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A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat

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Summary

1. A simple method of stimulating different segments of the autonomic outflow from the spinal column in the pithed cat and rat is described.

2. Using a movable Teflon-shielded electrode inserted into the vertebral canal and an indifferent electrode under the skin, reproducible responses were obtained from the adrenals, the bladder, the blood vessels, the colon, the heart and the vas deferens. The level and number of segments stimulated was altered by varying the depth of insertion of the Teflon tube and the length of central steel electrode protruding from it.

3. The degree of localization of the stimulus seemed satisfactory since it was possible by stimulating the sacral parasympathetic outflow to the bladder and colon or the lumbar sympathetic outflow to the vas deferens, to elicit responses from these organs with little effect on the cardiovascular system. Stimulation at higher levels of the thoracic sympathetic outflow permitted discrimination between the pressor and cardio-accelerator responses.

4. Hexamethonium (1 mg/kg) inhibited all the responses, but the colonic response, which occurred only after a latent period, was especially sensitive to ganglion blockade. Atropine (1 mg/kg) inhibited the bladder response by 50% but only prolonged the latency of the colonic response without affecting its magnitude. Physostigmine (1 mg/kg) enhanced the bladder and colonic responses and reversed the effects of hexamethonium on these organs. Phentolamine (1 mg/kg) abolished the pressor responses to stimulation of the thoraco-lumbar sympathetic outflow but did not affect the cardio-accelerator response.

Introduction

In investigating the responsiveness of an organ to electrical stimulation of its nerve supply, the functional integrity of the tissues should be unimpaired by the preliminary dissection. This is difficult to achieve if extensive operative interference is required to expose the nerves. The problem was avoided in a simple method of stimulating the entire sympathetic outflow from the spinal cord in the pithed rat (Gillespie & Muir, 1967). This technique required no dissection beyond that necessary to record the blood pressure, because the steel pithing rod itself acted as an electrode to stimulate the spinal nerve roots in the thoraco-lumbar region.

This technique has now been refined to permit electrical stimulation of different segments of the sympathetic and parasympathetic outflows. Animals were pithed

using a flexible Teflon tube through which was passed a fine steel electrode. The level of stimulation could be altered by moving the insulating Teflon tubing within the vertebral canal and the number of segments stimulated by varying the extent to which the inner steel electrode protruded beyond the insulation. Using this simple arrangement the adrenals, the bladder, the blood vessels, the colon, the heart and the vas deferens could all be stimulated.

Methods

Male rats (250–300 g) and cats (2–2·5 kg) of both sexes were anaesthetized with a mixture of halothane and nitrous oxide and respired artificially through a tracheal cannula. Rats were pithed by first inserting a short steel tube (13 s.w.g.) through the orbit and the foramen magnum and down into the spinal column to the level of the sixth cervical vertebra. Through this trocar were passed successively a Teflon tube (0·16 mm O.D.) and inside that a fine steel tube (26 s.w.g.) which was extruded at the sacral end to complete the pithing (Fig. 1A). A steel rod (13 s.w.g.)

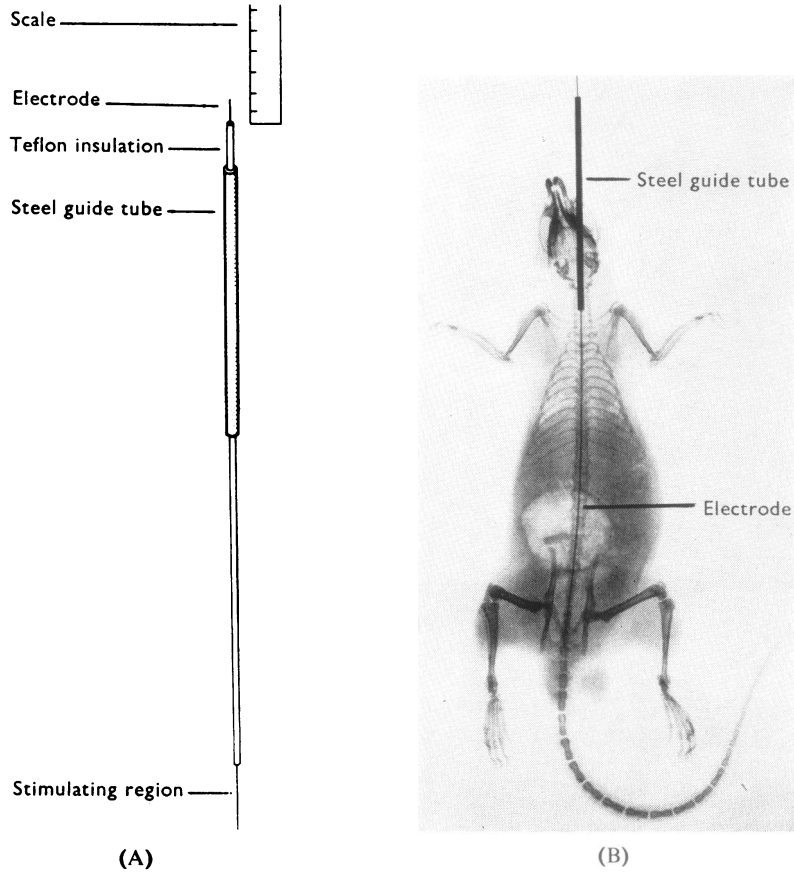

FIG. 1. A: Diagram of the pithing rod electrode assembly showing the calibrated scale, the central electrode, the Teflon insulation tube, the steel guide tube and the stimulating region. B: Radiograph of a pithed rat showing the steel guide tube inserted through the orbit, the foramen magnum and into the vertebral canal as far as C5 with the electrode inserted to the point of furthest penetration in the sacrum. The Teflon insulation is not radio-opaque and is therefore invisible.

inserted behind the skull and pushed down between the vertebral column and the skin acted as an indifferent electrode.

The level of stimulation was determined by varying the depth of insertion of the shielding Teflon tube. The number of segments affected was regulated by altering the length of central steel electrode exposed in the vertebral canal. The position of this stimulating electrode within the vertebral canal was investigated by radiography in nine rats (Fig. 1B). These radiographs showed that the length of the vertebral column and of its successive sections varied little, even in animals at the extremes of the weight range (Table 1). In pithing the animals a standard length of Teflon tubing and steel electrode was used and the position and length of electrode exposed in the vertebral column determined from the length of Teflon and steel protruding from the skull. A simple scale was arranged, as shown in Fig. 1A, to permit this calculation. The maximum penetration achieved was to the fourth sacral vertebra.

Cats were pithed using essentially the same technique, except that a cork borer was inserted through the upper table of the orbit rather than through the optic foramen. This avoided bleeding from the retinal artery which enters through this foramen with the optic nerve.

The temperature of each pithed animal was maintained at 37° C by a tungsten lamp and monitored with a rectal thermometer. In every animal blood pressure was recorded from one carotid artery and either the jugular or femoral vein was cannulated for drug administration. The spinal nerve roots were stimulated electrically by 1 ms pulses of supra-maximal voltage at the frequencies indicated in the text. Maximal responses from parasympathetic stimulation were obtained at low frequencies about 10 Hz, whereas about 50 Hz was required for the sympathetic outflow. Muscle twitching as a result of stimulating the motor fibres in the ventral roots was abolished either by tubocurarine (1 mg/kg intravenously) or by gallamine (5 mg/kg intravenously). The latter was preferable where organs (for example, the colon), particularly susceptible to ganglion blockade, were studied.

Seven measures of response were used, though not all in any given animal. The blood pressure (1 mmHg≡1·333 mbar) served as an integrated measure of the effect on the cardiovascular system. In addition, the separate effects on the heart and peripheral resistance were measured, the former from the change in heart rate

TABLE 1. *Intra-cranial distance together with the length of the vertebral column and its various sections penetrated by the pithing rod in rats.*

Rat No.	Weight (g)	Intra-cranial distance (cm)	7 cervical (cm)	13 thoracic (cm)	6 lumbar (cm)	4 sacral (cm)	Vertebral total (cm)
1	250	3·2	2·1	5·35	4·2	2·6	14·25
2	250	3·5	2·1	5·40	4·2	2·5	14·20
3	250	3·3	2·1	5·10	4·0	2·5	13·70
4	265	3·4	2·2	5·35	4·2	2·6	14·35
5	285	3·9	2·2	5·60	4·5	2·8	15·10
6	285	3·9	2·1	5·60	4·5	2·7	14·90
7	295	3·2	2·2	5·60	4·5	2·8	15·10
8	300	3·2	2·2	5·40	4·4	2·8	14·80
9	300	—	2·2	5·80	4·6	2·7	15·30
	Mean	3·45	2·16	5·47	4·34	2·67	14·63
	S.E.M.	0·10	0·017	0·068	0·067	0·041	0·18

The numbers in each column heading are the numbers of vertebrae.

recorded with a Grass cardiotachometer and the latter by the change in perfusion pressure in one hind limb perfused via the femoral artery with blood taken from a carotid artery using a Watson Marlow constant output pump. The output was initially adjusted to give a pressure of 100 mmHg. Clotting was prevented by injecting heparin (1,000–5,000 i.u./kg intravenously) before the perfusion started. The pressure response of the urinary bladder was measured via a saline-filled polythene cannula tied into the organ. The pressure response of the colon was recorded from a saline-filled polythene balloon inserted into the colon to a depth of 6 cm. The colon was first emptied by a saline enema. Two measures of the response of the vas deferens were used. Either longitudinal muscle contractions were recorded by a Grass FTO3 force displacement transducer or the rise in perfusion pressure was determined when oxygenated McEwen's solution was slowly perfused through the lumen by means of a Palmer slow injection apparatus. The perfusing cannula was inserted into the lumen through an incision in the testicular end and fluid allowed to escape through the urethra. Blood pressure and all perfusion pressures were recorded with Statham P23A pressure transducers and displayed on a Grass polygraph.

The following drugs, dissolved in normal saline, were used. Doses refer to the salts. Acetylcholine chloride (B.D.H.), atropine sulphate (B.D.H.), physostigmine sulphate (B.D.H.), gallamine triethiodide (May & Baker), guanethidine monosulphate (Ciba), heparin (Evans), hexamethonium bromide (May & Baker), hyoscine hydrobromide (Smith), noradrenaline bitartrate (B.D.H.), phentolamine mesylate (Ciba), propranolol hydrochloride (I.C.I.), (+)-tubocurarine chloride (Burroughs Wellcome).

Results

Stimulation of the sacral parasympathetic outflow

With the stimulating electrode fully inserted to sacral vertebrae S2-3, stimulation produced only small responses from the bladder and colon. When the electrode was raised to vertebral levels L5-6 or sometimes S1-2, supramaximal stimulation caused a rise in pressure in both the bladder and colon (Fig. 2). The optimum frequency of stimulation was 10 Hz and frequencies as low as 1 Hz produced clear rises in pressure. Maximum increases in bladder and colon pressure were achieved with no increase in blood pressure or heart rate (Fig. 2). Certain differences were observed in the response of the bladder as compared with the colon. The bladder response began after a short latent period and rapidly reached a maximum which was fairly well maintained for the period of stimulation. When stimulation was stopped the pressure quickly dropped. In contrast, the colonic response began only after a considerable latent period averaging 5 s, pressure developed slowly and when stimulation ended, decayed slowly. There were also differences in the drug-sensitivities of the two responses. Both responses were blocked by hexamethonium (1 mg/kg) but the colonic response was particularly sensitive to this drug (Fig. 3). A similar sensitivity to ganglion blockade by tubocurarine may explain the irregularity and variability of the responses of the colon in early experiments in which this drug was used to block skeletal muscle twitching. Gallamine, which has little ganglion-blocking action, was found to be free from these effects, and reproducible responses were obtained with this drug. Unfortunately, gallamine

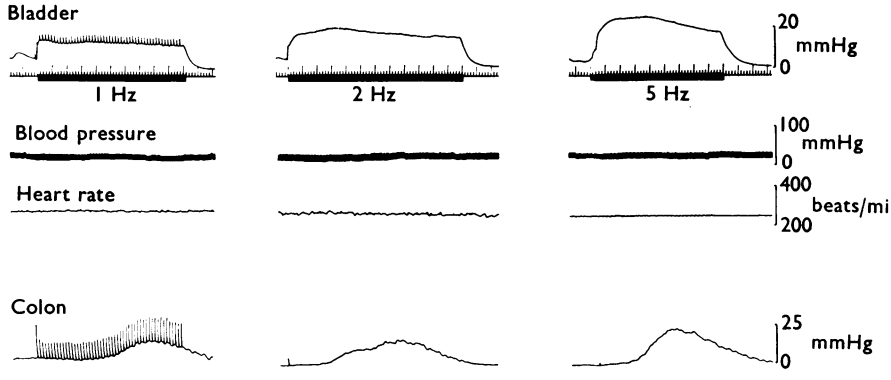

FIG. 2. Effects on the gallamine-treated pithed rat of supramaximal stimulation at 1 Hz, 2 Hz and 5 Hz of the sacral parasympathetic outflow opposite L5-6 for the periods indicated by the bars on the time trace (large interval=5 s). The records from above downwards show the responses of the bladder, the blood pressure, the heart rate and the intra-colonic pressure. Stimulation increased the bladder pressure and after a latency the intra-colonic pressure without affecting the heart rate. Despite the presence of gallamine (5 mg/kg) stimulation at 1 Hz caused contractions of the skeletal musculature corresponding to the frequency of stimulation. These were superimposed on the sustained contractions of the bladder and colon and disappeared at higher frequencies, leaving only a small initial "spike" which contributed little to the responses of these organs. The disappearance of the voluntary muscle contractions at frequencies greater than 1 Hz demonstrates the effectiveness of the neuromuscular blockade produced by gallamine.

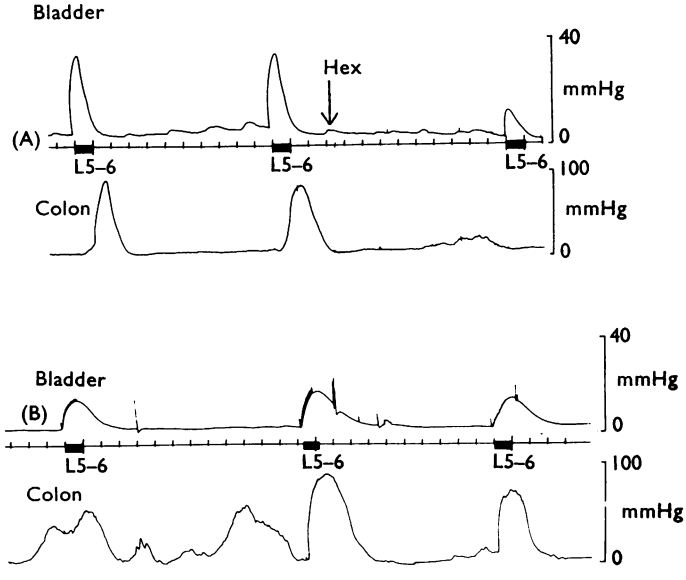

FIG. 3. Effects of hexamethonium (1 mg/kg) and physostigmine (2 mg/kg) on the responses of the gallamine-treated pithed rat to supramaximal stimulation at 10 Hz of the sacral parasympathetic outflow opposite L5-6 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards show in panel A the responses of the bladder pressure and the intra-colonic pressure before and after hexamethonium, which inhibited both responses, and in panel B the responses of the bladder pressure and intra-colonic pressure in the same animal after physostigmine, which restored the responses and increased the frequency of spontaneous contractions.

had a shorter duration of action and therefore had to be administered several times during each experiment.

The effect of stimulation was mediated by cholinergic nerve fibres and, as would be expected, physostigmine (1 mg/kg) enhanced the response. The amplitude of the rise in bladder pressure after physostigmine was not greatly altered but the duration was increased. The action of atropine is illustrated in Fig. 4. Both the bladder and colon were relatively resistant to this drug but, as Fig. 4 shows, 0·5 mg/kg reduced the responses of the bladder by some 50%. The effect on the colon differed. The latent period was greatly lengthened but the magnitude of the response was little affected.

Stimulation of the thoraco-lumbar sympathetic outflow

When the stimulating electrode was moved up into the region of the thoraco-lumbar outflow, responses from a variety of organs could be recorded and, by appropriate selection of the level and extent of stimulation, considerable discrimination between these was achieved. At the level of L3-4, stimulation excited the fibres to the vas deferens, resulting in its longitudinal contraction (Fig. 5) and an increase in perfusion pressure (Fig. 6). This contraction was observed at frequencies as low as 2 Hz, reached a maximum at about 50 Hz and was unaccompanied by any change in heart rate and only small or no rises in blood pressure. This ability to elicit separately, pressor responses and contractions of the vas deferens is more clearly illustrated in Fig. 6. In this experiment the stimulating electrode was moved progressively down the thoraco-lumbar region of the vertebral canal. Stimulation at T7-9 and T10-11 produced a large rise in blood pressure with little or no increase in vas perfusion pressure. At T12-13 the pressor response was considerably decreased with an increase in the response of the vas, at L1-2 the pressor response was very small and at L3-4 no pressor response was elicited but the rise in vas perfusion pressure reached its maximum.

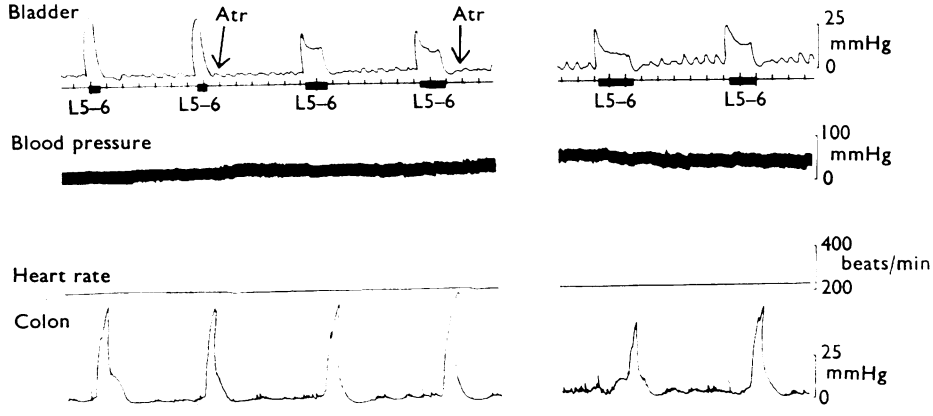

FIG. 4. Effects of atropine (Atr, 2×0·5 mg/kg) on the responses of the gallamine-treated pithed rat to supramaximal stimulation at 10 Hz of the sacral parasympathetic outflow opposite L5-6 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards are of the bladder pressure, the blood pressure, the heart rate and the intra-colonic pressure. Atropine inhibited the bladder response and prolonged the latency of the colonic response. The blood pressure and the heart rate were not affected either by stimulation or atropine.

At higher levels in the thoracic column it was possible to discriminate between the pressor response (T7-9) and the cardiac accelerator response (C7-T1) (Fig. 7). Stimulation at the upper lumbar region (L1-2) caused vasoconstriction in the hind limbs as shown by the increase in hind limb perfusion pressure, but little or no increase in blood pressure or heart rate (Fig. 8). Raising the level of stimulation to the lower thoracic outflow (T12-13) abolished the hind limb response but increased the rise in the systemic blood pressure and greatly increased heart rate. Phentolamine (1 mg/kg) abolished both the increase in hind limb perfusion pressure observed at L1-2 and the rise in blood pressure at T12-13, but did not alter the increase in heart rate produced at the latter position (Fig. 8). An interesting observation also seen in Fig. 8 was that when phentolamine was given during a

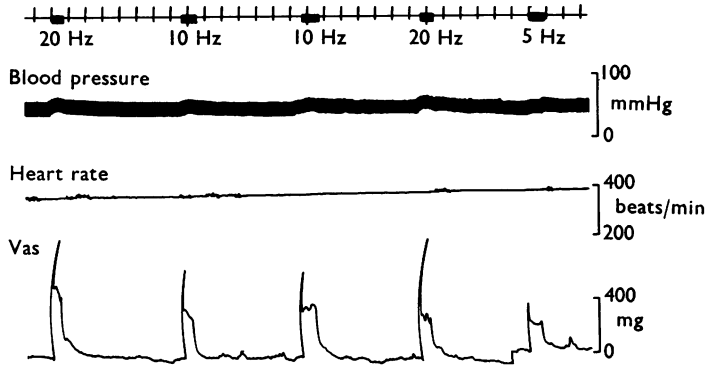

FIG. 5. Effects on the tubocurarine-treated pithed rat of supramaximal stimulation at 5 Hz, 10 Hz and 20 Hz of the lumbar sympathetic outflow opposite L3-4 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards are of the blood pressure, the heart rate and the isometric tension on the vas deferens. For a single frequency the response was reproducible and at all frequencies consisted of an immediate " spike " followed by a " plateau " at a lower level during the remaining period of stimulation. The heart rate was unaffected but the blood pressure was increased slightly, the response being more marked at higher frequencies.

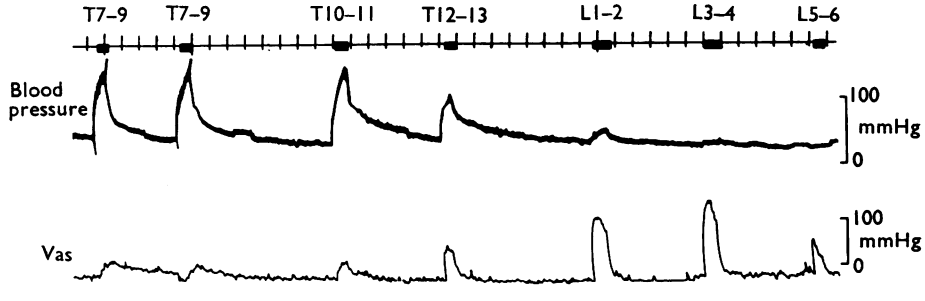

FIG. 6. Effects on the tubocurarine-treated pithed rat of supramaximal stimulation at 20 Hz of different levels of the thoraco-lumbar sympathetic outflow for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards are of the blood pressure and the resistance of the vas deferens to perfusion. Stimulation of the outflow opposite T7-9 produced a rise in the blood pressure with little effect on the vas deferens perfusion pressure. As the stimulating electrode was moved down the column the pressor response diminished while the vas deferens response increased to reach a maximum when the electrode was opposite L3-4. Stimulation in this position had little effect on the blood pressure.

long period of stimulation at L1-2, the increase in hind limb perfusion pressure was immediately abolished and at the same time a small but definite increase in heart rate was observed, presumably due to the block of uptake of noradrenaline by phentolamine resulting in an overflow into the general circulation and stimulation of the unblocked β-adrenoceptors in the heart.

As the stimulating electrode was raised, the blood pressure response increased to reach a maximum opposite vertebrae T7, 8 and 9. At this level, the heart rate was also increased but reached its peak somewhat later than the pressor response. The pressor response itself was often biphasic, suggesting a component due to the release of adrenaline from the adrenal glands (Fig. 9). This was confirmed when it was found that adrenalectomy reduced both the cardio-acceleration and the secondary rise in the blood pressure (Fig. 9). Still higher levels of stimulation, corresponding to C7-T1, caused a large increase in heart rate with little effect on the blood pressure (Fig. 7).

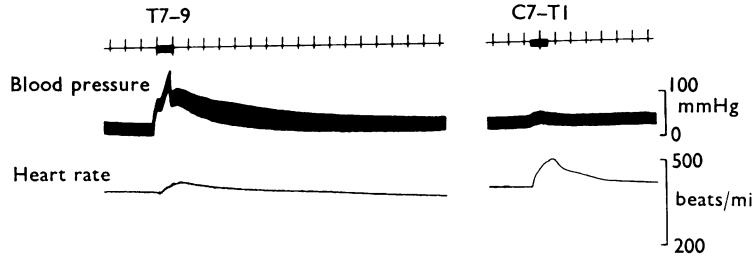

FIG. 7. Effects on the tubocurarine-treated pithed rat of supramaximal stimulation at 10 Hz of the sympathetic outflow opposite T7-9 and C7-T1 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards are of the blood pressure and the heart rate. Stimulation at T7-9 produced a large biphasic rise in the blood pressure and a small delayed increase in heart rate. In contrast, stimulation opposite C7-T1 increased the heart rate but had little effect on the blood pressure.

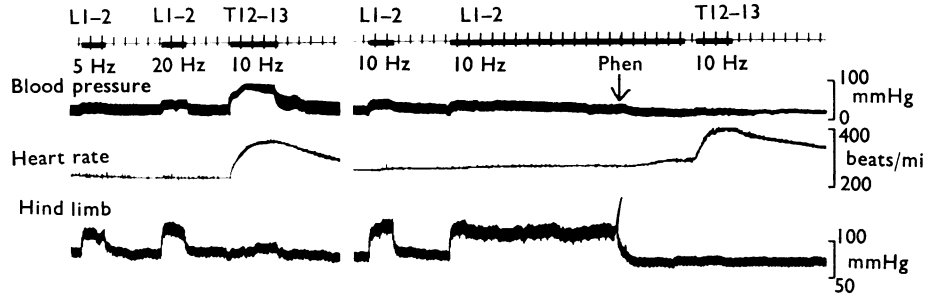

FIG. 8. Effects on the tubocurarine-treated pithed rat of supramaximal stimulation at 5 Hz, 10 Hz and 20 Hz of the thoraco-lumbar sympathetic outflow opposite T12-13 and L1-2 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards are of the blood pressure, the heart rate and the hind limb perfusion pressure. Stimulation opposite L1-2 at 5 Hz increased the hind limb perfusion pressure with little effect on the blood pressure and none on the heart rate. Stimulation in the same position at higher frequencies increased the blood pressure only slightly. In contrast, stimulation opposite T12-13 at 10 Hz increased both blood pressure and heart rate without affecting the hind limb perfusion pressure. During prolonged stimulation at L1-2 the hind limb perfusion response was well maintained but was abolished by phentolamine (1 mg/kg intra-arterially), which also blocked the pressor response produced by stimulation at T12-13. The cardio-accelerator component in this response was unaffected.

Stimulation of the cranial parasympathetic outflow

The outflow from the brain stem was stimulated in relatively few experiments. On those occasions the Teflon-shielded electrode could not be used because of the presence of the guide trocar in the cranium and the cervical column. Instead, the steel guide tube itself acted as the stimulating electrode. Using this modification, the vagal fibres to the heart were stimulated to produce cardiac slowing, which was blocked by atropine (1 mg/kg). Other signs of parasympathetic activity such as changes in pupil diameter and salivary secretion were no doubt also affected but these have not yet been measured.

Stimulation of the autonomic outflows in the cat

The cat has also proved to be a very suitable animal for this kind of stimulation. So far most observations have been on the cardio-accelerator and pressor responses, both of which were increased by stimulating the appropriate part of the thoraco-lumbar outflow. Stimulation of the sacral parasympathetic outflow produced contractions of the urinary bladder. A more complete investigation of the responses and pharmacological sensitivity of this animal is under way.

Discussion

The sensitivity to hexamethonium of all responses elicited by this method of stimulating the autonomic outflow confirms that stimulation is pre-ganglionic. The finding that the sacral parasympathetic is optimally stimulated opposite vertebrae

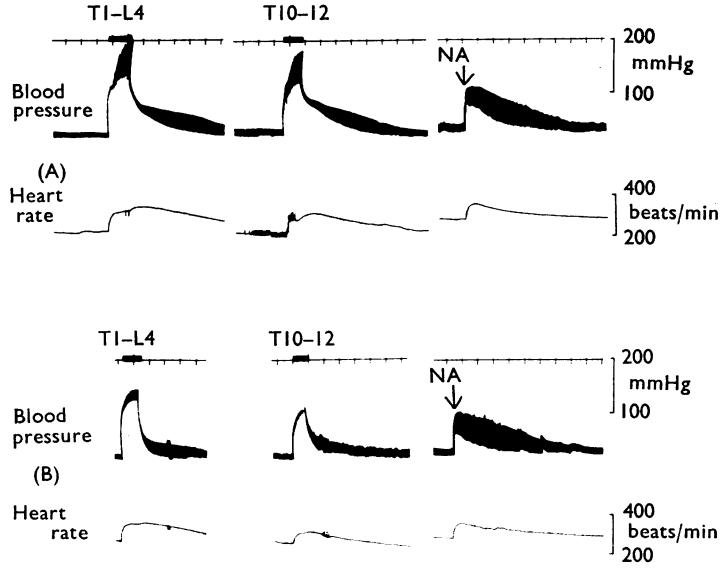

FIG. 9. Effects on the tubocurarine-treated pithed rat of supramaximal stimulation at 10 Hz of the thoraco-lumbar sympathetic outflow opposite T1-L4 and T10-12 for the periods indicated by the bars on the time trace, which shows 30 s intervals. The records from above downwards show in panel A the blood pressure and heart rate responses to stimulation and to injected noradrenaline (NA, 500 ng intravenously) and in panel B the blood pressure and heart rate responses to stimulation and to injected noradrenaline in the same animal (500 ng intravenously) following acute bilateral adrenalectomy, which reduced both the cardio-acceleration and the secondary sustained pressor response. The response to injected noradrenaline was unaffected by adrenalectomy.

L5-6, and sometimes extending to S1-2, suggests that at least in this region the preganglionic fibres in the nerve roots as they run within the vertebral canal may be the region of stimulation rather than their point of egress through the vertebral foramina. If this is so, there could be a discrepancy between the position of the stimulating electrode in terms of vertebrae and the nerve root stimulated. This discrepancy would be maximal in the sacral region and our findings suggest that in the rat, in this region, it is not greater than one or two vertebral segments. At higher levels, therefore, it is probably safe to equate the nerve roots stimulated with the position of the electrode.

The degree of localization of the stimulus seems highly satisfactory. This was particularly clear when stimulating the thoraco-lumbar outflow, where it was possible to select regions which activated a significant part of the vaso-motor outflow without stimulating either the fibres to the adrenals or the heart (Fig. 8); or to stimulate the vas deferens without affecting blood pressure (Fig. 6); or, finally, to stimulate the cardio-accelerator fibres without increasing peripheral resistance or blood pressure (Fig. 7).

This paper is intended primarily to describe the preparation and some simple pharmacological effects on it. It is clear, however, that several of the responses themselves will repay further study. For example, it was surprising to find vaso-constriction confined to the hindquarters from stimulation at L1-2, and cardio-acceleration from stimulation at C7-T1, while there was no rise in the general blood pressure, though the same animals when stimulated between T7-9 showed large rises in blood pressure. In this last position, the electrode stimulated the nerves to the adrenal glands with a consequent widespread effect on peripheral blood vessels and on the heart. It may be that in the pithed animal, either limited vaso-constriction of the vasculature or cardiac stimulation alone are insufficient to cause a marked rise in blood pressure. Either nerve stimulation must be widespread, as in the original technique of Gillespie & Muir (1967) or, if localized, must involve the fibres to the adrenal medulla.

The responses of the bladder and colon to parasympathetic stimulation also showed interesting differences. The rise in colonic pressure began after a long latent period, was slow and irregular and, when stimulation stopped, decayed slowly. The response was susceptible to fatigue if elicited more often than once every 5 min and, finally, it was very sensitive to ganglion blockade, so that even the dose of tubocurarine used was sufficient to interfere. The bladder, by contrast, contracted after a short latent period, pressure rose rapidly, was maintained for the period of stimulation, and declined rapidly when stimulation ceased. The bladder response was more resistant to fatigue, and less sensitive to ganglion blockade. These differences may well be due to the interposition of Auerbach's plexus, with its capacity for independent reflex activity, in the nervous outflow to the colon. It is likely that nerve stimulation triggers off the peristaltic reflex and it is the contraction resulting from this reflex which appears as the response. The time taken for the wave of contraction to travel from the point of excitation to the recording balloon would explain the long latent period. The slow rise and decay of pressure would correspond to the development and decay of the peristaltic reflex contraction, rather than the period of extrinsic nerve stimulation; and the greater sensitivity to ganglion blockade would indicate the involvement of other synapses. Waves of contraction consistent with such an explanation were observed during stimulation

with the abdomen open. The bladder, on the other hand, organizes its reflex emptying centrally, so that the parasympathetic outflow has a relatively simple, two-neurone path to the effector cells.

By stimulating the autonomic fibres at their origin, this simple technique avoids the confusing picture due to the anatomical mingling of sympathetic and parasympathetic fibres which often takes place in the periphery. It is therefore ideally suited for the study of regional variations in the sensitivity to drugs, and for the examination of the origin of the many cholinergic fibres which are often found in anatomically sympathetic peripheral nerves. In physiological studies the ability to examine separately the components of responses which are often centrally organized into a complex reflex may prove valuable ; thus, the ability to separate cardiac stimulation from sympathetic vasoconstriction and to vary the extent and localization of the latter may prove of interest.

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