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Studies on renal vasomotion

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1. The present investigation was made on the left kidney of the dog. The animals were anaesthetized intravenously with pentobarbitone (30 mg/kg) and the kidneys were perfused with saline at room temperature (20°–22° C). The renal innervation was untouched.

2. Stimulation of the left splanchnic major nerve at T10–T12, and of the renal nerves, consistently caused renal vasoconstriction.

3. Repeated stimulation of both supradiaphragmatic vagi failed to induce any vasomotion in the kidney.

4. The vasoconstrictor effect was not blocked by either nicotine or hexamethonium even in enormous doses (30,000 μg). This may indicate that renal ganglia do not exist, for these ganglion blockers would prevent transmission across the ganglia.

5. Kidney perfusate, re-injected into the kidney after vasoconstriction induced by stimulation of the renal nerves, brought about a notable reduction in outflow. This effect was not observed when perfusate from a non-stimulated kidney was used. This points to the release of a vasoconstrictor substance after nervous stimulation.

6. Acetylcholine (ACh) in concentrations ranging from 0.001 μg/ml. caused a reduction in renal outflow. Thresholds were extremely variable. Higher concentrations of ACh (100–1,000 μg/ml.) often induced vasodilatation. The vasoconstrictor effect of ACh was not blocked by atropine.

7. Nicotine and hexamethonium (10,000–30,000 μg) induced blockade which elevated the threshold for ACh to values of 1,000 μg/ml.

8. Noradrenaline (0.0001 μg/ml.) induced a strong renal vasoconstriction.

9. Hydergine (5–10 ml. solutions in concentrations ranging from 15 to 30 μg/ml.) blocked the renal response to nerve stimulation. This suggests that the nature of the renal innervation is adrenergic.

10. In diseased kidneys which show reduction of the lumen of the arterioles, the thresholds for ACh, nicotine and noradrenaline are greatly increased, which might explain why we failed to show any effect of these drugs on renal vasomotion in several kidneys, many of which were not examined histologically.

11. The collision technique was applied in an attempt to discover the nature of the fibres activated by ACh. It was found that ACh greatly reduced the size of the action potentials generated by splanchnic stimulation. This would seem to indicate that these impulses are conducted antidromically by sympathetic postganglionic fibres.

12. These findings are discussed in relation to the hypothesis that the renal innervation is chiefly adrenergic and that ACh acts as a sympathetic transmitter, liberating noradrenaline, and that this effect is blocked at postganglionic endings, or at some structure intervening between adrenergic nerve endings and the effector cells, or at sensory nerve endings.

Revision of the literature leads to the conclusion that the work performed so far on renal innervation has not yet thrown a clear light on the nature of the nervous supply to the kidneys. In the anatomical field, the work of Mitchell (1950b), De Muylder (1962) and Stöhr (1952) shows that the kidney receives contributions from widespread sources, both sympathetic and parasympathetic, and vagal fibres are known to pass on to the coeliac plexus, which gives rise to renal branches. From a physiological point of view, the results of Kaplan, West & Fomon (1953), Yamagishi & Azuma (1963), and Page & McCubbin (1953) contribute to the hypothesis that the control of the kidney is predominantly sympathetic, but they do not exclude the presence of vasodilator fibres. Moreover, the renal mechanism of action of transmitter substances has not been established, and we therefore considered it of great interest to undertake the study of the effect of the autonomic nervous system and of the renal nerves on renal outflow, and to investigate the mechanism by which renal responses to nerve stimulation are brought about.

It has become evident that acetylcholine (ACh) has a sympathetic effect on several organs (Hoffmann, Hoffmann, Middleton & Talesnik, 1945; Thompson, 1958; Burn, Rand & Thompson, 1959; Douglas & Ritchie, 1960; Blakeley, Brown & Ferry, 1963; Ferry, 1963; and Aström, Crafoord & Samelius-Broberg, 1964); and this drug was therefore used in an attempt to analyse the vasomotor response of the kidney.

Methods

Experiments were carried out on fifty-eight mongrel dogs of either sex, weighing between 5 and 25 kg. The dogs were anaesthetized intravenously with sodium pentobarbital (30 mg/kg body weight). The left kidneys of the animals were approached through a midline incision and the renal artery and vein were cannulated as close as possible to their respective origins from aorta and vena cava with polythene cannulae. The nervous supply was untouched.

The renal artery was perfused with saline of the following composition (mM): NaCl 136.8; glucose 5.5; KCl 2.7; $NaHCO_3$ 11.9; $MgCl_2.6H_2O$ 4.9; $CaCl_2$ 1.8; NaH_2PO_4 0.4; at room temperature (20°–22° C) at pH 7.4 and bubbled continuously with O_2.

Perfusion was carried out by means of a Mariotte bottle at constant pressure, which was usually adjusted between 60 and 100 mm Hg. The bottle and the arterial cannula were connected by a rubber tube. The rate of renal venous outflow was recorded on a kymograph by means of a drop interval recorder, a Palmer rheotachograph.

All drugs were given in saline into the renal artery at a distance of 7 cm from the kidney through the arterial cannula. Perfusion was continued during injection

of the drugs, which were always administered in the same volume of saline and at the same speed (1 ml. in 30 sec) to avoid mechanical artefacts. The records were controlled each time with equal volumes of saline. All concentrations indicated in the paper refer to the amount injected into the perfusion fluid of the kidney, and the final concentration in the kidney itself is unknown to us.

The following drugs were used: acetylcholine chloride (Merck); nicotine tartrate (Hopkin and Williams Ltd.); hydergine (Sandoz, a preparation of dihydrocornine, dihydroergocristine and dihydroergokryptine); and atropine sulphate. Fresh solutions were prepared daily from the stock solutions.

In another group of animals experiments were made on the isolated left kidney, which was placed in a shielded chamber, and also perfused with saline under the same conditions as in the previous experiments. The left splanchnic major nerve was isolated after thoracotomy performed under artificial respiration and stimulated between T10 and T12 with square pulses of 3.5 V intensity and 5 msec duration at a rate of 10 c/s by means of a Grass S-4 electronic stimulator through an isolation unit using a pair of Ag-AgCl electrodes. Both supradiaphragmatic vagi and the renal nerves were also isolated and stimulated in the same way.

Renal venous outflow was measured by means of a photoelectric flowmeter which has a linear scale of greater sensitivity than the Palmer rheotachograph. Records of venous flow were made by a Grass Model 5E polygraph amplifier and

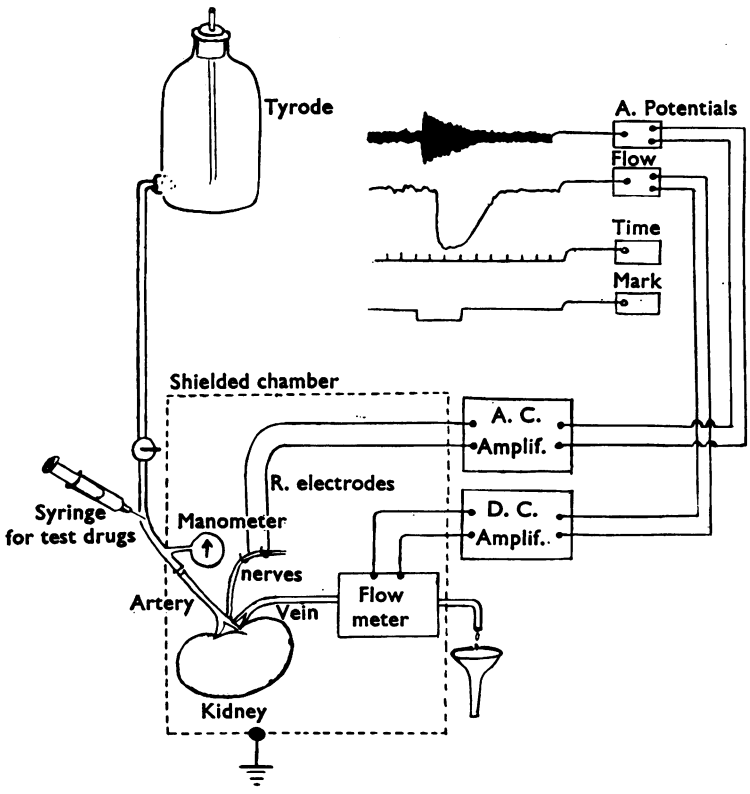

FIG. 1. Diagram illustrating the method and recording unit employed.

displayed on a kymograph. Nerve action potentials were recorded by electrodes, led to a 5-P3B a.c. pre-amplifier and driver amplifier and displayed on a kymograph through a Grass electromagnetic oscillograph (Fig. 1).

In this group of experiments the drugs were also given in saline into the renal artery at a distance of 20 cm from the kidney with the object of obtaining more effective homogenization with the saline; the volume injected was increased to 5 ml. and the duration of the injection was increased to 1 or 2 min.

A third group of experiments was made on two dogs and two cats anaesthetized intravenously with a mixture of sodium pentobarbital (15 mg/kg) and chloralose (40 mg/kg). The left splanchnic major nerve was stimulated through a Grass Model S-4 stimulator with pulses of 0.1 sec duration at a rate of 3/sec, and at the same time 50–200 μg ACh was injected into the renal artery to investigate the possibility of collision, using the technique described by Douglas & Ritchie (1957).

The following technique was used. The abdominal cavity was opened by a midline incision and the left kidney exposed; the renal nerves were then carefully isolated. The abdominal cavity was filled with paraffin oil and recording electrodes were placed over one of the renal nerve trunks which course along the renal artery, at a distance of 1 cm from the kidney. ACh solution was injected into the renal artery through a fine needle connected to a thin polythene cannula and positioned in such a way that injections could be made without interrupting the blood supply to the kidney.

The left splanchnic major nerve was reached by opening a breach in the left diaphragm under artificial respiration. A bipolar electrode was used for stimulating and the nerve was isolated from its surroundings by using cotton wool soaked in paraffin oil.

Action potentials were recorded by means of a Grass Model 5-P3B AC pre-amplifier and displayed on a two-channel Cossor oscillograph.

All these experiments were performed on the left kidney *in situ*.

In eight experiments using noradrenaline, arteriolar diameters were measured using the following technique. Immediately after the experiment, the kidney was injected arterially with 10% formalin. Five minutes after the injection of the fixative, the kidney was cut into small pieces with razor blades and the pieces were soaked in formalin for 24 hr, then sectioned, and stained with haematoxylin and eosin. The sections were then examined in a Leitz histologic section projector and the arteriolar diameters were measured directly from the projector screen.

Results

Effects of splanchnic nerve stimulation on renal outflow

Stimulation of the left splanchnic major nerve at T10–T12 induced a notable vasoconstriction.

This finding is in accordance with the results of several authors (Goodwin, Sloan & Scott, 1949; Kaplan *et al.*, 1953; Yamagishi & Azuma, 1963). Stimulation of isolated renal nerves induced vasoconstriction (Block, Wakim & Mann, 1952), but only at a higher voltage due to partial short-circuit of the current by surrounding fluid; furthermore, it is presumed that only a part of the renal vascular bed was affected.

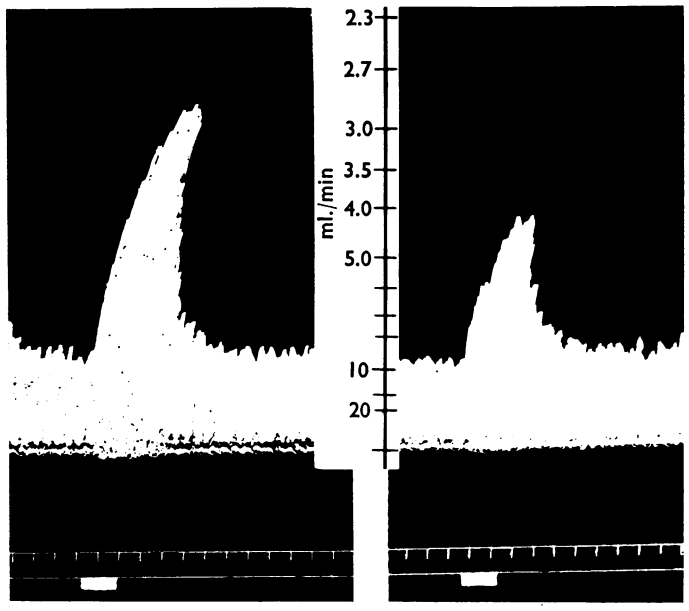

FIG. 2. Dog, pentobarbitone. Saline. Perfused left kidney *in situ*. Left: Effect on renal venous outflow of stimulation of a renal nerve. Right: Effect on renal venous outflow of the stimulation of same nerve 5 min after the injection of 30 mg of hexamethonium into the perfusion fluid. Time, 30 sec. The duration of the stimulation is signalled on bottom trace.

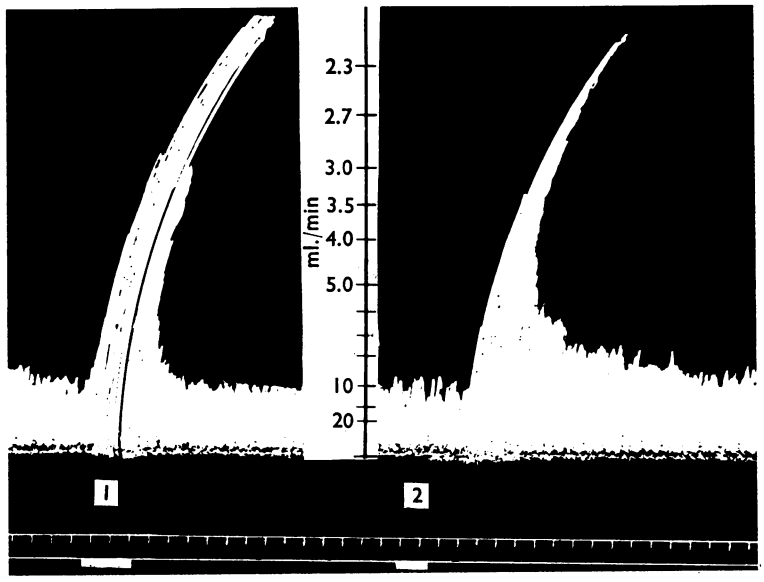

FIG. 3. Dog, pentobarbitone. Saline. Perfused left kidney *in situ*. (1) Effect on renal venous outflow of the stimulation of a renal nerve. (2) Effect on venous outflow of the injection of 1 ml. kidney perfusate into the perfusion fluid. This perfusate was collected during the preceding stimulation of the renal nerve. Time, 30 sec. Bottom trace, duration of injections.

Stimulation of the left supradiaphragmatic vagus nerve failed to show any disturbance of the renal circulation.

The vasoconstrictor effect of the stimulation of the splanchnic nerves and of the renal nerves was not blocked by either nicotine or hexamethonium even in enormous doses (30,000 μg). Only a slight reduction was occasionally found (Fig. 2).

On re-injecting into the kidney the perfusate obtained during stimulation of the renal nerves, a strong vasoconstrictor effect was repeatedly observed (Fig. 3). This finding points to the release of a vasoconstrictor substance by the kidney, due to stimulation of the nerves. A clear demonstration of the existence of this substance was obtained by the following experiment: kidney perfusate, re-injected after a control period during which the nerves were not stimulated, had no effect on the outflow ; whereas the perfusate re-injected after stimulation had induced vasoconstriction brought about a notable reduction in outflow. A control injection of saline had no effect (Fig. 4).

Effect of acetylcholine on renal outflow

Numerous papers have been concerned with the role of acetylcholine-like substances in the sympathetic innervation of several organs. Experiments were therefore carried out to determine whether ACh might have a similar effect on kidney innervation. The effect of ACh on renal venous outflow is seen in Fig. 5.

The lowest threshold for ACh was 0.0001 μg/ml., although most kidneys responded only to doses between 0.001 μg/ml. and 0.01 μg/ml. The strongest

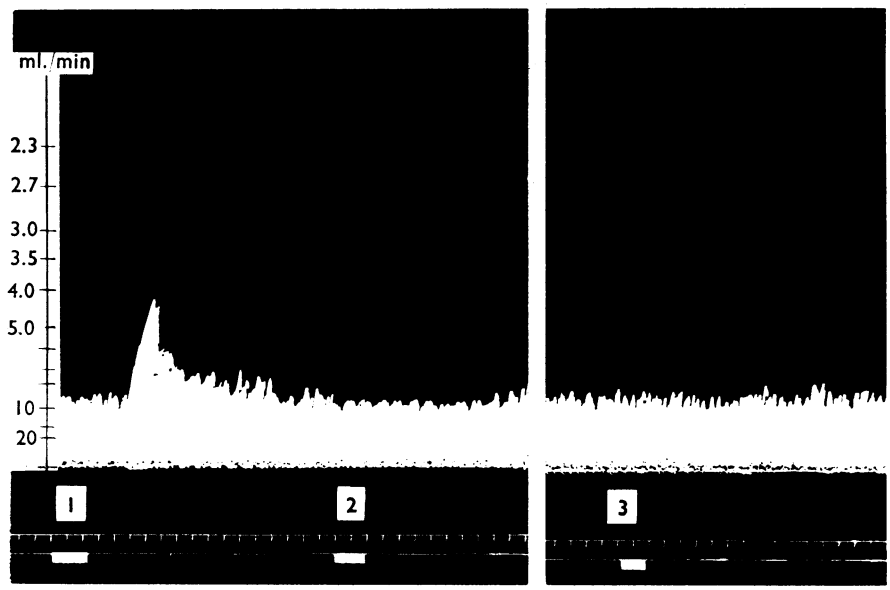

FIG. 4. Dog, pentobarbitone. Saline. Perfused left kidney *in situ*. (1) Effect on renal venous outflow of the injection of 1 ml. kidney perfusate collected during stimulation of a renal nerve. (2) Effect on venous outflow of the injection of 1 ml. kidney perfusate collected during a control period in which the nerve was not stimulated. (3) Effect on venous outflow of the injection of 1 ml. of saline into the perfusion fluid. Time, 30 sec. The duration of the injections is signalled on bottom trace.

concentration, 0.1 μg/ml., reduced kidney venous outflow from 8.6 ml./min to 5.5 ml./min, a 37% reduction; and at 0.0001 μg/ml. ACh induced a barely perceptible vasoconstriction.

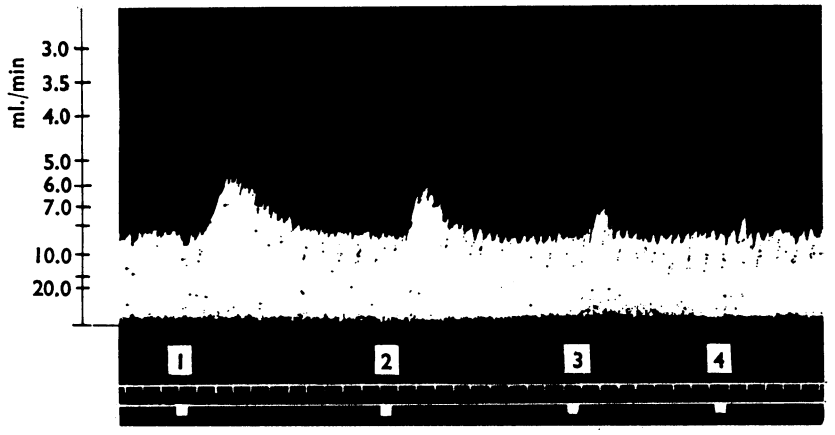

FIG. 5. Dog, pentobarbitone. Saline. Perfused left kidney *in situ*. The effect of decreasing doses of acetylcholine on renal venous outflow. Effect on renal venous outflow of (1) 0.1 μg ACh; (2) 0.01 μg ACh; (3) 0.001 μg ACh; (4) 0.0001 μg ACh. Time, 30 sec. The duration of the injection is signalled on bottom trace.

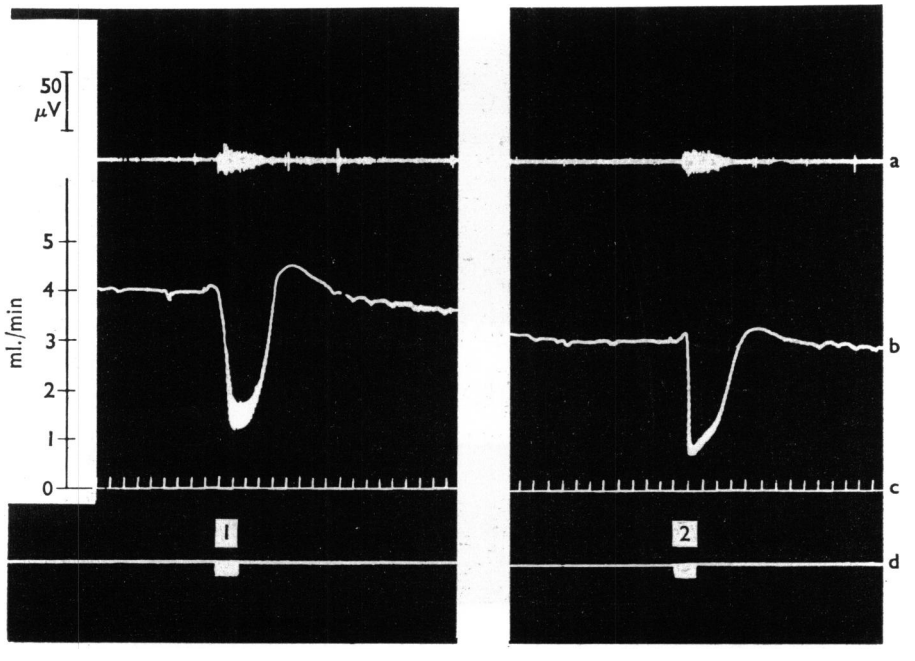

FIG. 6. Dog, pentobarbitone. Saline. Perfused isolated left kidney. The effect of acetylcholine on venous outflow before and after injection of atropine. a, Record of activity of an isolated renal nerve bundle; b, record of venous outflow; c, time trace, 30 sec; d, trace indicating duration of injections. (1) Response of venous outflow to ACh 10 μg/ml. (2) Response of venous outflow to ACh 10 μg/ml. after injection of 500 μg of atropine.

These thresholds were very variable and we did not find two kidneys which responded in a similar degree to a same concentration of ACh. The vasoconstrictor effect of ACh was uninfluenced by atropine. This may be seen in Fig. 6. At (1) the effect of ACh 10 μg/ml. is to produce an evident reduction in outflow ; between (1) and (2) 500 μg atropine was injected and 2 min later at (2) another dose of ACh 10 μg/ml. induced the same degree of vasoconstriction as before atropine.

We are certain that atropine is adequately picked up by the kidney following a single injection. In eleven experiments we found that atropine induced a vasoconstrictor effect within 30–60 sec. We did not investigate this effect any further, but it will be included in another paper.

A few ostensibly healthy kidneys showed no alteration in their outflow after ACh at 1 μg, 10 μg and 50 μg/ml., nor did they respond to other drugs, such as nicotine and noradrenaline.

Higher concentrations of ACh (100–1,000 μg/ml.) often induced vasodilatation.

Some authors (De Muylder, 1952 ; Page & McCubbin, 1953 ; and Mitchell, 1950a) indicate the existence of intrarenal ganglia. To investigate whether the

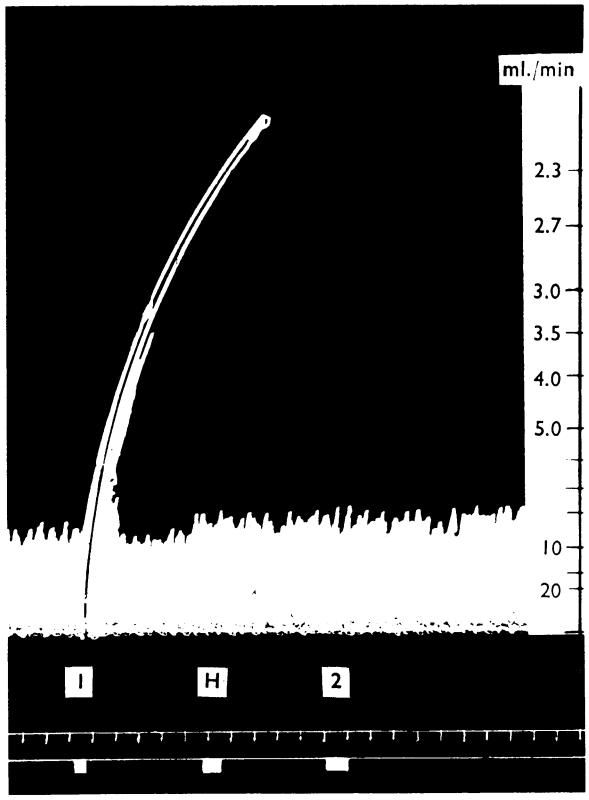

FIG. 7. Dog, pentobarbitone. Saline. Perfusion of the isolated left kidney. Effect of the injection of 300 μg hydergine into the perfusion fluid, on the nervous activity of the kidney. (1) Stimulation of a renal nerve and its effect on venous outflow ; (H) injection of 300 μg hydergine into the perfusion fluid ; (2) stimulation of the same nerve had no effect on venous outflow. Time, 30 sec. The duration of the stimulation and of the injection is indicated on bottom trace.

mode of action of ACh might be stimulation of intrarenal ganglia which would then liberate noradrenaline, nicotine was used in concentrations strong enough to block autonomic ganglia (30,000 μg). Small doses of nicotine (1–100 μg/ml.) induced renal vasoconstriction through stimulation either of ganglia or of post-ganglionic fibres. We are inclined to consider the second possibility more likely, because stimulation of the left splanchnic major nerve induced intense vasoconstriction which was not blocked by nicotine (30,000 μg) or by hexamethonium (30,000 μg) injected into the renal artery. Nicotine (2,000 μg) induced complete blockade of the effect of ACh, so that this substance in concentrations of 10 μg/ml. and 100 μg/ml. had no effect whatsoever on outflow: ACh 1,000 μg/ml. was necessary to show a minimal vasoconstrictor effect.

This blocking effect of nicotine, however, was never permanent: usually after 5 or 10 min ACh induced vasoconstriction in concentrations less than 10 μg/ml. and even less than 1 μg/ml.

Noradrenaline (0.0001 μg/ml.) induced strong vasoconstriction in every kidney which responded to ACh and to nicotine.

These experiments allow us to presume that renal innervation is adrenergic. This hypothesis was also tested in the following way. Vasoconstriction was induced by renal nerve stimulation: hydergine (150–300 μg/ml.) was then given: and on repeating stimulation no effect was obtained (Fig. 7). Adrenergic blocking agents cannot, however, be used to distinguish conclusively between adrenergic and cholinergic nerves (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1963) and to conclude that renal innervation is adrenergic the release of noradrenaline from the kidney must be shown, and this has yet to be done.

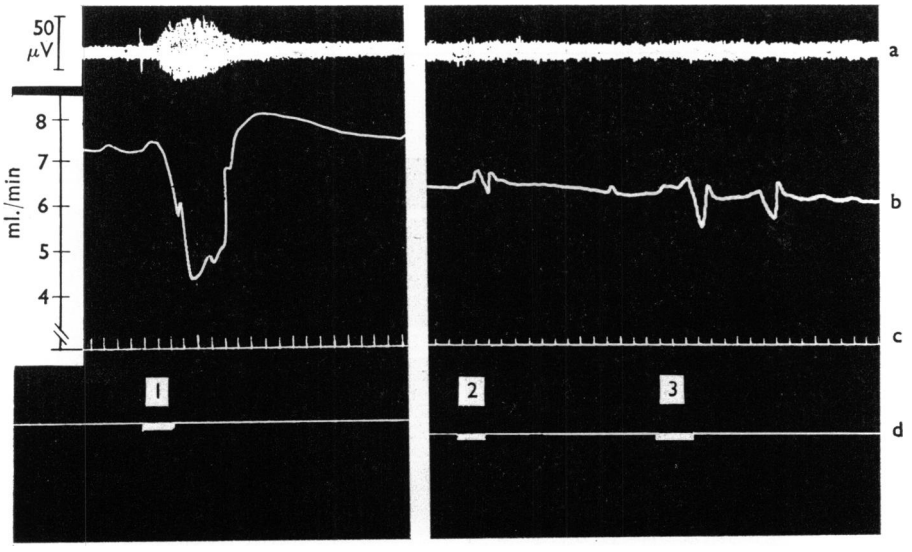

FIG. 8. Dog, pentobarbitone. Saline. Perfused left kidney. The effect of acetylcholine on nervous activity and venous outflow before and after nicotine. a, Record of activity of an isolated renal nerve bundle ; b, record of renal venous outflow ; c, time trace, 30 sec ; d, bottom trace signals duration of injection. (1) Effect of acetylcholine 20 μg/ml. ; (2) effect of acetylcholine 20 μg/ml. after injection of 10 mg of nicotine ; (3) effect of acetylcholine 50 μg/ml. after injection of 10 mg of nicotine.

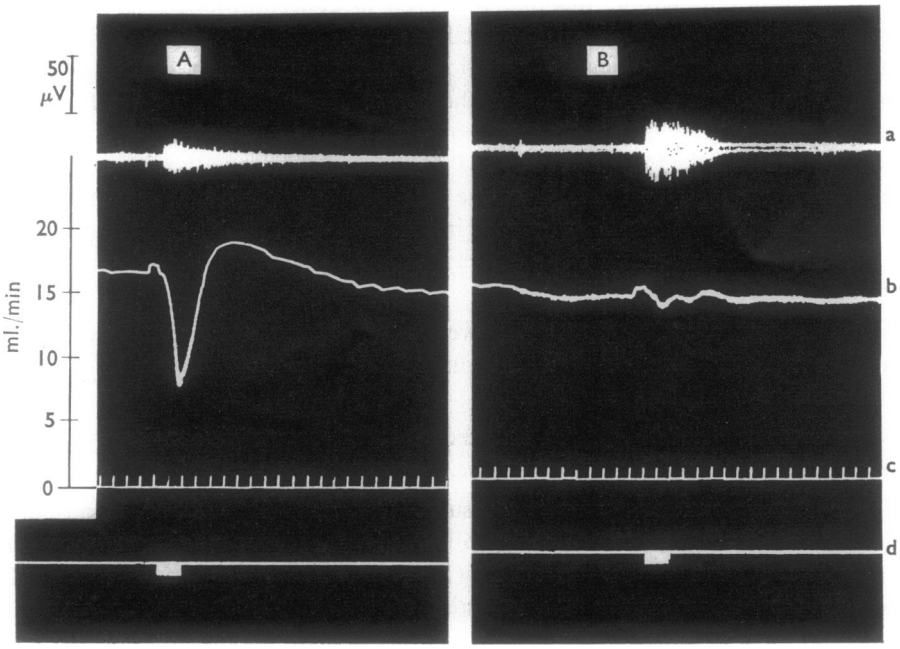

FIG. 9. Dog, pentobarbitone. Saline. Perfused isolated left kidney. The effect of acetylcholine on nervous activity and venous outflow before and after injection of hydergine. a, Record of activity of an isolated renal nerve bundle; b, record of venous outflow; c, time trace, 30 sec; d, trace which signals duration of injections. A, Effect of acetylcholine 10 μg/ml.; B, effect of acetylcholine 10 μg/ml. after injection of 300 μg of hydergine.

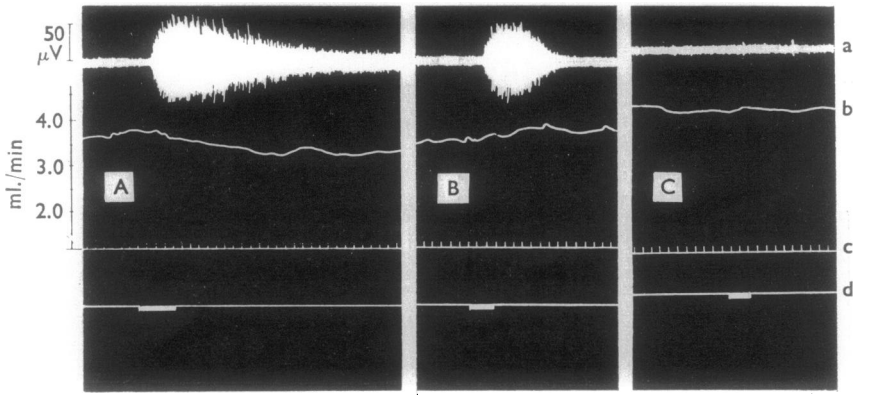

FIG. 10. Dog, pentobarbitone. Saline. Perfused isolated left kidney. The effect of nicotine, acetylcholine and noradrenaline on the nervous activity and on the venous outflow of a kidney showing chronic pyelonephritis. a, Record of activity of a renal nerve bundle; b, record of venous outflow; c, time trace, 30 sec; d, trace signalling duration of injections. A, Effect of nicotine 20 μg/ml.; B, effect of acetylcholine 20 μg/ml.; C, effect of noradrenaline 5 μg/ml.

Effect of ACh on renal nerve activity

In concentrations ranging from 20–50 μg/ml. ACh evoked a notable increase of nervous activity. At the same time a reduction in renal venous outflow was observed. This effect was blocked by nicotine (10 mg.) (Fig. 8). Hexamethonium (6 mg/ml.) also abolished both nervous and vasomotor responses to ACh and to nicotine. These ganglion blocking agents did not abolish the vasoconstrictor response to noradrenaline.

Hydergine (30 μg/ml.) abolished the vasomotor response to noradrenaline, to ACh and to nicotine, but did not alter the outburst of nervous activity following ACh (Fig. 9).

Figure 10 depicts the effect of nicotine and of ACh on nervous activity and on the vasomotor response in one of several instances where outbursts of nervous discharges were not accompanied by any disturbance of the vascular system. That this was probably due to an alteration of the effector was shown by the fact that doses of noradrenaline (5 μg/ml.) had no effect on venous outflow, and, moreover, histological examination of these kidneys revealed the existence of chronic pyelonephritis. It was also found that, as the arteriolar walls gradually thickened during the disease process, the threshold for noradrenaline became higher.

If we take the ratio between external diameter/internal diameter of the vessel wall, and plot this ratio against the logarithm of the concentration of noradrenaline necessary to induce vasoconstriction, it may be seen that, as the ratio increases, the threshold for noradrenaline is greatly augmented (Fig. 11).

Nature of the fibres activated

In order to discover the nature of the fibres activated, we used the collision technique on two dogs and two cats.

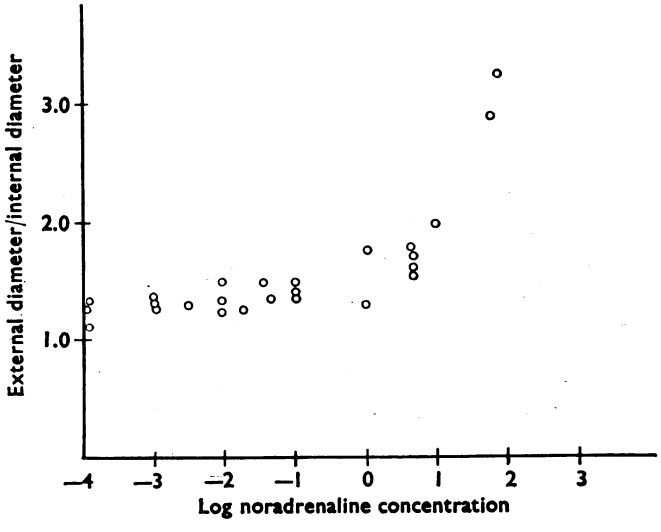

FIG. 11. Plot showing relation between the ratio external diameter/internal diameter of the vascular wall and the logarithm of the concentration of noradrenaline needed to induce vasoconstriction. As the ratio increases, there is a sudden and very marked increase of the threshold for noradrenaline.

Acetylcholine (50–100 μg/ml.) evoked an outburst of activity in the fibres. It was found that the action potentials generated by splanchnic stimulation were greatly reduced in size (Fig. 12). This would seem to indicate that these impulses are conducted antidromically by sympathetic post-ganglionic fibres.

Fibres with conduction velocities of 4.4 m/sec were not affected, but fibres with conduction velocities of 2.4 m/sec disappeared entirely. Fibres with slower con-

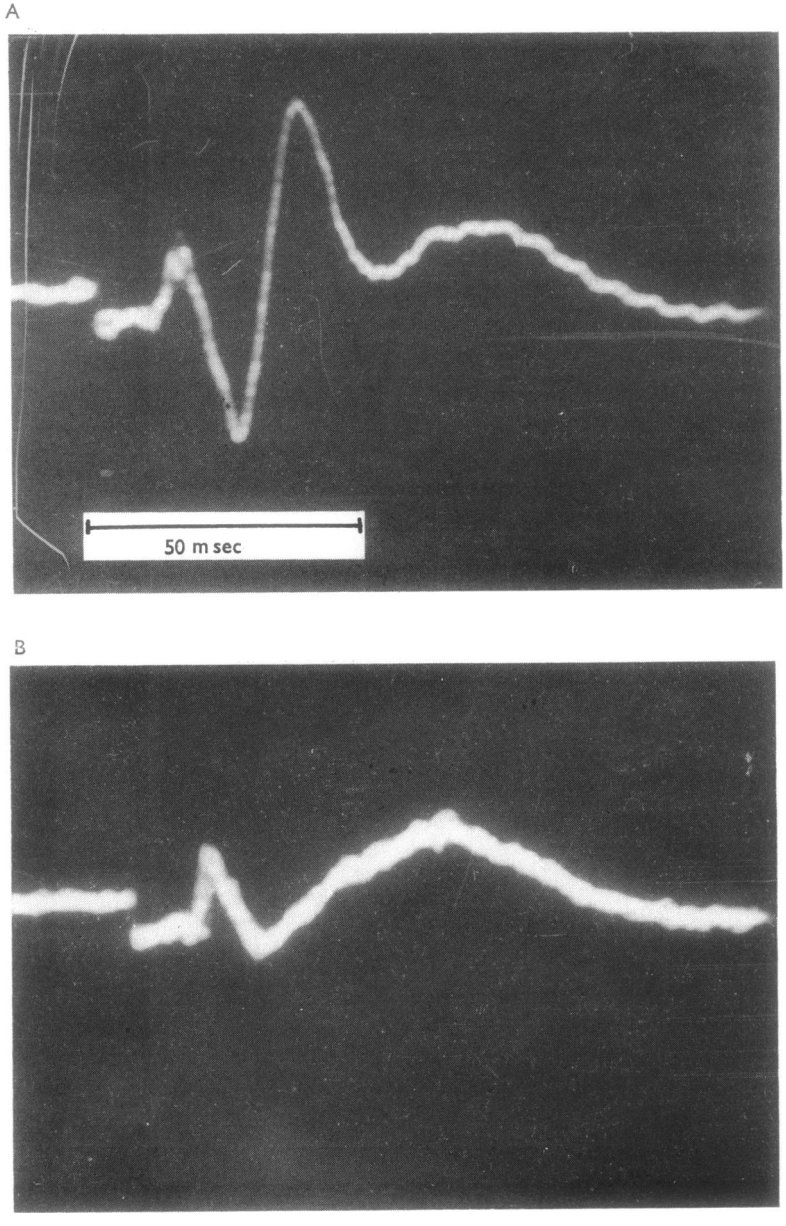

FIG. 12. Dog, pentobarbitone and chloralose. Left kidney *in situ*. The action potential of a renal nerve recorded 1 cm from the kidney before and after collision by means of the close arterial injection of 200 μg of acetylcholine. Above, control potential of nerve ; below, action potential of the same nerve after collision.

duction velocities (1.18 m/sec) did not disappear. It may be supposed that these are C fibres which do not travel along the splanchnic major nerve, but pass on to the dorsal roots, that is to say, they are sensory fibres.

Hexamethonium injected into the systemic circulation blocks all discharges evoked by stimulation of the splanchnic major nerve.

Discussion

Confirming results found in the literature, these findings provide further evidence that the nervous control of the kidney is mainly sympathetic, for we were unable to elicit any response to repeated vagal stimulation, and stimulation of the renal nerves consistently induced sympathetic effects, as also did the various drugs employed. Although Mitchell (1950b) shows clearly that the vagal fibres are distributed to the coeliac plexus, which sends branches to the kidneys, we were unable to demonstrate vagal effects on these organs. Mitchell (1950b), however, pointed out that renal nerves arising from the lower ends of the intermesenteric fibres or from the superior hypogastric plexus might carry vasodilator fibres, and these were not stimulated. On the other hand, stimulation of all the renal nerves produced vasoconstriction and never vasodilatation, and some of these nerves may arise from these presumed vasodilator fibre-carrying bundles.

It was shown that the vasoconstrictor effect of nervous stimulation was only slightly affected by enormous doses of hexamethonium (30,000 μg/ml.) and nicotine (30,000 μg/ml.). These doses are capable of completely blocking any ganglia and they abolish the response to ACh and to nicotine. This suggests that these kidneys do not possess ganglia, for, if so, hexamethonium would prevent the transmission of nervous impulses across the ganglia, thus abolishing the effect of stimulation.

The vasoconstrictor effect of ACh and of nicotine suggests that they both act by releasing noradrenaline, as in several other sympathetically innervated organs.

Excitation of renal nerves by acetylcholine

Acetylcholine injected arterially provokes an increase in the nervous activity of the kidney, and at the same time acts to reduce renal venous outflow, thus indicating a sympathomimetic effect. This effect could not be blocked by atropine. It is concluded that the effect of ACh is nicotinic.

These effects could be prevented by nicotine and by hexamethonium, but these blocking agents did not prevent the vasoconstrictor response induced by stimulation of the renal nerves. It is therefore postulated that ACh and nicotine act either on (1) sympathetic postganglionic fibres, (2) some structure (Mitchell, 1958) intervening between the adrenergic nerve endings and the effector cells such as Cajal's " neurones sympathiques intersticiels " which would normally release catecholamines ; or (3) sensory nerve endings, setting up an axon reflex (Hilton, 1954).

In an attempt to analyse these possibilities, the collision technique was used : our experiments are not conclusive, but collision was evident. From our results we feel that the activity induced in the renal nerves by ACh is predominantly antidromic and is due to the stimulation of post-ganglionic fibres.

The presence of centripetal fibres cannot be discarded in spite of the fact that we were unable to show a pupillary dilator response to renal nerve stimulation. More work is needed before these points can be clarified.

The present work is further evidence in support of the hypothesis of the cholinergic link. In agreement with the review by Ferry (1966), however, we cannot confirm or deny the existence of a cholinergic process in the release of a sympathetic transmitter until further investigations are made.

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REFERENCES

ASTRÖM, A., CRAFOORD, J. & SAMELIUS-BROBERG, U. (1964). Vasoconstrictor action of acetylcholine on kidney blood vessels. *Acta physiol. scand.*, **61**, 159–164.

BLAKELEY, A. G. H., BROWN, G. L. & FERRY, C. B. (1963). Pharmacological experiments on the release of the sympathetic transmitter. *J. Physiol., Lond.*, **167**, 505–514.

BLOCK, M. A., WAKIM, K. G. & MANN, F. G. (1952). Renal function during stimulation of renal nerves. *Am. J. Physiol.*, **169**, 670–677.

BOYD, H., BURNSTOCK, G., CAMPBELL, G., JOWETT, A., O'SHEA, J. & WOOD, M. (1963). The cholinergic blocking action of adrenergic blocking agents in the pharmacological analysis of autonomic innervation. *Br. J. Pharmac. Chemother.*, **20**, 418–435.

BURN, J. H., RAND, M. J. & THOMPSON, J. W. (1959). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol., Lond.*, **156**, 246–259.

DE MUYLDER, C. G. (1952). *The "Neurility" of the Kidney.* Oxford: Blackwell Scientific Publications.

DOUGLAS, W. W. & RITCHIE, J. M. (1957). A technique for recording functional activity in specific groups of medullated and non-medullated fibres in whole nerve trunks. *J. Physiol., Lond.*, **138**, 19–30.

DOUGLAS, W. W. & RITCHIE, J. M. (1960). The excitatory action of acetylcholine on cutaneous non-myelinated fibres. *J. Physiol., Lond.*, **150**, 501–514.

FERRY, C. B. (1963). The sympathomimetic effect of acetylcholine on the spleen of the cat. *J. Physiol., Lond.*, **167**, 487–504.

FERRY, C. B. (1966). Cholinergic link hypothesis in adrenergic neuroeffector transmission. *Physiol. Rev.*, **46**, 420–456.

GOODWIN, W. E., SLOAN, R. D. & SCOTT, W. W. (1949). The " Trueta " renal vascular " shunt." *J. Urol.*, **61**, 1010–1027.

HILTON, S. M. (1954). The effects of nicotine on the blood vessels of skeletal muscle in the cat. An investigation of vasomotor axon reflexes. *J. Physiol., Lond.*, **123**, 289–300.

HOFFMANN, F., HOFFMANN, E. J., MIDDLETON, S. & TALESNIK, J. (1945). The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. *J. Physiol., Lond.*, **144**, 189–198.

KAPLAN, S. A., WEST, C. D. & FOMON, S. J. (1953). Effects of unilateral division of splanchnic nerve on the renal excretion of electrolytes in unanaesthetised and anaesthetised dogs: the mechanism of " crossed stimulation." *Am. J. Physiol.*, **175**, 363–374.

MITCHELL, G. A. G. (1950a). The nerve supply of the kidneys. *Acta Anat.*, **10**, 1–37.

MITCHELL, G. A. G. (1950b). The renal nerves. *Br. J. Urol.*, **22**, 269–279.

MITCHELL, G. A. G. (1958). The innervation of vessels. *Jl R. Coll. Surg., Edinburgh*, **4**, 1–18.

PAGE, I. H. & MCCUBBIN, J. W. (1953). Renal vascular and systemic arterial pressure response to nervous and chemical stimulation of the kidney. *Am. J. Physiol.*, **173**, 411–420.

STÖHR, P. (1952). *Handbuch der microscopischen anatomie des menschen.* Berlin: Springer Verlag.

THOMPSON, J. W. (1958). Studies on the response of the isolated nictitating membrane of the cat. *J. Physiol., Lond.*, **141**, 46–72.

YAMAGISHI, S. & AZUMA, T. (1963). The innervation of the renal blood vessels of the toad. *Jap. J. Physiol.*, **13**, 399–412.

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