensive rabbits were studied using the method developed for quantification of the baroreceptor heart rate reflex in Chapters 3 and 4.

METHODS

Stimulus response curves relating decreases in MAP to baroreceptor induced rises in HVR were constructed from the results of experiments in 10 normotensive and 5 hypertensive rabbits of Series A with all autonomic effectors intact. Using the previously implanted vena caval balloon, five or six balloon inflations were made at 5 minute intervals in order to produce a range (2 to 40 mmHg) of reductions in the prevailing MAP. MAP and HVR were averaged over 10-15 secs. before inflating the balloon and during the last 5-7 secs. of a 30 second inflation in a manner similar to that described for balloon inflations in Chapter 4 (Fig. 30). The duration of the inflation period was sufficient for a steady resistance response to be reached following a step change in MAP. MAP-HVR curves and their parameters were derived as described in Chapter 2.

TABLE 19

Series B. Parameters of Dose Response Curves to Angiotensin II before and after α - block with phentolamine.

	PRE SYNAPTIC BLOCK *	POST SYNAPTIC BLOCK
1. Slope		
sham operated (6) †	61 ± 11	68 ± 17
hypertensive (6)	113 ± 24	117 ± 5
2. Intercept		
sham operated	-5 ± 9	-11 ± 5
hypertensive	4 ± 10	5 ± 10

* Experiments performed after presynaptic sympathetic block with guanethidine, atropine and propranolol.

† Number of animals in each group.

TABLE 20

Series B. Parameters of Dose Response Curves to angiotensin II and to vasopressin following bleeding.

	BEFORE BLEEDING *	AFTER BLEEDING
1. Slope		
vasopressin (6) †	359 ± 110	346 ± 115
angiotensin II (6)	113 ± 24	152 ± 53
2. Intercept		
vasopressin	-328 ± 112	-352 ± 130
angiotensin II	4 ± 10	22 ± 9

* Experiments performed in 6 hypertensive animals after autonomic block (with guanethidine, atropine and propranolol) and restoration of blood pressure. After bleeding blood pressure reduced to level of normotensive animals.

† Number of animals.

RESULTS

The changes in HVR following falls in MAP were reflex in origin since autonomic block effectively prevented the constrictor responses (Fig. 30). The arterial baroreceptors probably play the major role in the response with small contributions from cardiopulmonary receptors (Korner, 1971; Dampney, Taylor and McLachlan, 1971).

The 'steady state' relationship between reductions in MAP and rises in HVR form the upper half of a sigmoid curve (Fig. 36) in agreement with the findings of previous investigators (Koch, 1931; Kendrick, Öberg and Wennergren, 1972). With caval balloon inflation HVR rose to a maximum plateau level in both normotensive and hypertensive groups. The lower half of the sigmoid curve relating the dilator response of the hindlimb bed to rises in MAP was not investigated. The value of HVR after autonomic block (Section (i)) provided an estimate of the maximal vasodilation, when all autonomic effects have been abolished but myogenic tone had become stable again. Whether or not this degree of vasodilation would have been achieved during maximal stimulation of the baroreceptors is not certain, but the non-autonomic block value of HVR probably provides a reasonable first approximation of this value.

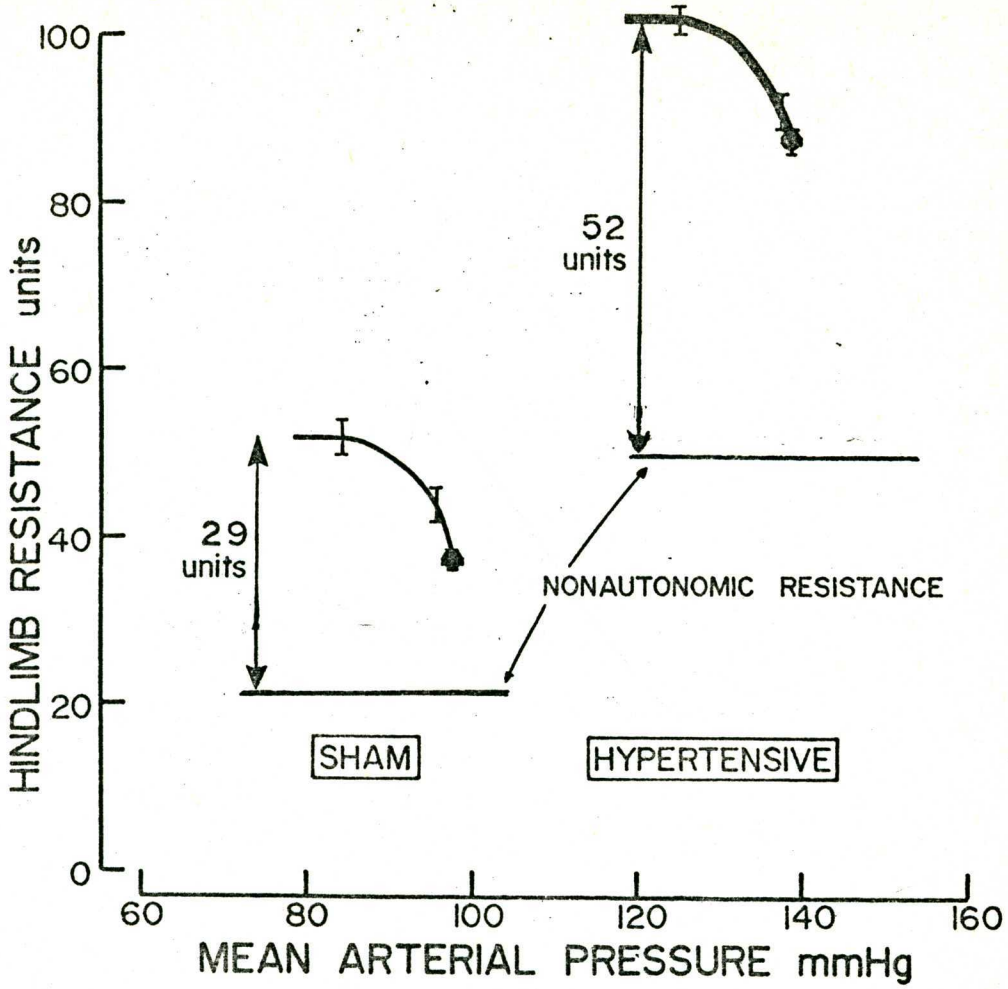

FIGURE 36

Relationship between the change in hindlimb vascular resistance and mean arterial pressure in 10 sham and 5 hypertensive animals. Curves were constructed similarly to the MAP-HP curves described in Chapter 2. The construction in the present experiment was based on responses to inflation of the vena caval balloon alone. Three parameters were derived for each curve, the pressure-dependent constrictor range (29 and 52 units respectively), the median blood pressure (mmHg), and the average gain ($\bar{G}$, units/mmHg). The values of the latter parameters are given in Table 21. The values of the nonautonomic resistance were those obtained after presynaptic block and are given in Table 16.

The constrictor range of the reflex calculated from the value of HVR after autonomic block to the plateau level at maximum vasoconstriction was greater in the hypertensive than in the sham-operated animals in the ratio of 1.8 to 1 ($P < 0.001$) while the average gain of the constrictor response in each group was about the same (Table 21).

DISCUSSION

At 4 to 5 weeks after renal wrapping there was a significant level of hypertension in both series of animals which was associated with a rise in hindlimb vascular resistance. Similar findings have been previously reported for limb vascular resistance in experimental renal hypertension in the dog and rat (Page, Kaneko, and McCubbin, 1966; Overbeck, Swindall, Cowan and Fleck, 1971; Laverty, McGregor and McQueen 1968), and in human essential hypertension (Doyle and Fraser 1961; Overbeck, Daugherty and Haddy, 1969).

There was a lower resting hindlimb blood flow in the hypertensive animal which persisted after autonomic block. Systematic differences in diameter within the transducers may have accounted for the lower flow in hypertensive animals compared to the normotensives, however there were no differences in the calibrations between the transducers of

TABLE 21

Mean resting values and parameters of MAP-HVR curves in renal hypertensive and normotensive rabbits. Series A.

	NORMOTENSIVE	HYPERTENSIVE
Number of animals	10	5
Mean arterial pressure (mmHg)	97.8 ± 6.8 *	138 ± 14.9
Hindlimb vascular resistance (units)	37 ± 6 *	88 ± 20
Hindlimb blood flow (kHz)	2.8 ± 0.4 *	1.6 ± 0.3
Constrictor range (units)	29 ± 2 †	52 ± 5
BP_{50} (mmHg) T	99 ± 1 †	143 ± 1.5
$\bar{G}$ (units/mmHg)	1.6 ± 0.1 †	2.6 ± 0.6

* SE between animals

† SE within animals. Curve parameters calculated using within animal regression equations.

T BP_{50} - median blood pressure. $\bar{G}$ - average gain between median blood pressure and -ISD. Parameters calculated as described in Chapter 2.

a group of hypertensive and normotensive animals despite small individual differences in transducer calibration (see Chapter 2). Since the calibration lines of the Doppler flow transducers were independent of pressure in the range 50 to 150 mmHg, the difference in flow was not due to differences in the resting blood pressure. Previous studies have shown that the forelimb blood flow in renal hypertension in the dog, in the early and chronic stages of hypertension, is normal (Overbeck, Swindall, Cowan and Fleck, 1971; Overbeck, 1972a). THe difference in blood flow was also present following autonomic block which suggests that cardiac failure in the hypertensive animal was not a contributory factor. The present finding of reduced blood flow in the hindlimb of the hypertensive rabbit was thus unexpected and needs further study. If this finding is confirmed, it is difficult to invoke overperfusion of at least the hindlimb vascular bed as one of the early mechanisms in the development of hypertension.

The nonautonomic component of resting HVR was estimated under two sets of conditions following autonomic block (series A and B, Section (i)). Firstly in series A, due to the prolonged fall in blood pressure which was relatively greater in the hypertensive animals, there was the possibility that vasoconstrictor substances (e.g. noradrenaline, angiotensin II

and vasopressin) were liberated and were contributing to the nonautonomic HVR. For example, it has been shown in the areflexic decapitated dog that activation of the renin-angiotensin system by hypotension could produce a 65% compensation in blood pressure within 20 minutes of the fall in blood pressure (Cowley, Miller and Guyton, 1971; Cowley and Guyton, 1972). For this reason, the blood pressure in Series B was controlled for long enough so that the effects from any substances liberated at the initial fall in pressure would have disappeared. The findings in Series B however were opposite to those expected had the renin-angiotensin system or vasopressin system been contributing to nonautonomic HVR in Series A. Since α-adrenergic block had no significant effect on HVR, circulating catecholamines were probably not involved either. The second possibility was that differences in intravascular pressure after autonomic block between Series A and Series B could have contributed to the different values of nonautonomic HVR obtained for each series. This effect was demonstrated following bleeding of the hypertensive animals in Series B where virtually the same responses as in Series A after autonomic block were reproduced. The reason why such a relatively small fall in blood pressure should influence the resistance is not clear although autoregulatory effects due to the myogenic Bayliss mechanism may be involved (Folkow and

Neil, 1971). In any event control of the blood pressure in the open-loop preparation of Series B avoided the problem. For these reasons nonautonomic HVR as estimated in Series B was considered to be more closely representative of the nonautonomic component of HVR in the resting animal.

The increase in nonautonomic resistance in the hindlimb bed of renal hypertensive rabbits is in agreement with the raised nonautonomic total peripheral resistance in renal hypertension determined in the previous chapter and in studies of human essential hypertension (Korner, Shaw, Uther, West, McRitchie and Richards, 1973). The findings of increased HVR in the hypertensives when muscle tone was completely abolished supports Folkow's hypothesis that adaptive changes can occur which result in physical hypertrophy of the walls (Folkow, 1971). This suggests that part of the increased nonautonomic HVR is due to structural narrowing of the hypertensive vessels. The role of hormonal factors and electrolyte changes in the vessel walls has not been examined in the present experiments.

The autonomic resistance component of HVR under resting conditions was the same in renal hypertensive and normotensive animals as indicated by the results of Series B following

autonomic block. This contrasts with an apparent increased autonomic component of resistance in the hypertensives compared to the normotensive animals as determined in Series A during uncontrolled conditions of blood pressure. Thus conclusions concerning the autonomic component of total peripheral resistance in renal hypertension in the previous chapter and in the human essential hypertensive (Korner, Shaw Uther, West, McRitchie and Richards, 1973) should be made with reservations since in those experiments the blood pressure was also uncontrolled after autonomic block.

An estimate of the autonomic reflex resistance effect in the intact animal may be made from the range of the baroreceptor constrictor reflex. This was increased in the hypertensive animals in the ratio of 1.8 to 1. However, the increased sensitivity of vascular smooth muscle to a given dose of neurotransmitter in hypertension (ratio of 1.9 to 1) suggests that the greater reflex constrictor range may be entirely accounted for by the altered effector 'responsiveness'. Thus in renal hypertension constrictor nerve activity is probably normal but there is a greater autonomic effect due to greater muscle reactivity.

There was an increased sensitivity of the hindlimb vessels to all pressor substances tested over a wide constrictor range which is in agreement with the findings of other workers (e.g. Doyle and Fraser, 1961; Mendlowitz, Gitlow, Wolf and Tuckman, 1965; Overbeck, 1972b). Structural or functional increase in the vessel wall to lumen ratio is probably a contributing factor (Folkow and Öberg, 1959; Sivertsson, 1970). The present experiments contrast with the absence of any significant increase in vascular sensitivity for the whole animal determined in the previous chapter. Anatomical data from Suwa and Takahashi (1971) in hypertension show there are nonuniformities in vascular resistance between the vascular beds which may account for these differences. In support of their studies, Folkow, Hallbäck, Lundgren, Sivertsson and Weis (1973) have found differences in the resistance between the hindlimb and renal beds in genetic hypertensive rats.

CONCLUSION

The major factor contributing to the rise in HVR in renal hypertensive rabbits was the nonautonomic component of the resistance, with alterations in structure participating in this rise of nonautonomic HVR. The resting autonomic

component of HVR appears to be the same in normotensive and hypertensive animals. However increased autonomic reflex effects in hypertension are probably due to increased vascular responsiveness to the neurotransmitter stimuli.

CHAPTER 8

GENERAL DISCUSSION

In this chapter the main findings of the present work are summarized briefly and related to findings in other species. The work has been principally concerned with the analysis of cardiovascular mechanisms in experimental renal hypertension in the rabbit. One part has dealt with the haemodynamic changes occurring during the development of renal hypertension following bilateral renal cellophane wrapping, but the major part of the study has examined the properties of the circulation once hypertension has become established 4 to 6 weeks after the wrapping procedure. The main finding has been that the non-autonomic component of the rise in peripheral resistance accounts entirely for the elevated blood pressure of this type of hypertension in the rabbit, and is also an important determinant of the enhanced 'responsiveness' to pressor stimuli in these animals. Quantitative comparisons have been made between hypertensive and normotensive animals of the properties of the baroreceptor-heart rate reflex and the properties of the baroreceptor-hindlimb reflex. The former appears to be definitely impaired in established renal hypertension, whilst the results with the latter suggest that a normal change in baroreflex activity

in the renal hypertensive animal will evoke an abnormally large peripheral constrictor response because of the enhanced responsiveness to pressor stimuli of the peripheral blood vessels in renal hypertension.

Development of Experimental Renal Hypertension

The haemodynamic changes occurring during the development of renal hypertension in the rabbit differ from those previously described in the dog and the rat (Ferrario Page and McCubbin, 1970; Ledingham and Pelling, 1967). In the rabbit serial studies have indicated that in the renal hypertensive animal there is little change or a slight fall in cardiac output from 10 to 33 days after bilateral cellophane wrapping of the kidneys compared with a control group of sham-operated animals. During this time total peripheral resistance and blood pressure rise steadily in the hypertensive rabbits at an even rate, and the changes appear to depend entirely on non-autonomic factors. At corresponding times following induction of renal hypertension in the dog and rat cardiac output has been definitely raised, and has shown little tendency to return to normal, whilst peripheral resistance rose progressively. Some preliminary data obtained in rabbits suggests that the cardiac output may be elevated at a time 6 to 10 days after induction of hypertension, but this point

requires further confirmation. However the present results suggest that in the rabbit the phase of elevated cardiac output is very much more transient than in the species mentioned above.

It remains to examine how the present data relates to current theory concerning the pathogenesis of renal hypertension. It has been proposed that in the development of renal hypertension the rise in total peripheral resistance is preceded by a phase of increased cardiac output (Ledingham and Cohen, 1963). Perfusion of the peripheral tissues in excess of their metabolic requirements is believed to provide the stimulus for the gradual elevation of vascular resistance through local vascular ('autoregulatory') mechanisms (Ledingham and Cohen, 1963; Ledingham and Pelling, 1967 and Guyton and Coleman, 1967). According to this view once peripheral resistance increases the cardiac output should return towards normal.

In the present study the brief period of elevated cardiac output was followed by a small gradual decline in this variable, which was not significantly different from the response observed in the sham-operated group. The data in the

rabbit is thus consistent with a return in cardiac output to 'normal' level. There is a suggestion that in the hypertensive animal cardiac output may fall somewhat below the value in the sham-operated group although the difference was not statistically significant. The results thus do not provide either unequivocal support or firm refutation of the above 'hyperperfusion' theory of the pathogenesis of renal hypertension. The results do however indicate clearly that there are at least quantitative differences in the changes in the different haemodynamic variables between different species or between the types of renal hypertension.

Whilst the rise in blood pressure was similar to that observed in the rat (Ledingham and Pelling, 1967) the blood pressure achieved in the present series was less than that obtained in hypertensive rabbits in Chapters 4 and 7 using identical methods for the induction of hypertension. It is thus possible that the use of the presynaptic blocking agent guanethidine in this series may be responsible for the reduced rise in blood pressure. A further difference was that the present work has considered the changes occurring when renal function of both kidneys is disturbed whereas the major part of the observations in the rat and dog followed unilateral renal hypertension with removal of the other kidney (Ledingham

and Pelling, 1967; Ferrario, Page and McCubbin, 1970). The entire rise in the non-autonomic component of total peripheral resistance occurs at a time when the cardiac output of the renal hypertensive and sham-operated groups behave in an absolutely identical manner (Fig. 26), suggesting that much of the resistance changes are not related to an oversupply with blood of the peripheral tissues. The 'hyperperfusion' theory of the pathogenesis of renal hypertension would thus appear to be somewhat of an oversimplification in the light of the present findings.

Established Renal Hypertension

1. Autonomic and non-autonomic components of resting peripheral resistance

The most detailed analysis of the role of non-autonomic and autonomic factors on the elevated vascular resistance in established renal hypertension has been performed in the hindlimb. Following an initial determination of blood pressure, hindlimb blood flow and vascular resistance in the resting animal, the sympathetic constrictor nerves were blocked pharmacologically with guanethidine. The circulation was then allowed to stabilize over a period of 2 hours without experimental intervention (Figs. 29, 31; series A). At that time the blood pressure was below the initial control

value in both renal hypertensive and normotensive animals, blood flow exceeded control values in both groups, whilst the hindlimb resistance was below control in both groups. In the hypertensive animal both the non-autonomic component of resistance, and the autonomic component of resistance (i.e. the difference between initial control and the value 2 hours after block) were significantly greater than in normotensives. It seemed possible that the magnitude of the non-autonomic component might be greater than its 'true' value in the intact animal since the blood pressure was less than the control values. As a result of the relative hypotension humoral factors such as adrenal catecholamines, angiotensin and vasopressin might be released and accentuate the vasoconstriction. The contribution of the catecholamines to the resistance appeared to be negligible since the resistance after guanethidine was similar to that after phentolamine.

For this reason a second study was performed (series B) where after the pharmacological blockade of the sympathetic constrictor nerves the blood pressure was restored to the initial control value in both groups by infusion of dextran. Under these conditions the magnitude of the non-autonomic component was still markedly enhanced in the renal hypertensive

animal, but the estimated autonomic resistance effect was now much smaller than before and was the same as in the sham-operated preparation. It therefore seemed that reducing the blood pressure below initial control value tended to underestimate (rather than as had been expected, to overestimate) the non-autonomic component of the hindlimb resistance, with consequent overestimation of the autonomic component. This was supported by experiments in the totally blocked preparation where the non-autonomic resistance fell significantly from its previous value when blood pressure was at control level. Whether this reduction in non-autonomic resistance effect following fall in blood pressure was due to the myogenic Bayliss mechanism or was the result of local metabolic factors has not been determined in the present study. However what the study suggests strongly is that in order to assess the magnitude of the non-autonomic resistance component in the intact animal, using the technique of autonomic block it is essential to control the 'open-loop' preparation from the point of view of initial blood pressure. Thus the main conclusion from the experiments where pressure was controlled (Figs. 29 and 32; series B) is that in established renal hypertension the non-autonomic component of the peripheral vascular resistance is raised, but the resting autonomic effect is the same as in the sham-operated

normotensive animal.

These studies are relevant to several studies performed in patients with essential hypertension where after appropriate pharmacological blockade a rise in both non-autonomic and autonomic components of the peripheral resistance was found in experiments similar to those performed in Series A (Korner, Shaw, Uther, West, McRitchie and Richards, 1973). The present study in the rabbit suggests that this conclusion is probably qualitatively correct as far as the non-autonomic resistance effect is concerned. However the magnitude of the non-autonomic component will be underestimated unless the blood pressure is maintained at the initial pre-blockade control value, assuming that the vascular responses of man resemble those of the rabbit. It is likely that these studies in man have also overestimated the magnitude of the autonomic effect, and it therefore seems worthwhile to reinvestigate this problem in patients subject to autonomic blockade before and after control of their arterial pressure in a manner analogous to that of the present study.

2. Resistance effect when hindlimb bed is fully dilated

Folkow (1971) has suggested that in any type of

established hypertension structural changes in the vascular wall contribute considerably to the raised resistance compared with that of normotensive controls. He has pictured that in accord with anatomical fact there is hypertrophy of the media, with some encroachment on the vascular lumen produced by infolding of the intima.

The present findings in the hindlimb suggest that when the limb vascular smooth muscle is fully dilated by means of glyceryl trinitrate there is still a marked difference between hypertensive and normotensive animals. These results are very much in accordance with Folkow's hypothesis. In the hindlimb the resistance ratio of the fully dilated hypertensive/normotensive limb is approximately the same as the ratio of the non-autonomic component of the two preparations when muscle tone is present (2.3 and 2.2 respectively). The atonic hypertensive/normotensive ratio in the rabbit is greater than observed by Folkow, Hallbäck, Lundgren, Sivertsson and Weiss (1973) in renal hypertensive rats (2.3 and 1.2 respectively). The reason for this difference was not obvious but the findings suggest that the reduction in vascular cross-section even in the virtually complete absence of muscle tone is important in renal hypertension. The anatomical basis of this cross-sectional

narrowing was not determined in the present study.

3. Vascular responsiveness to pressor stimuli in renal hypertension

Previous studies in man and experimental animals with both essential and renal hypertension have shown that vascular responsiveness is enhanced in hypertension (Doyle and Fraser, 1961; Page and McCubbin, 1968; Smirk, 1970). This has been examined in the present work using the guanethidine blocked preparation, where reflex changes in cardiac output have also been prevented by using atropine plus propranolol. Under these conditions reflex changes in constrictor activity are abolished (Chalmers, Korner and White, 1967b; Gaffney, Chidsey and Braunwald, 1963), though there may be enhancement of endorgan sensitivity owing to catecholamine depletion from the nerve endings by guanethidine (Vane, 1962; Trendelenburg, 1963). The importance of the latter does not account for the greater responsiveness to catecholamines observed in the renal hypertensive rabbit, since a similar enhanced responsiveness occurs under conditions of baroreflex mediated hindlimb vasoconstriction which was induced by physiological means.

The present work has extended previous studies by

demonstrating (i) that the enhanced responsiveness in renal hypertension is quite non-specific in nature, and the magnitude of hypertensive/normotensive resistance change is the same in response to sympathetic constrictor nerve stimuli, infused catecholamines, infused angiotensin and injected vasopressin (ii) that the enhanced vascular responsiveness is probably not uniform in all vascular beds since the responsiveness differs in the hindlimb and total peripheral resistance, the former showing a much greater difference in response between hypertensive and normotensive rabbits.

An additional finding of the present study is that the enhanced responsiveness of the hindlimb bed in the renal hypertensive animal is not critically related to the exact value of the non-autonomic component of the peripheral vascular resistance. Thus the ratio of hypertensive/normotensive responsiveness was about the same when the non-autonomic resistance in the hindlimb of hypertensive animals averaged 63 units and 45 units (i.e. 71% of the former) after reducing the blood pressure by means of bleeding. This again supports the view that it is not the exact muscle tone at the moment that determines the vascular responsiveness but some fundamental change in property of the vessel.

The hindlimb data is relevant only to the problem of established hypertension. In the total peripheral resistance data (Chapter 6) an attempt was made to follow the development of the increased responsiveness to catecholamines during the development of renal hypertension. However the results showed that the difference in responsiveness between hypertensive and normotensive animals were not nearly as large as in the hindlimb. Elevated responsiveness of total peripheral resistance appeared to be greatest about 2 weeks after renal wrapping, but it will be necessary to perform a longitudinal study on the alterations in responsiveness in the hindlimb and various other vascular beds.

4. Autonomic function in renal hypertension

The present findings are that there is no increase in the autonomic component of hindlimb vascular resistance in renal hypertension when the experimental conditions are carefully controlled. Assessment of an increased resting autonomic component of resistance has been made in the past under conditions when blood pressure was not controlled, as in series A of the present series of hindlimb experiments (Chapter 7). The present conclusion is in agreement with the direct observations made in patients with different types

of hypertension by Wellin, Delius and Hagbarth (1973) who showed that there was little if any alteration in resting discharge activity in the muscle and skin sympathetic constrictor nerves.

The results have also shown that the resting heart rate is the same in renal hypertensive as in normotensive animals. No detailed investigation has been made concerning the relative magnitude of vagal and cardiac sympathetic effector drives, but in a recent study from this laboratory it was observed that there was no difference between the two groups in the level of resting excitation of each effector (Korner and Oliver, unpublished data).

Two reflex pathways have been studied: the baroreceptor - heart rate reflex and the baroreceptor - hindlimb reflex. As far as the baroreceptor - heart rate reflex is concerned, the evidence suggests that reflex function is impaired in relation to the performance of normotensive control animals, and there is certainly no evidence of enhanced performance. The resetting of the pressure-dependent heart period response about a higher pressure level can be entirely explained by the general property of adaptation observed in every type of

mechanoreceptor, and to some extent this can also account for the reduction in gain in the hypertensive animal, as discussed in Chapter 4. The reduction in heart period range provides definite evidence of some impairment of autonomic effector output for equivalent changes in intravascular pressures in the hypertensive animal. This may again be at least partly accounted for by some loss of baroreceptor function (Angell James, 1973). The changes in renal hypertension are almost identical to the changes observed in patients with essential hypertension, suggesting that it is the chronic elevation of blood pressure which is primarily responsible for the alteration in reflex properties.

With so much of the change in steady-state properties of the baroreceptor - heart rate reflex dependent on the changes in function of the arterial baroreceptors it is difficult to assess from experiments such as those performed in Chapter 4 whether there is in fact any alteration in central excitability of the baroreflex pathways. Such a change in excitability would in any case be small, since it has not been large enough to alter the level of resting excitation of vagus and cardiac sympathetic with resulting affects on the resting heart rate. One possible approach to the problem of studying excitability changes in renal

hypertension has been suggested in Chapter 5 by the various ways in which the properties of the mean arterial pressure-heart period curve can be altered by (i) interactions between the baroreceptor system and other afferent inputs and (ii) baroreflex-independent effects of other afferent inputs as described in the chapter on hypoxia. If there was a systematic small change in, for example, sympathetic excitability in renal hypertension the changes in baroreflex-dependent curve parameters during hypoxia might be significantly different in the two groups of rabbits, as might the magnitude of the baroreflex - independent shifts in plateau level. This has not been studied in the present work, but the approach outlined in Chapter 5 affords a possible approach for the study of such reflex excitability in unanaesthetized animals. Recently instead of using hypoxia, the effects of clonidine on the MAP-HP curve have been compared between renal hypertensive and normotensive rabbits, and a small increase in sympathetic and vagal excitability has been demonstrated in renal hypertension (Korner and Oliver, unpublished data).

The experiments described in Chapter 7 concerning the baroreceptor - hindlimb reflex has indicated a greater constrictor response range mediated through the baroreceptor

system in the hypertensive than in the normotensive animal. This is due to the greater responsiveness of the vascular smooth muscle to constrictor stimuli. If the findings in the hindlimb applied to the other vascular beds such as the kidney the hypertensive animal would constrict its blood vessels more for a given level of autonomic constrictor nerve activity. If in addition there is a small enhancement of central sympathetic (constrictor) neuronal excitability as is suggested from studies on cerebral amine metabolism (Nakamura, Gerold and Thoenen, 1971), and from the results referred to above for the cardiac sympathetic, the impairment of receptor function in hypertension would tend to be offset to a degree. There seems however little doubt that the major factor resulting in increased constriction in established hypertension is due to alteration in the properties of the vessels themselves. This appears to be far more important than a small increase in sympathetic excitability.

CHAPTER 9

SUMMARY

Reflex function and the role of autonomic and non-autonomic factors in circulatory haemodynamics have been studied in unanaesthetized rabbits with renal hypertension which was produced by bilateral wrapping of the kidneys with cellophane. During the development of renal hypertension there is a steady rise in blood pressure and total peripheral resistance which depends on non-autonomic factors. The cardiac output is not raised above control levels from 10 to 33 days after the induction of hypertension. In established hypertension when the experimental conditions are carefully controlled there is an increased non-autonomic hindlimb resistance, however the autonomic component of hindlimb resistance is the same as in the sham-operated preparation. A significant fall in non-autonomic hindlimb resistance occurs in the blocked hypertensive animal following reduction in blood pressure with bleeding. The greater vascular resistance of the hindlimb bed in the hypertensive animal after full dilation with glyceryl trinitrate suggests the presence of narrowing of the vessel lumen independently of muscle tone after 4-6 weeks of hypertension. The open loop vascular responsiveness to pressor agents and the responsiveness to sympathetic

constrictor stimuli in the hindlimb of the hypertensive animal are both greater than that in the normotensive animal in the ratio of 1.9 to 1. The enhanced responsiveness is not uniform in all beds since the rise is less in the total vascular bed.

The steady state properties of the baroreceptor-heart rate and constrictor reflexes were assessed by the construction of S-shaped curves relating the changes in mean arterial pressure to the changes in heart period (pulse interval) or hindlimb vascular resistance. There is impairment of the baroreceptor-heart rate reflex in hypertensive compared to normotensive animals. Resetting occurs about a higher pressure which is probably due to adaptation of the baroreceptors. These changes in renal hypertension are almost identical to those in patients with essential hypertension. Arterial hypoxia was used to alter the properties of the mean arterial pressure-heart period curves. Interactions between the baroreceptor system and other afferent inputs on the one hand and baroreflex independent effects on the other contributed to the shift in curves. The study suggested a means of assessing the excitability of the central autonomic motoneurone pools. The range of the hindlimb constrictor response mediated through the baroreceptor system was greater

in hypertensive than in normotensive animals. This appears to be due to the enhanced responsiveness of the hypertensive vascular bed to constrictor stimuli.

APPENDIX
COMPUTER PROCESSING OF BARORECEPTOR HEART RATE DATA

Programmes in FORTRAN IV language were written to facilitate the computation involved in the construction of baroreceptor heart period curves from the raw data. In the first programme (Tables 22 and 23) after transferral of the raw data to punched cards (top set of figures in Table 24) the means, percentage steps and gains of several variables for each pair of balloon inflations (or drug injections) are calculated and reproduced on a set of punched cards (lower set of figures in Table 24). A multiple regression analysis was then carried out on combinations of the variables. The output from this regression program is given in Table 25 and illustrates the significance of the linear relationship between the gain (variable 8) and log ΔBP, ΔRAP and ΔPP (variables 9, 5 and 6 respectively). The programme in Tables 26 and 27 uses the above results to compute the values of the points of the MAP-HP curves and their standard errors. An example of the output is given at the bottom of Table 27.

TABLE 22

```
      $FORTRAN
      C      BAROTESTING  CALCULATIONS   PART 1    FOR HUMAN DATA
      C      PROGRAM CALCULATES MEANS, PERCENT STEPS AND GAINS FROM RAW DATA.
      C      IT THEN WRITES AND PUNCHES OUT CARDS FOR USE IN REGRESSION
      C      ANALYSIS
      C      WRITTEN BY M WEST 26/3/73.
      C      ********************************************************************
      C        DATA(      RAP,PP,BP AND HP) IS PUT INTO A 2BY4 MATRIX FOR
      C      UP AND 2 BY 4 MATRIX FOR DOWN FOR EACH SET OF BALLOON INFLATIONS
      C      CARD ARRANGEMENT IS AS FOLLOWS-IDENTIFICATION, NO OF OBSERVATIONS,
      C      DATA, END.
      C      DECLARATION OF VARIABLES
1             REAL UDATA,DDATA,MEAN,SU,SD,GD,BPLOGU,BPLOGD,SSD
2             INTEGER K,NN
      C      DECLARE DIMENSIONS
3             DIMENSION UDATA(2,4),DDATA(2,4),UP(2,4),DP(2,4)
4             DIMENSION MEAN(10,4),SU(10,4),SD(10,4),SSD(10,4)
5             DIMENSION GU(10),GD(10),CD(10),BPLOGU(10),BPLOGD(10)
6             DIMENSION STRING(80),FINIS(3)
7             DATA FINIS/1HE,1HN,1HD/
      C      READ IN DATA AND CHECK.
      C      FIRSTLY IDENTIFICATION.
10         10 READ(5,30) STRING
11            WRITE(6,35) STRING
12            DO 15 KOL=1,3
13            IF(STRING(KOL).NE.FINIS(KOL))GO TO 20
14         15 CONTINUE
15            STOP
      C      WRITE OUT IS -IDENTIFICATION, NO OF OBSERVATIONS, DATA (EACH ROW
      C      1OBSERVATION) FOLLOWED BY CALCULATIONSFOR EACH ROW,THEN WRITE OUT
      C      OF SELECTED CALCULATIONS IN 2 GROUPS 1 FOR UP AND 1 FOR DOWN.
      C      PUNCHED CARDS ARE PRODUCED FOR THE SAME WRITTEN 2 GROUPS. THESE
      C      2GROUPS SHOULD BE NUMBERED.
      C      NO OF OBSERVATIONS
16         20 READ(5,40)K
17            WRITE(6,50)K
20            WRITE(6,60)
21            WRITE(6,61)
22            DO 80N=1,K
23            NN=N
      C        DATA
24         25 READ(5,55) UDATA, DDATA
25         30 FORMAT(80A1)
26         35 FORMAT(1H1,80A1)
27         40 FORMAT(I5)
30         50 FORMAT(1HO,I5,14H  OBSERVATIONS)
31         55 FORMAT(2F5.1,6F5.0/2F5.1,6F5.0)
32         60 FORMAT(1HO,4HDATA,44X,5HMEANS,23X,5HSTEPS,20X,4HGAIN,8X,8H LOGSTEP
             1)
33         61 FORMAT(1H ,3HRAP,7X,2HPP,8X,2HBP,8X,2HHP,16X,4HRAP ,4HPP  ,2HBP,4X
             1,2HHP,12X,4H RAP,4H PP ,5H BP  ,5H HP  )
      C      CALCULATIONS ARE MEANS, STEPS FOR RAP(ABSOLUTE UNITS), PERCENT
      C      STEPS FOR PP, BP, HP AND R, GAINS FOR HP AND R STEPS AND
      C      LOGARITHMS OF BP STEPS
      C
      C      STEPS FOR RAP
34         65 MEAN(N,1)=(UDATA(1,1)+DDATA(1,1))/2.0
35            SU(N,1)=UDATA(2,1)-UDATA(1,1)
36            SD(N,1)=DDATA(2,1)-DDATA(1,1)
      C
```

TABLE 23

```
      C       STEPS FOR PP, BP AND HP
   37         DO 75J=2,4
   40            MEAN(N,J)=(UDATA(1,J)+DDATA(1,J))/2.0
   41         DO 70I=1,2
   42            UP(I,J)=UDATA(I,J)/MEAN(N,J)*100.0
   43            DP(I,J)=DDATA(I,J)/MEAN(N,J)*100.0
   44        70 CONTINUE
   45            SU(N,J)=UP(2,J)-UP(1,J)
   46           SSD(N,J)=DP(2,J)-DP(1,J)
   47        75 CONTINUE
      C       TAKE ABSOLUTE VALUES OF STEPS FOR BP AND HP
   50         SD(N,2)=SSD(N,2)
   51         SD(N,3)=ABS(SSD(N,3))
   52         SD(N,4)=ABS(SSD(N,4))
      C
      C
      C       GAINS
   53         GU(N)=SU(N,4)/SU(N,3)
   54         GD(N)=SD(N,4)/SD(N,3)
   55         BPLOGU(N)=ALOG10(SU(N,3))
   56         BPLOGD(N)=ALOG10(SD(N,3))
      C       WRITE OUT CALCULATIONS
   57         WRITE(6,125)UDATA,(MEAN(N,J),J=1,4),(SU(N,J),J=1,4),GU(N),
             1BPLOGU(N),DDATA,(SD(N,J),J=1,4),GD(N),BPLOGD(N)
   60        80 CONTINUE
      C       NOW WRITE OUT AND PUNCH THE ACCUMULATED RESULTS OF ALL N CARDS
      C       FOR UP STEPS FIRST THEN DOWN STEPS
   61        82 WRITE(6,127)
   62        85 WRITE(6,130)
   63           DO 90 I=1,NN
   64           WRITE(6,135)(MEAN(I,J),J=1,4),(SU(I,J),J=1,3),GU(I),BPLOGU(I),I
   65        90 CONTINUE
   66        95 WRITE(6,150)
   67           DO 100 I=1,NN
   70           WRITE(6,155)(MEAN(I,J),J=1,4),(SD(I,J),J=1,3),GD(I),BPLOGD(I),I
   71       100 CONTINUE
   72           PUNCH 137,STRING
   73       105 PUNCH 140
   74           DO 110 I=1,NN
   75           PUNCH 145,(MEAN(I,J),J=1,4),(SU(I,J),J=1,3),GU(I),BPLOGU(I),I
   76       110 CONTINUE
   77       115 PUNCH 160
  100           DO 120 I=1,NN
  101           PUNCH165,(MEAN(I,J),J=1,4),(SD(I,J),J=1,3),GD(I),BPLOGD(I),I
  102       120 CONTINUE
  103           GO TO 10
  104       125 FORMAT(1HO,2F5.1,4F5.0,2F6.0,6X,F4.1,F4.0,F6.1,F7.1,7X,F4.1,F4.0,2
               1F5.1,5X,F7.3,7X,F7.4/1H ,2F5.1,4F5.0,2F6.0,34X,F4.1,F4.0,2F5.1,5X,
               2F7.3,7X,F7.4)
  105       127 FORMAT(1H1,5HMEANS,50X,5HSTEPS,25X,4HGAIN,13X,7HLOGSTEP/1H ,7X,3HR
               1AP,8X,2HPP,8X,2HBP,7X,2HHP,15X,3HRAP,8X,2HPP,8X,2HBP)
  106       130 FORMAT(1H ,7HUPSTEPS)
  107       135 FORMAT(1H ,F10.1,3F10.0,10X,F10.1,F10.0,F10.1,F10.3,10X,F10.4,3X,2
               1HUP,5X,I3)
  110       137 FORMAT(80A1)
  111       140 FORMAT(7HUPSTEPS)
  112       145 FORMAT(F5.1,2F5.0,F6.0,9X,F5.1,F5.0,F5.1,F7.3,7X,F7.4,6X,2HUP,3X,I
               13)
  113       150 FORMAT(1HO,9HDOWNSTEPS)
  114       155 FORMAT(1H ,F10.1,3F10.0,10X,F10.1,F10.0,F10.1,F10.3,10X,F10.4,3X,4


               1HDOWN,3X,I3)
  115       160 FORMAT(9HDOWNSTEPS)
  116       165 FORMAT(F5.1,2F5.0,F6.0,9X,F5.1,F5.0,F5.1,F7.3,7X,F7.4,6X,5HDOWN ,I
               13)
  117           END
           $ENTRY

OBJECT PROG          969
DATA STORAGE         332
UNUSED CORE         9641
SYMBOL TABLE         352
```

TABLE 24

13A CONT

5 OBSERVATIONS

DATA RAP	PP	BP	HP	MEANS RAP	PP	BP	HP	STEPS RAP	PP	BP	HP	GAIN	LOGSTEP
2.0	3.5	27.	32.	83.	92.	250.	290.	2.0	30.	86.0	262.5	1.5	17.	10.5	15.2	1.456	1.0197
2.0	-0.5	33.	25.	89.	80.	275.	227.					-2.5	-27.	10.5	18.3	1.747	1.0197
5.0	6.5	25.	30.	86.	102.	300.	325.	3.8	25.	85.5	282.5	1.5	20.	18.7	8.8	0.473	1.2722
2.5	-0.0	25.	20.	85.	81.	265.	230.					-2.5	-20.	4.7	12.4	2.648	0.6701
1.5	2.6	25.	35.	85.	91.	260.	280.	1.0	29.	87.0	272.5	0.5	35.	6.9	7.3	1.064	0.8386
0.5	-1.0	32.	23.	89.	81.	285.	230.					-1.5	-32.	9.2	20.2	2.195	0.9636
1.0	5.0	30.	35.	85.	102.	255.	300.	1.5	29.	84.5	260.0	4.0	17.	20.1	17.3	0.860	1.3036
2.0	-0.5	28.	23.	84.	80.	265.	225.					-2.5	-17.	4.7	15.4	3.250	0.6752
4.0	5.0	30.	40.	93.	106.	260.	305.	3.5	33.	91.5	260.0	1.0	31.	14.2	17.3	1.218	1.1525
3.0	0.5	35.	30.	90.	85.	260.	225.					-2.5	-15.	5.5	13.5	2.463	0.7375

MEANS	RAP	PP	BP	HP	STEPS RAP	PP	BP	GAIN	LOGSTEP		
UPSTEPS											
	2.0	30.	86.	263.	1.5	17.	10.5	1.456	1.0197	UP	1
	3.8	25.	86.	283.	1.5	20.	18.7	0.473	1.2722	UP	2
	1.0	29.	87.	273.	0.5	35.	6.9	1.064	0.8386	UP	3
	1.5	29.	85.	260.	4.0	17.	20.1	0.860	1.3036	UP	4
	3.5	33.	92.	260.	1.0	31.	14.2	1.218	1.1525	UP	5
DOWNSTEPS											
	2.0	30.	86.	263.	-2.5	-27.	10.5	1.747	1.0197	DOWN	1
	3.8	25.	86.	283.	-2.5	-20.	4.7	2.648	0.6701	DOWN	2
	1.0	29.	87.	273.	-1.5	-32.	9.2	2.195	0.9636	DOWN	3
	1.5	29.	85.	260.	-2.5	-17.	4.7	3.250	0.6752	DOWN	4
	3.5	33.	92.	260.	-2.5	-15.	5.5	2.463	0.7375	DOWN	5

TABLE 25

COMPUTE,SIVE CONTROL DOWN
DD MATRIX AND SELECTIONS 51 OBSERVATIONS 9 VARIABLES

MATRIX OF SUMS OF CROSS PRODUCTS

140.8016								
-127.9295	1649.1764							
-471.5627	1151.1764	4712.5094						
408.4627	1161.8233	-3543.5094	21068.5056					
-11.9745	35.1471	61.9804	-243.9804	30.5392				
60.4764	590.9412	896.9412	404.0586	167.1176	5798.3522			
-18.7826	-223.5646	-92.9980	-1363.9018	-0.5539	-1136.1116	986.2203		
5.3769	8.8163	10.5013	347.4926	-10.0371	6.0842	-97.2508	23.5584	
-1.7924	-8.9093	-6.4452	-47.9026	0.3951	-43.4361	38.2151	-4.0494	

CORRELATION MATRIX

1.0000								
-0.2655	1.0000							
-0.5789	0.4129	1.0000						
0.2325	0.1971	-0.3556	1.0000					
-0.1826	0.1566	0.1634	-0.3042	1.0000				
0.0669	0.1911	0.1716	0.0366	0.3971	1.0000			
-0.0504	-0.1753	-0.0431	-0.2992	-0.0032	-0.4751	1.0000		
0.0934	0.0447	0.0315	0.4932	-0.3742	0.0165	-0.6380	1.0000	
-0.1199	-0.1741	-0.0745	-0.2620	0.0567	-0.4528	0.9659	-0.6622	1.0000

MULTIPLE REGRESSION

SELECTION..... 2

REGRESSION 8 ON 9,5,6 NEXT

VARIABLE NO.	MEAN	STANDARD DEVIATION	CORRELATION X VS Y	REGRESSION COEFFICIENT	STD. ERROR OF REG.COEF.	COMPUTED T VALUE
9	0.97714	0.17816	-0.66225	-2.89731	0.42448	-6.82557
5	-1.13725	0.78153	-0.37420	-0.21152	0.09401	-2.24993
6	-23.41176	10.76880	0.01646	-0.01456	0.00764	-1.90566
DEPENDENT						
8	1.82106	0.68642				

INTERCEPT 4.07075

MULTIPLE CORRELATION 0.76444

STD. ERROR OF ESTIMATE 0.45643

ANALYSIS OF VARIANCE FOR THE REGRESSION

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F VALUE
ATTRIBUTABLE TO REGRESSION	3	13.76680	4.58893	22.02703
DEVIATION FROM REGRESSION	47	9.79160	0.20833	
TOTAL	50	23.55840		

TABLE 26

```
      $FORTRAN
      C       PROGRAM TO CALCULATE GAIN AND HP WITH ERRORS
      C       FOR CONSTRUCTION OF BARORECEPTOR CURVES
      C       ABSOLUTE HP IS CALCULATED FOR VARIOUS BP STEPS USING REGRE
      C       COEFFICIENTS FROM RELATION BETWEEN G AND LOG BP
      C       ERRORS ARE CALCULATED USING THE STANDARD STATISTICAL
      C       FORMULA FOR THE SAMPLE STANDARD DEVIATION OF G AS AN ESTIM
      C       POPULATION LINE .THE ERROR IN G IS THEN CONVERTED TO ABSOL
      C       UNITS OF HP
      C       PROGRAM WRITTEN BY M WEST 30/3/73
      C       ************************************************************
      C
      C       DECLARATION OF VARIABLES
1             REAL A,B,HPR,BPR,VAR,EANLOG,SSQ,EHPR
2             REAL G,HPPC,BPPC,BPABS,HPABS,EG,EHPAB,OBS
3             INTEGER I
4             DIMENSION STRING(20),DOWN(4),FINIS(3)
5             DIMENSION BPPC(8),BPLOG(8)
      C       READ IN DATA AND CHECK
      C       IDENTIFICATION WITH UP OR DOWN STARTING IN COLUMN 15
6          10 READ(5,100)STRING
7             WRITE(6,101)STRING
10            DATA FINIS/1HE,1HN,1HD/
11            DO 20 KOL=1,3
12            IF(STRING(KOL).NE.FINIS(KOL))GO TO 30
13         20 CONTINUE
14            STOP
      C       REGRESSION CONSTANTS A AND B
15         30 READ(5,103)A,B
16            WRITE(6,104)A,B
      C       RESTING BP AND HP
17            READ(5,105)HPR,BPR
20            WRITE(6,106)HPR,BPR
      C       ERRORS
21            READ(5,107)VAR,OBS,EANLOG,SSQ,EHPR
22            WRITE(6,108)VAR,OBS,EANLOG,SSQ,EHPR
23        100 FORMAT(20A1)
24        101 FORMAT(1H1,20A1)
25        103 FORMAT(2F10.5)
26        104 FORMAT(1HC,2F10.5)
27        105 FORMAT(2F10.1)
30        106 FORMAT(1H ,2F10.1)
31        107 FORMAT(F10.5,F10.0,F10.5,F10.4,F10.2)
32        108 FORMAT(1H ,F10.5,F10.0,F10.5,F10.4,F10.2)
33            WRITE(6,110)
34        110 FORMAT(1HO,5H STEP,5X,5H GAIN,5X,5H  SEG,5X,5HHPABS,3X,7HS
             1X,5HBPABS)
35            DATA BPPC/2.5,5.0,7.5,10.0,15.0,20.0,25.0,30.0/
36            DATA BPLOG/0.3979,0.699,0.875,1.0,1.1761,1.301,1.3979,1.47
37            DATA DOWN/1HD,1HO,1HW,1HN/
      C       TEST FOR UP OR DOWN
40        112 DO 115 KOL=15,18
41            KKOL=KOL-14
42            IF(STRING(KOL).NE.DOWN(KKOL)) GOTO 120
43        115 CONTINUE
44            GOTO 160
      C       CALCULATE GAIN ETC FOR EACH STEP UP
45        120 DO 150 I=1,8
46            G=A+B*BPLOG(I)
47            HPPC=G*BPPC(I)
```

TABLE 27

```
      50        130 HPABS=(100.0+HPPC)/100.0*HPR
      51        140 BPABS=(100.0+BPPC(I))/100.0*BPR
                C     ERRORS FOR UP
      52            EG=SQRT(VAR*(1.0/OBS+(BPLOG(I)-EANLOG)**2/SSQ))
      53            EHPAB=SQRT((EG*BPPC(I)*HPR/100.0)**2+EHPR**2)
                C     PRINT RESULTS
      54            WRITE(6,220)BPPC(I),G,EG,HPABS,EHPAB,BPABS
      55         150 CONTINUE
      56            GOTO 10
                C     CALCULATE GAIN ETC FOR EACH STEP DOWN
      57        160 DO 200 I=1,8
      60            G=A+B*BPLOG(I)
      61            HPPC=G*BPPC(I)
      62        170 HPABS=(100.0-HPPC)/100.0*HPR
      63        180 BPABS=(100.0-BPPC(I))/100.0*BPR
                C     ERRORS FOR DOWN
      64            EG=SQRT(VAR*(1.0/OBS+(BPLOG(I)-EANLOG)**2/SSQ))
      65            EHPAB=SQRT((EG*BPPC(I)*HPR/100.0)**2+EHPR**2)
                C     PRINT RESULTS
      66            WRITE(6,220)BPPC(I),G,EG,HPABS,EHPAB,BPABS
      67        200 CONTINUE
      70        220 FORMAT(1H ,F5.1,2F10.3,3F10.1)
      71            GO TO 10
      72            END
                $ENTRY

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UNUSED CORE          10369
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NORM CONT      UP

   4.23268  -2.27887
     258.4      95.6
   0.53029       51.   1.10845    2.2932      2.87
 STEP       GAIN        SEG       HPABS    SEHPABS      BPABS
  2.5      3.326      0.357       279.9        3.7       98.0
  5.0      2.640      0.222       292.5        4.1      100.4
  7.5      2.239      0.152       301.8        4.1      102.8
 10.0      1.954      0.115       308.9        4.1      105.2
 15.0      1.553      0.107       318.6        5.0      109.9
 20.0      1.268      0.138       323.9        7.7      114.7
 25.0      1.047      0.173       326.0       11.5      119.5
 30.0      0.867      0.205       325.6       16.1      124.3
```

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