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EFFECT OF ACETYLCHOLINE ON RENAL VASOMOTION

Revision of the literature leads to the conclusion that the work done 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 works of Mitchell (1950), de Muylder (1952) and Stöhr (1957) show that the kidney receives contributions from widespread sources, both sympathetic and parasympathetic, and vagal fibres are known to pass on the coeliac plexus, which gives rise to renal branches. From a physiological point of view, the works of Kaplan et al (1953) Yamagishi and Azuma (1963) and Page and McCubbin (1963) contribute to the feeling 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 have not been established, and we therefore considered it of great interest to undertake the study of the effect of the autonomic system and of the renal nerves on renal outflow, and to investigate the mechanisms by which renal responses to nerve stimulation are brought about.

It has become evident that ACh has a sympathomimetic effect on several organs (Hoffmann et al, 1945: Burn and Rand, 1959 Daly and Scott, 1961: Douglas and Ritchie, 1960: Blakeley, 1963: and Ferry, 1963) and this drug was therefore used in these experiments in an attempt to analyse the vasomotor responses of the kidney.

Methods

Experiments were carried out on 50 mongrel dogs of either sex, weighing between 5 and 25 Kgs. The dogs were anaesthetised with sodium pentobarbital (30 mgr. per Kg. weight). The animals' left kidney was approached through a midline incision and the renal artery and vein were cannulated as close as possible to their respective origins from aorta or vena cava with a polyethylene catheter. The nervous supply was untouched.

The renal artery was perfused from a Mariotte bottle, which could be placed at any desired level. The perfusion of the kidney was carried out at a constant pressure, which was adjusted usually between 60 and 100 mm. Hg. The bottle and the cannula were connected by a rubber tube. The rate of renal venous outflow was recorder on smoked paper by means of a drop interval (Palmer Rheotachograph, Fig. I).

The perfusion fluid used was Tyrode's solution for dogs, adjusted to pH 7.4.

The drugs were given in Tyrode into the renal artery by means of the rubber tubing which connected the arterial cannula to the perfusion bottle. The volume was always 1 ml. and the injection speed was 15 seconds. Control volumes of Tyrode were used in every experiment.

The following drugs were used:

Acetylcholine 0.1 mgr.‰ (Colliere), nicotine (Hopkin and Williams Ltd.), noradrenaline (Instituto Bioquímica "Beta"), hexamethonium from Burroughs, Wellcome and Co., and Hydergine (Sandoz, a preparation of dihydrocornine, dihydroergocristine and dihydroergokryptine). Fresh solutions were prepared daily from these stocks.

The left splanchnic major nerve was isolated after thoracotomy performed under artificial respiration and stimulated between T 10 and T 12 with square pulses by means of an electronic stimulator through a pair of Ag—AgCl electrodes.

Both supradiaphragmatic vagii and the renal nerves were also isolated and stimulated in the same way.

Results

Stimulation of the left splanchnic nerve at T 10 - T 12 with a square wave pulse of 3.5 V intensity and 5 msec. duration at a rate of 10 c/sec. induced a notable vasoconstriction (Fig. II).

This finding is in accordance with the results of many authors (Kaplan, 1953; Yamagishi, 1963). Stimulation of isolated renal nerves also induced vasoconstriction, but only at a higher voltage due to partial short-circuit of the current by surrounding fluid.

Stimulation of both supradiaphragmatic vagii 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 ugr): only a slight reduction was occasionally found (Fig. III).

On re-injecting into the kidney the perfusate obtained during stimulation of the renal nerves, a strong vasoconstrictor effect was repeatedly observed (Fig. IV). 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 Tyrode had no effect (Fig. V).

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 is seen in Fig. VI A. The lowest threshold was 0.0001 ugr/ml. although most kidneys responded only to doses between 0.001 ugr/ml. and 0.01 ugr/ml. The strongest concentration, 0.1 ugr/ml., reduced kidney outflow from 8.6 ml/min. to 5.5 ml/min., a 37% reduction: and 0.0001 ugr/ml. ACh induced a barely perceptible vasoconstriction.

These thresholds were very variable and we did not find two kidneys which responded in a similar degree to a same concentration of acetylcholine.

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

Higher concentrations of ACh (100-1000 ugr/ml often induced vasodilatation.

Some authors (De Muylder, Page and McCubbin, Mitchell) indicate the existence of intrarenal ganglia. Investigating whether the mode of action of ACh might be stimulation of intrarenal sympathetic ganglia which would then liberate noradrenaline, nicotine was used in concentrations strong enough to block autonomic ganglia. Small doses of nicotine (1-100 ugr) induced renal vasoconstriction due to stimulation of either ganglia or post-ganglionic fibres. We are inclined to consider the latter possibility more likely, since stimulation of the left splanchnic nerve induced intense vasoconstriction which was not blocked by nicotine or by hexamethonium injected into the renal artery. Both these drugs block the stimulating effect of ACh on intrarenal post-ganglionic fibres but do not block the conduction of impulses through these fibres. These findings have been previously confirmed by Douglas and Ritchie (1960).

Small doses of nicotine or of ACh stimulate post-ganglionic fibres, whereas similar doses of hexamethonium have no effect. Two thousand ugr. nicotine induced complete blockade of the effect of ACh (Figs. VI B and VI V), and this substance in concentrations of 10 ugr/ml. and 100 ugr/ml. had no effect whatsoever on outflow: 1000 ugr/ml had to be given to show a minimal vasoconstrictor effect.

This effect, however, has never permanent: usually after five or ten minutes ACh induced vasoconstriction in concentrations under 10 ugr/ml. and even under 1 ugr/ml.

Noradrenaline (0.0001 ugr/ml) induced strong vasoconstriction in every kidney which responded to ACh and nicotine. These experiments allow us to presume that renal innervation is adrenergic. To test possibility further, vasoconstriction was induced by renal nerve stimulation. Hydergine (150-300 ugr) was then given, and on repeating stimulation no effect was obtained. Hydergine also blocked the response to ACh and to noradrenaline (Fig. VII).

Discussion

Confirming results found in the literature, these findings provide further evidence that the nervous control of the kidney is mainly sympathetic, since we were unable to elicit any response to repeated vagal stimulation, and stimulation of the renal nerves consistently induced sympathetic effects, as did also the various drugs employed. Although Mitchell (1950) shows clearly that vagal fibres are distributed to the coeliac plexus, which sends branches to the kidneys, we were unable to demonstrate vagal effects on these organs. However, Mitchell points 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 and nicotine (30,000 ugr.) which are capable of completely blocking any ganglia and which abolish the response to ACh and nicotine. This suggests that these kidneys do not possess ganglia, for if so, hexamethonium would prevent the transmission of the nervous impulses across the blocked ganglia, thus abolishing the effect of stimulation.

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

Both hexamethonium and nicotine abolish the effect of ACh, and since it is very doubtful that they do so by acting on a hypothetical ganglion, the following blocking mechanisms may be proposed:

- 1.—Blockade of sympathetic post-ganglionic nerve fibres.
- 2.—Blockade of some structure intervening between the adrenergic nerve endings and the effector cells: this structure (Gillespie and Mackenna, 1960), would liberate catecholeamines normally. They were described by Cajal as "neurones sympathiques intersticiels".
- 3.—Blockade of an axon reflex set up by sensory nerve endings in the kidney.

To test these hypotheses further work is being carried out in this laboratory and will be the object of other communications.

Summary

- 1.—Stimulation of the left splanchnic major nerve at T10-T12, and the renal nerves, consistently caused renal vasoconstriction.
- 2.—Repeated stimulation of both supradiaphragmatic vagi failed to induce any vasomotion in the left kidney.
- 3.—The vasoconstrictor effect was not blocked by either nicotine or hexamethonium even in enormous doses (30,000 ugr). This may indicate that intrarenal ganglia do not exist, as there ganglion blockers would prevent transmission across the ganglia.
- 4.—Kidney, perfusate, re-injected into the kidney after vasoconstriction induced by stimulation of the renal nerves, brought 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.

5.—Acetylcholine in concentration ranging from 0.001 ugr/ml. to 0.01 ugr/ml. caused a reduction in renal outflow. Thresholds were extremely variable. Higher concentrations of ACh (100-1000 ugr/ml.) often induced vasodilatation.

6.—Nicotine and hexamethonium (1000-2000 ugr/ml.) induced blockade which elevated the threshold for ACh to values of 1000 ugr/ml.

7.—Noradrenaline (0.0001 ugr/ml.) induced strong renal vasoconstriction.

8.—Hydergine (5-10 ml. solutions in concentrations ranging from 15 to 30 ugr/ml), blocked the renal response to nerve stimulation, to ACh and to noradrenaline. This suggests that the nature of the renal innervation is adrenergic.

9.—These findings are discussed in relation to the hypothesis that renal innervation is mainly adrenergic and that ACh acts as sympathetic transmitter, liberating noradrenaline, and that this effect is blocked at post-ganglionic endings, or at some structure intervening between adrenergic nerve endings and the effector cells, or at sensory nerve endings.

Bibliography

- 1.—Blakeley, A. G. H., Brown, G. L. and Ferry, C. B., Pharmacological Experiments On The Release Of The Sympathetic Transmitter. *J. Physiol.* (1963) **167**: 505-514.
- 2.—Block, M. A., Wakim, K. G. and Mann, F. C., Renal Function During Stimulation Of Renal Nerves. *Am. J. Physiol.* **169**: 670, 1952.
- 3.—Burn, J. H. and Rand, M. J., The Cause Of The Supersensitivity Of Smooth Muscle To Noradrenaline After Sympathetic Degeneration. *J. Physiol.* (1959) **147**: 135-143.
- 4.—Burn, J. H., Leach, E. H., Rand M. J. and Thompson, J. W. Peripheral Effects Of Nicotine And Acetylcholine Resembling Those Of Sympathetic Stimulation *J. Physiol.* (1959) **148**: 332-352.
- 5.—Daly, M. de B. and Scott, M. J., The Effects Of Acetylcholine On The Volume And Vascular Resistance Of The Dog's Spleen. *J. Physiol.* (1961), **156**: 246-259.
- 6.—De Muylder, C. G., The "Neurality" Or The Kidney. Blackwell Scientific Publications. Oxford, 1952.
- 7.—Douglas, W. W. and Ritchie, J. M., The Excitatory Action Of Acetylcholine On Cutaneous Non-Myelinated Fibres. *J. Physiol.* (1960), **150**: 501-514.
- 8.—Goodwing, W. E., Sloan, R. D. and Scott, W. W., The "Trueta" Renal Vascular "Shunt". An Experimental Demonstration Of Neurovascular Control Of The Renal Circulation In The Rabbit, Cat, Dog and Monkey. *J. Urol.* **61** (6): 1010, 1949.
- 9.—Harrison, J. S. and B. A. McSwiney, The Chemical Transmitter Of Motor Impulses To The Stomach. *J. Physiol.* (1936), **87**: 79-86.
- 9.—Harrison, J. S. and B. A. McSwiney, The Chemical Transmitter Of Motor Impulses To The Stomach. *J. Physiol.* (1936), **87**: 79-86.
- 10.—Hilton, S. M., The Effects Of Nicotine On The Blood Vessels Of Skeletal Muscle In The Cat. An Investigation Of Vasomotor Axon Reflexes. *J. Physiol.* (1954), **123**: 289-300.
- 11.—Hoffmann, F., Hoffmann, E. J., Middleton, S. and Talesnik, J., The Stimulating Effect Of Acetylcholine On The Mammalian Heart And The Liberation Of An Epinephrine-like Substance By The Isolated Heart. *J. Physiol.* (1945), **144**: 189-198.
- 12.—Kaplan, S. A., West, C. D. and Fomon, S. J., Effects Of Unilateral Division Of Splanchnic Nerve On The Renal Excretion Of Electrolytes In Unanes-

- thetized And Anesthetized Dogs: The Mecanism Of "Crossed Stimulation".
Am. J. Physiol. **175** : 363, 1953.
- 13.—**Mitchell, G. A. G.**, The Nerve Supply Of The Kidneys. Acta Anat. **10** : 1, 1950.
- 14.—**Mitchell, G. A. G.**, The Innervation Of Vessels. J. cf. The Royal Coll. of Surgeons Edinburgh (1958), **4** : 1-18.
- 15.—**Page, I. H.** and **McCubbin, J. W.**, Renal Vascular And Systemic Arterial Pressure Response To Nervous And Chemical Stimulation Of The Kidney. Am. J. Physiol. **173** : 411, 1953.
- 16.—**Stöhr, Philipp**, Hndbuch Der Mikroskopischen Anatomie Des Menschen. Springer Verlag. Berlin, 1952.
- 17.—**Thompson, J. W.**, Studies On The Response Of The Isolated Nictitating Membrane Of The Cat. J. Physiol. (1958), **141** : 46-72.
- 18.—**Yamagishi, S.** and **Azuma, T.**, The Innervation Of The Renal Blood Vessels Of The Toad. Jap. J. Physiol. **13** (4) : 399, 1963.

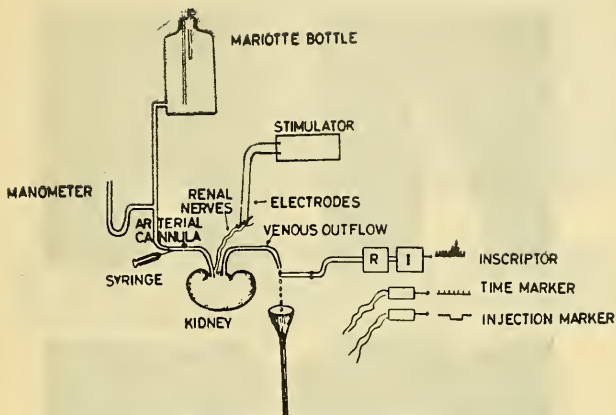


Fig. 1.— Schema illustrating the method and recording unit employed

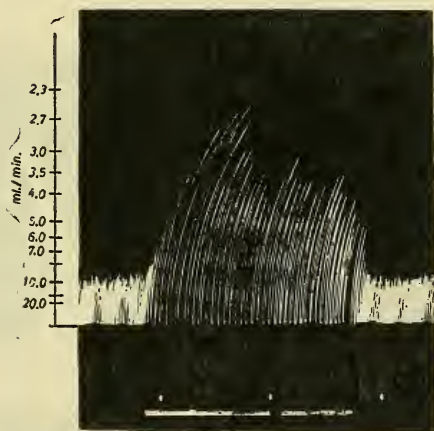


Fig. II.— Dog. Pentobarbitone. Tyrode's solution for dogs. Pertused left kidney in situ. Effect on renal venous outflow of the stimulation of the left splanchnic major nerve between T10 and T12 with a square wave pulse of 3.5 V intensity and 5 msec duration at a rate of 10 c/sec Time, 30 seconds. The duration of the stimulation is signaled on bottom trace.

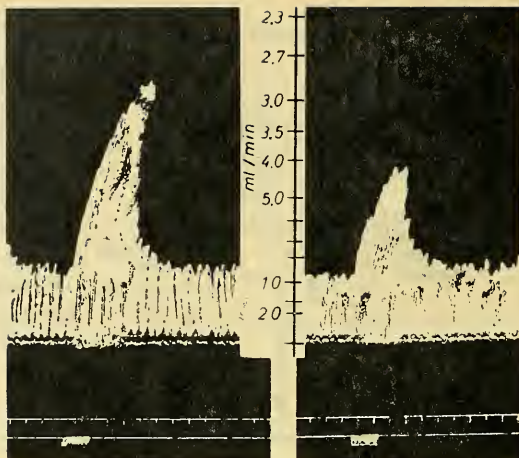


Fig. III.—Dog. Pentobarbitone. Tyrode's solution for dogs. Perfused left kidney in situ. **Left:** Effect on renal venous outflow of a renal nerve. **Right:** Effect on renal venous outflow of the stimulation of same nerve five minutes after the injection of 30 mgr hexamethonium into the perfusion Time, 30 seconds. The duration of the stimulation and of the injection is signaled on bottom trace.

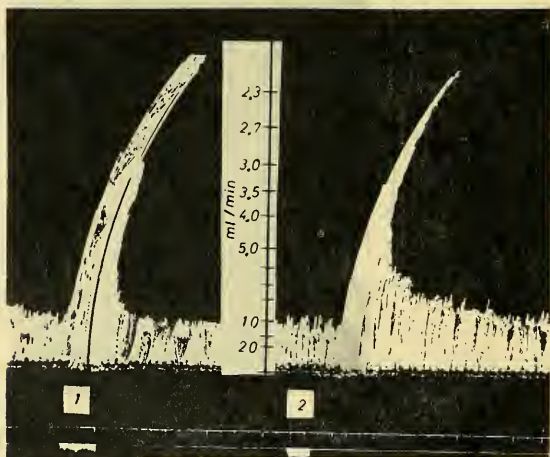


Fig. IV.—Dog. Pentobarbitone.—Tyrode's solution for dogs. Perfused left kidney in situ. 1) Effect on renal venous outflow of the stimulation of a renal nerve. 2) Effect on venous outflow of the injection of 1 ml kidney perfusate into the perfusion fluid. This perfusate was collected during the preceding stimulation of the renal nerve.

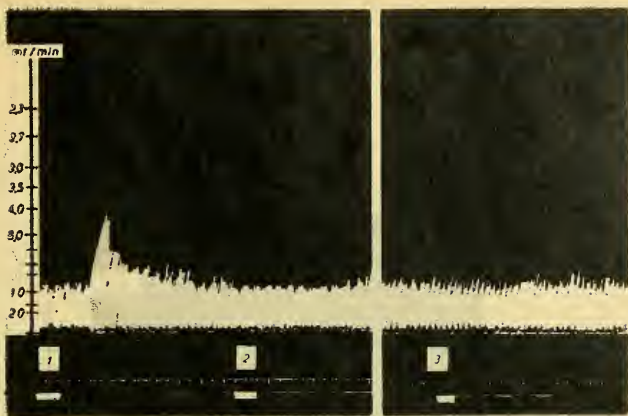


Fig. V.—Dog.—Pentobarbitone. Tyrode's solution for dogs. Perfused left kidney in situ. 1) Effect on renal venous outflow of the injection of 1 ml kidney perfusate collected during stimulation of a renal nerve. 2) Effect on venous outflow of the injection of 1 ml kidney perfusate collected before stimulating the renal nerves. Time, 30 seconds. The duration of the injections is signalled on bottom trace. 3) Effect on venous outflow of the injection of 1 ml of Tyrode's solution into the perfusion fluid.

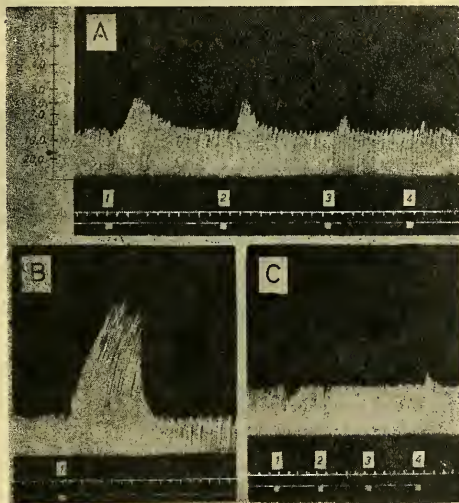


Fig. VI.—Dog. pentobarbitone. Tyrode's solution for dogs. Perfused left kidney in situ. A) Effect on renal venous outflow of the injection of: 1) 0.1 ugr ACh; 2) 0.01 ugr ACh; 3) 0.001 ugr ACh; 4) 0.0001 ugr ACh into the perfusion fluid. B) Effect on renal outflow of the injection of 100 ugr nicotine into the perfusion fluid. C) Effect of the injection of nicotine into the perfusion fluid, on the threshold of ACh: 1) 2000 ugr nicotine; 2) 10 ugr ACh; 3) 100 ugr ACh; 4) 1000 ugr ACh. Time, 30 seconds. The duration of the injection is signalled on bottom trace.

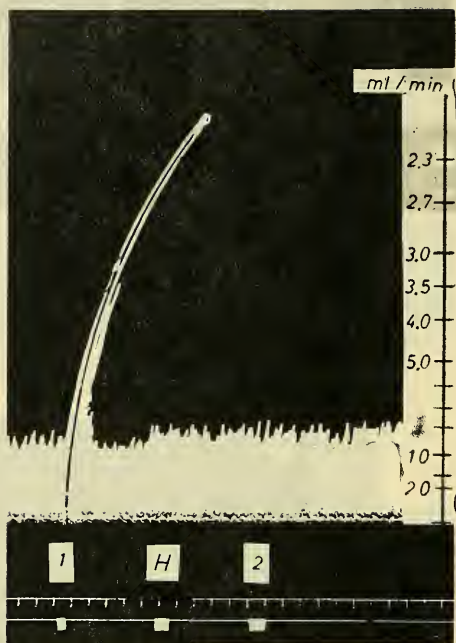


Fig. VII.—Dog. Pentobarbitone.—Tyrode's solution for dogs. Perfusion of the isolated left kidney. Effect of the injection of 300 ugr hydergine into the perfusion fluid, on the nervous activity of the kidney:

At 1) stimulation of a renal nerve.

At H) injection of 300 ugr hydergine into the perfusion fluid.

At 2) stimulation of the same nerve had no effect on venous outflow.

Time, 30 seconds. The duration of the stimulation and of the injection is indicated on bottom trace.