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Pharmacological studies on the hypogastric ganglion of the rat and guinea-pig

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Summary

1. Preparations were developed whereby the hypogastric ganglion of the rat or guinea-pig was perfused through its vasculature with saline solutions. Drugs were injected into the perfusion stream, and their effects were indicated by contractions of the vas deferens. The base of this organ could be ligated to prevent the drugs reaching the smooth muscle via the blood vessels. For comparison, experiments were also performed on fully isolated preparations of the rat and guinea-pig hypogastric nerve-vas deferens, where the vas deferens was held in an inner bath, so that drugs added to the outer bath could act only on the ganglion. Attempts were made to demonstrate non-nicotinic receptors in these preparations.

2. It was shown that the perfused hypogastric ganglion of the guinea-pig would respond repeatedly to several nicotinic stimulants, though autodesensitization eventually occurred. The fully isolated preparation behaved similarly, but desensitized much more rapidly. In contrast, the rat ganglion, either perfused or fully isolated, was remarkably insensitive to nicotinic stimulant drugs.

3. Neither species responded well to non-nicotinic stimulants; that from the guinea-pig gave small contractions to methacholine in about 33% of cases, but did not respond to (4-m-chlorophenyl-carbamoyloxy)-2-butynyl trimethylammonium chloride (McN-A-343). With the rat, the situation was opposite.

4. The guinea-pig ganglion did not become more sensitive to non-nicotinic stimulants after some treatments known to sensitize the cat superior cervical ganglion. These include preganglionic tetanization, chronic decentralization, and removal of all potassium. Sensitization did occur, however, in the presence of physostigmine, after tachyphylaxis to dimethyl phenyl piperazinium iodide (DMPP) had developed, and when the preparation was perfused with a suspension of washed erythrocytes.

5. It is concluded that the responses of sympathetic ganglia vary from one species to another, and according to whether the organs are perfused or fully isolated.

Introduction

Perry & Reinert (1954) showed that after its preganglionic nerve had been cut, the superior cervical ganglion of the cat became super-sensitive to intra-arterially injected acetylcholine, and that this response was not blocked by methonium compounds such as hexamethonium, but was frequently potentiated. In addition, several of the

methonium compounds had stimulant actions on the chronically decentralized ganglion. In the same paper these authors also showed that when the normal ganglion was perfused with saline containing no potassium, it behaved as if it had been chronically decentralized, and became resistant to the blocking action of methonium compounds. Trendelenberg (1954, 1956) showed that histamine, pilocarpine, and 5-hydroxytryptamine all caused stimulation of the cat superior cervical ganglion. This action was resistant to nicotinic blocking drugs and was much more marked when the organ was perfused with blood than when saline was used. Since then the non-nicotinic receptors of sympathetic ganglia have been extensively studied and there is much evidence that these organs contain cholinoceptors which are resistant to nicotinic blocking drugs but which are blocked by atropine or raised calcium (Komalahirinya & Volle, 1962) and which can be revealed after a preganglionic tetanus (Trendelenberg & Jones, 1965), or treatment with anticholinesterase drugs (Takashige & Volle, 1962, 1963) or after chronic decentralization. Flacke & Gillis (1968) have suggested that muscarinic receptors play a part in normal ganglionic transmission. Holman, Muir, Szurszewski & Yonemura (1971) reported studies on the isolated hypogastric ganglion of the guinea-pig, the cells of which were impaled with microelectrodes. Both nerve stimulation and the iontophoretic application of several nicotinic drugs could cause depolarization, usually with the firing of action potentials, but no response was obtained to methacholine. The responses to nerve stimulation and to nicotinic stimulants were blocked by dihydro-β-erythroidine, and surprisingly, by atropine.

In 1967, Bentley & Smith described a preparation in which the vas deferens and the hypogastric ganglion of the guinea-pig could be perfused with saline solution via the vascular supply. In the present paper experiments will be described in which similar preparations from guinea-pigs and rats were used to study muscarinic and other non-nicotinic responses in the perfused hypogastric ganglion. For comparison other experiments were also done with fully isolated hypogastric nerve-vas deferens preparations (Hukovic, 1961 ; Graham, al Katib & Spriggs, 1968). A preliminary report of some of these findings has been published (Bentley, 1968).

Methods

Isolated hypogastric nerve-vas deferens preparations of the rat and guinea-pig

These were very similar to the preparations described by Hukovic (1961) and Graham *et al.* (1968) except that a modification was added to confine the direct action of added drugs to the ganglion. This was done by having a small, inner organ bath of about 10 ml capacity, made from a length of glass tubing sealed at the lower end by a piece of fine sheet rubber. This was held inside a larger bath of about 50 ml capacity. Both baths were filled with physiological saline of a composition given in a previous publication (Bentley, 1966). A needle-hole was made in the centre of this rubber membrane, and the distal end of the vas deferens was drawn through so that the major part of the organ lay within this small bath, while the lowermost 5 mm, plus the hypogastric ganglion and nerve, remained in the outer bath. A cotton tie was stitched through the vas deferens at the level of the rubber diaphragm, and attached to the holder (see Fig. 1). In this way, contractions were measured only from those parts of the organ within the inner bath. The hypogastric nerve was held between annular platinum electrodes, and stimulated for periods of 5 s every 2 min with supramaximal voltage. The frequency of stimulation was

alternated between 50 and 20 Hz for the guinea-pig and 50 and 10 Hz for the rat. Pulse duration was 0·5 mseconds. The upper end of the vas deferens was attached by a cotton tie to a frontal-writing lever, and contractions were recorded on a smoked drum. Both baths were maintained at 36° C, and were aerated with 95% oxygen, 5% carbon dioxide.

Perfused preparations

The method of setting up the perfused hypogastric ganglion, or perfused hypogastric ganglion plus vas deferens, is a modification of that previously described by Bentley & Smith (1967).

Young adult male guinea-pigs of 300–500 g weight, or rats of 200–300 g weight were stunned and bled. The posterior part of the body, from the lower border of the ribs, was rapidly skinned, and a midline incision was made into the abdominal cavity. The rectum was ligated and the whole intestinal canal was removed. Both iliac arteries were ligated just central to the origin of the femoral arteries.

A fine polyethylene cannula was then tied into the abdominal aorta so that the tip lay just distal to the origin of the inferior mesenteric artery. Perfusion with physiological saline was commenced at a rate of 0·5 ml/min using a Palmer micro-perfusion pump. The animal was then bisected immediately below the level of the kidneys and the anterior half was discarded. Both seminal vesicles were ligated close to

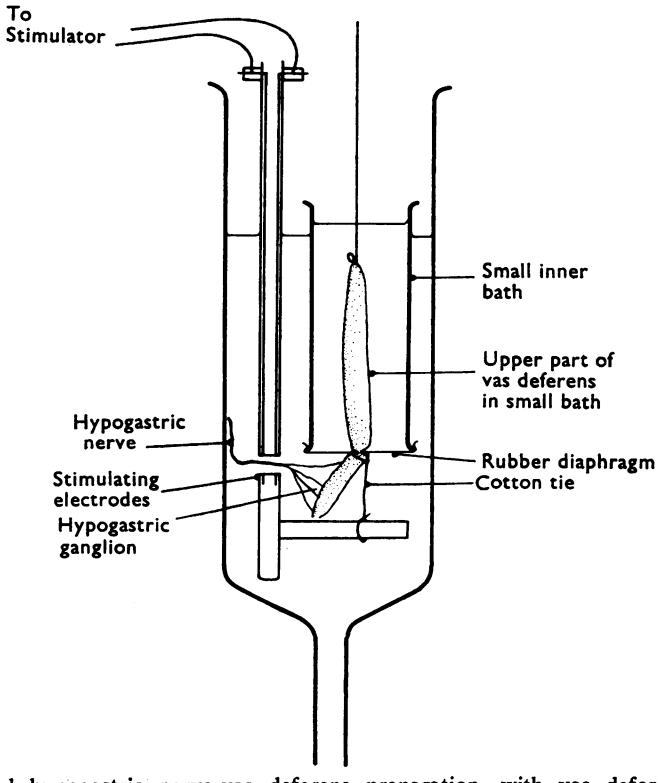

FIG. 1. Isolated hypogastric nerve-vas deferens preparation, with vas deferens held in a small inner bath. Drugs are added to the outer bath, and contractions are recorded only from that part of the vas deferens within the inner bath.

their base and were removed, and both ureters were ligated at their junction with the bladder. The testes were discarded, and a cotton tie was placed around the epididymal end of each vas deferens. The cremaster muscle was dissected away from the vas deferens, and the hypogastric nerve was identified and dissected free to about the base of the seminal vesicle.

A cotton ligature was then tied around the base of one vas deferens so as to prevent the perfusion fluid reaching this organ, but still permitting perfusion of the hypogastric ganglion. This ligature was then stitched into the dorsal wall of the abdomen, so that only the upper, unperfused part of the vas deferens was free to move, and it was from this that contractions were recorded. The vena cava was cannulated with a length of polyethylene tubing to carry off the venous effluent from the preparation. The contralateral vas deferens was usually not ligated, so that the perfusion fluid reached both the vas deferens and the hypogastric ganglion on this side. This acted as a control for the ligated preparation. In some experiments, this vas deferens was ligated and removed.

The whole preparation was then tied to a flat piece of Perspex and placed in a 1 litre vessel of physiological saline, bubbled continuously with 5% carbon dioxide, 95% oxygen. This was in turn placed in a thermostatically controlled bath at 37° C. The perfusing fluid, which was also aerated as above, was passed from the pump through a long coil of fine polyethylene tubing, immersed in the warming bath, before going to the preparation. The upper end of the tie from the top of the vas deferens was attached to the frontal writing lever, by which contractions were recorded on a smoked drum.

For most experiments, the physiological saline was the same as that used for the fully isolated preparations. In some cases, however, this was modified by the addition of 5% dextran plus washed cattle erythrocytes. This suspension was prepared from heparinized cattle blood, which had been centrifuged and the cells washed four times with physiological saline. They were then resuspended in saline containing 5% dextran, so that their concentration was 50% of the original level in the whole blood. This suspension was bubbled with 5% carbon dioxide–95% oxygen at 37° C for 1 h before use. The preparation was held in a bath of normal physiological saline without erythrocytes.

It was found that in most cases the vas deferens of the guinea-pig would still contract in response to electrical stimulation of the hypogastric nerve after the ligature had been tied around its base. Contractions were smaller than when the whole organ was perfused, because a shorter length of smooth muscle was involved in the response. Occasionally, however, no responses could be obtained, presumably because the intramural nerves had been damaged by the tie, and these preparations were discarded. With the rat, failures were more frequent.

All drugs were given in a volume of 0·1 ml, and were dissolved in physiological saline. Nicotinic stimulants were never given at less than 4 min intervals, and with non-nicotinic drugs, a minimum period of 30 min was allowed between doses, as these substances are notorious for causing auto- and cross-desensitization (Trendelenburg, 1956 ; Jones, 1963 ; Smith, 1966).

At the conclusion of all experiments a small quantity of ink was injected into the perfusion stream. Only those experiments were accepted in which there were no leaks from the preparation, and the ink was seen to perfuse the hypogastric ganglion.

Decentralized hypogastric ganglion

Rats or guinea-pigs were anaesthetized with either halothane or ether. The abdominal cavity was opened under aseptic conditions by a low midline incision, and the hypogastric nerves were identified. The nerve on one side, usually the left, was cut about 1 cm below the inferior mesenteric ganglion. The abdominal wound was sutured with cotton, and the animals were allowed to recover. They were used 2–5 weeks later. In all cases, the seminal vesicles on the side of the sectioned nerve were markedly distended, and no response could be obtained from the vas deferens when a stimulus was applied to the stump of the nerve proximal to the cut.

In some cases, recordings were made from both innervated and decentralized sides, while in a few cases only the latter was used.

Drugs used were: acetylcholine chloride (B.D.H.); acetyl β-methyl choline chloride (Methacholine) (L. Light & Co); angiotensin, val-5-hypertensin 11-asp-β-amide (Hypertensin) (Ciba); atropine sulphate (Drug Houses of Australia); bradykinin triacetate trihydrate (Koch-Light); carbamyl choline chloride (CCh) (B.D.H.); dimethyl phenyl piperazinium iodide (DMPP) (K & K Laboratories); hexamethonium bromide (Koch-Light); histamine acid phosphate (B.D.H.); 5-hydroxytryptamine creatinine sulphate (Sigma Chemicals); McN-A-343 (4-m-chlorophenylcarbamoyloxy)-2-butynyl trimethylammonium chloride (McNeil Laboratories); nicotine tartrate (B.D.H.); physostigmine sulphate (B.D.H.); tetramethyl ammonium chloride (TMA) (B.D.H.).

Results

Electrical stimulation

Isolated hypogastric nerve-vas deferens preparations, with inner bath

Electrical stimulation of the hypogastric nerve with supramaximal voltage at frequencies of 50 Hz elicited large regular contractions with both rat and guinea-pig preparations. The rat also responded well to stimuli at 10 Hz, giving contractions only slightly smaller than at 50 Hz. The guinea-pig, however, seldom responded adequately to stimuli at 10 Hz, and the contractions always became much smaller after a short time. In most cases, a frequency of 20 Hz was necessary to provide reliable and reproducible responses.

TABLE 1. *Guinea-pig isolated hypogastric nerve–vas deferens preparation with inner bath. Effects of nicotinic and muscarinic stimulant drugs added to outer bath (that is acting on ganglion only)*

Stimulant drugs	No. of preparations contracting	Mean response (% of hg. stim.)
(a) Nicotinic stimulant		
DMPP, 1×10^{-4}	10/14	$\bar{X}_{10}=71{\cdot}2$
DMPP, 1×10^{-4}+atropine 1×10^{-6}	9/9	$\bar{X}_{9}=73{\cdot}3$
CCh, 1×10^{-4}	3/4	$\bar{X}_{3}=92$
CCh, 1×10^{-4}+atropine 1×10^{-6}	13/13	$\bar{X}_{13}=67{\cdot}6$
TMA, 1×10^{-4}	16/22	$\bar{X}_{16}=67{\cdot}2$
(b) Muscarinic stimulant		
Methacholine 1×10^{-4}	2/17	$\bar{X}_{2}=10{\cdot}3$
McN-A-343	0/6	—

Responses expressed as percentage of maximal contractions produced by stimulation of the hypogastric nerve (hg).

Nicotinic stimulant drugs. Nicotinic stimulant drugs, added to the outer bath, usually caused strong contractions of the vas deferens of the guinea-pig. DMPP, carbamyl choline and tetramethyl ammonium chloride (TMA) all gave contractions averaging 64–92% of the maximal responses to hypogastric nerve stimulation. These results are set out in Table 1. In the presence of atropine (1 μg/ml), the mean responses to DMPP were not altered, while with carbamyl choline there was a small reduction. Tachyphylaxis was always very marked, and it was most unusual to obtain any response after the third or fourth addition of the drug, even when these were spaced at 5 or 10 min intervals (Fig. 2). However, when the preparation had become completely resistant to nicotinic drugs, it would still contract maximally in response to stimulation of the hypogastric nerve. The isolated hypogastric nerve-vas deferens from the rat was completely unresponsive to TMA (100 μg/ml) in nine experiments, and to DMPP (100 μg/ml) in another experiment.

Nicotinic blocking drugs. When hexamethonium was added to preparations isolated from the guinea-pig and stimulated via the hypogastric nerve alternately at 20 and 50 Hz, the depressant effect was always greater on the low frequency response. Hexamethonium (100 μg/ml) caused complete block of the responses to stimulation at 20 Hz in three out of five cases, but failed to block responses to stimulation at 50 Hz, though varying degrees of depression occurred. The addition of atropine (2 μg/ml) did not increase this block at all.

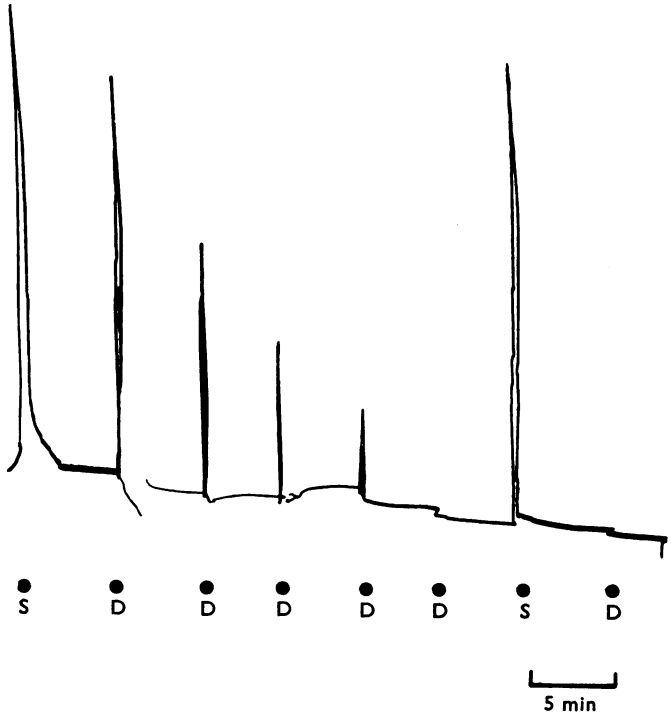

FIG. 2. Guinea-pig isolated hypogastric nerve-vas deferens, with inner bath. At S, electrical stimulation of the hypogastric nerve at 50 Hz for 5 s with supramaximal voltage. At D, DMPP added to outer bath to a concentration of 100 μg/ml. Tachyphylaxis to DMPP is rapid and complete, but the response to nerve stimulation (50 Hz for 5 s) is undiminished.

Four preparations isolated from the rat were tested. Hexamethonium (100 μg/ml) caused 25, 82 and 92% reduction, and at 200 μg/ml it caused only a 16% reduction in the fourth experiment.

Non-nicotinic stimulant drugs. Non-nicotinic stimulant drugs were always tested 2 min after a 20 s preganglionic stimulus applied to the hypogastric nerve, at a frequency of 50 Hz. This procedure potentiates the effects of these drugs (Jones, 1963; Trendelenburg & Jones, 1965).

McN-A-343 (100 μg/ml) had no stimulant action at all on the guinea-pig hypogastric ganglion in the six cases tested. Methacholine (100 μg/ml) was similarly quite inactive in a further five experiments, and in ten others where the preparations had been soaked for 15–20 min in physostigmine (2 μg/ml). The only effect of methacholine noted was that in three cases it caused a marked increase in the size of the responses to electrical stimulation at 20 Hz. In another four experiments, where hexamethonium had reduced the response to electrical stimulation, this was partly reversed by the addition of methacholine.

The preparation isolated from the rat was also quite unresponsive to methacholine and to McN-A-343 both at concentrations of 100 μg/ml, even in the presence of physostigmine, 2 μg/ml (6 experiments) although a small contraction of the vas deferens occurred in one out of 2 cases with McN-A-343 250 μg/ml plus physostigmine.

Perfused preparations

The perfused preparations of both the rat and guinea-pig responded to electrical stimulation of the hypogastric nerve in much the same way as the fully isolated preparation. The rat gave adequate and repeatable responses to stimulation at 10 Hz, while in the guinea-pig stimulation at a minimum of 20 Hz was required. In most guinea-pig preparations, ligating the base of the vas deferens had little effect on the responses, although, because a shorter length of muscle was involved in the recorded contraction, the responses were always somewhat smaller than on the unligated side. With the rat, however, ligatures adequate to occlude the perfusion sometimes seriously depressed the electrically induced response. However, sufficient experiments were obtained to test several drug responses.

Nicotinic stimulants. In the guinea-pig, acetylcholine, carbamyl choline, DMPP, nicotine and TMA at doses of 20–40 μg all produced strong contractions of the ligated vas deferens when injected at 5 min intervals into the fluid perfusing the hypogastric ganglion. The contractions were seldom less than 50% of the maximal response to stimulation of the hypogastric nerve, and usually were larger. In the presence of atropine, the response to TMA was not reduced, though with carbamyl choline the mean response was somewhat less. These results are summarized in Table 2.

With all these drugs tachyphylaxis almost always appeared though its onset was slower than in the isolated preparation. All of the nicotinic stimulants were capable of causing complete auto-desensitization, but the number of doses required varied considerably and sometimes there were residual responses after twenty doses of the drug. TMA was noticably slower to cause complete tachyphylaxis, while the other drugs were all about equally effective. Cross-tachyphylaxis to other nicotinic stimulants was always present when tested for, and no spontaneous recovery was seen even after withholding the drugs for up to one hour.

As with the isolated preparations, the response to hypogastric nerve stimulation was unaltered after auto-desensitization to the nicotinic drugs had occurred (Fig. 3).

The rat preparation behaved differently from the guinea-pig, and seldom responded to nicotinic stimulants. In six experiments using the ligated vas deferens, there were no responses to DMPP, 20 μg, and in eight other cases, there were only two responses to TMA 20 μg (7 and 30% of the maximal electrically-induced contraction). Where the vas deferens was not ligated, there were only three contractions

TABLE 2. *Guinea-pig perfused hypogastric ganglion, with ligated vas deferens. Effects of nicotinic and non-nicotinic stimulant drugs injected into perfusion stream*

Stimulant drugs	No. of preparations responding	Mean response (% of hg. stim.)
1. Nicotinic stimulant (20-40 μg)		
ACh	6/6	67·3
CCh	10/10	75·3
CCh+atropine 1×10^{-6}	4/5	53
DMPP	10/10	98·5
Nicotine	10/10	96·3
TMA	10/10	86·0
TMA+atropine 1×10^{-6}	8/8	98·7
2. Non-nicotinic stimulant		
Methacholine (100-200 μg)	13/38	18·6
McN-A-343 (200-1,000 μg)	0/13	—
Histamine (1,000 μg)	2/10	15·5
5-hydroxytryptamine (100 μg)	0/2	—
Angiotensin (100 μg)	0/2	—
Bradykinin (50 μg)	0/2	—

Responses expressed as percentage of maximal contraction in response to electrical stimulation of hypogastric nerve (hg).

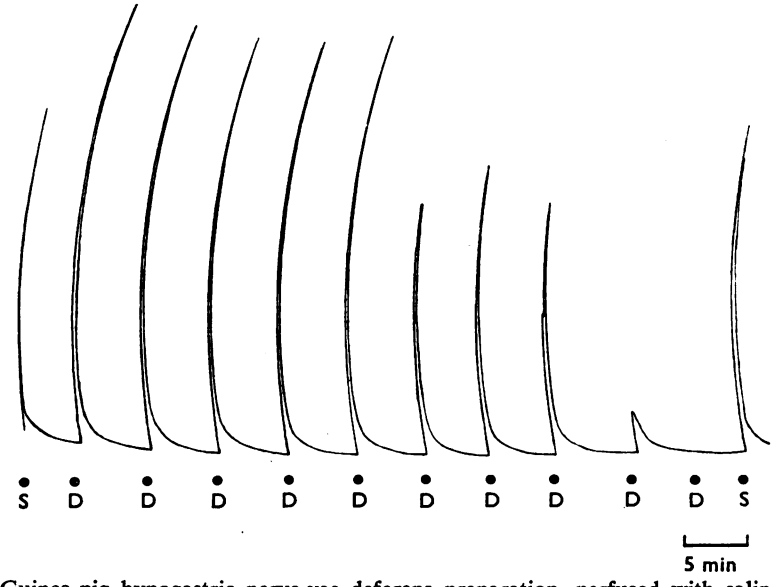

FIG. 3. Guinea-pig hypogastric nerve-vas deferens preparation, perfused with saline through its vasculature. At S, electrical stimulation of the hypogastric nerve at 50 Hz, with supramaximal voltage for 5 s. At D, DMPP 20 μg injected into perfusion stream. Tachyphylaxis is much slower than in the isolated preparation, and nerve stimulation still elicits a large contraction.

in six experiments and the preparation invariably developed complete auto-desensitization after one dose of the stimulant drug. However, in all these experiments, large contractions were repeatedly obtained in response to electrical stimulation of the hypogastric nerve, both before and after the nicotinic drugs (Fig. 4).

Nicotinic blocking drugs. The guinea-pig perfused hypogastric ganglion was more susceptible to blockade by hexamethonium than was the isolated preparation. When perfused with this drug at 10 μg/ml, the response to nerve stimulation was completely blocked in five experiments, and depressed to varying degrees in four others. The response to TMA was completely blocked by hexamethonium (10 μg/ml) in

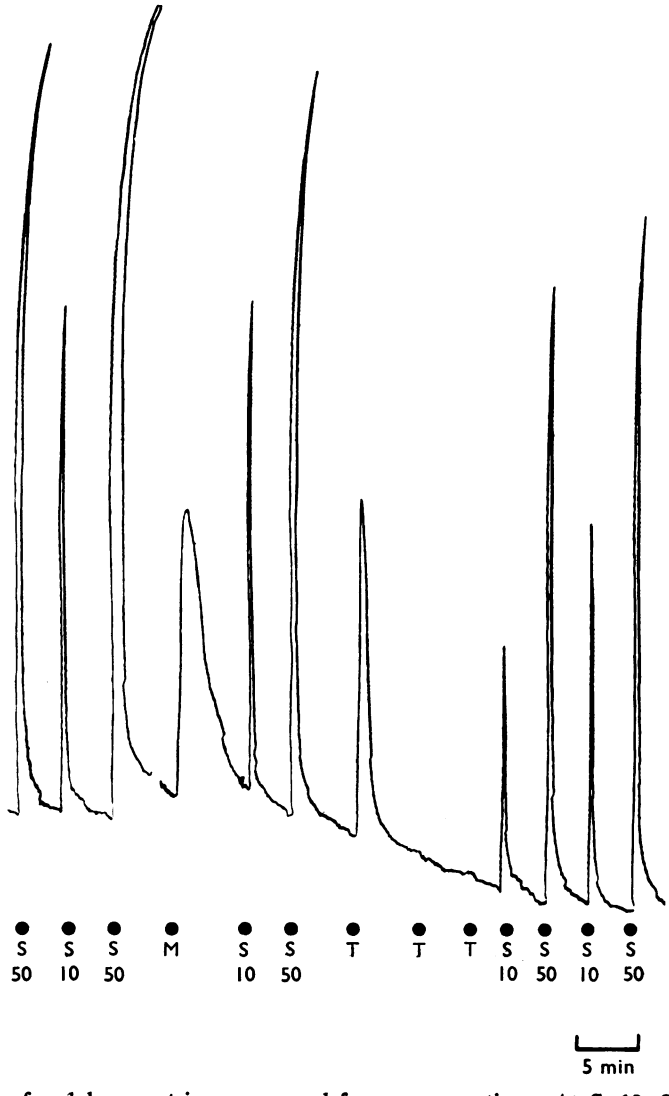

FIG. 4. Rat perfused hypogastric nerve-vas deferens preparation. At S, 10, S, 50 electrical stimulation of hypogastric nerve at 10 and 50 Hz respectively, with supramaximal voltage for 5 seconds. At M, injection of 200 μg McN-A-343 into perfusion fluid. At T, injection of TMA, 20 μg. Moderate but prolonged response to McN-A-343, only one response to TMA, but nerve stimulus still effective.

six experiments and by 20 μg/ml in another three. Nicotine (20 μg/ml) also caused complete block in each of four experiments.

The rat preparation behaved differently. Hexamethonium (10 μg/ml) had no effect on the response to hypogastric nerve stimulation in three experiments and caused only a 14% depression in a fourth. A concentration of 100 μg/ml caused a mean depression of 60% (range 18–93%, four experiments).

Non-nicotinic stimulants. The non-nicotinic stimulants were given 1–2 min after a 20 s tetanic stimulus applied to the hypogastric nerve.

The guinea-pig perfused hypogastric preparation was completely insensitive to McN-A-343 in doses from 200 μg to 1 mg in thirteen experiments. Furthermore, this drug had no effect on the response to hypogastric nerve stimulation in five experiments, nor did it restore responses blocked by hexamethonium, in two others. Methacholine (100–200 μg) caused the vas deferens to contract in only thirteen of thirty-eight experiments. These responses were usually small, averaging only 18·6% of the maximal response to nerve stimulation (range 2·5–80%). Methacholine, when given during electrical stimulation of the hypogastric nerve had varying effects on the contractions. In four out of nine cases the responses to 50 Hz stimulation were increased from 6 to 200% while with stimulation at 20 Hz, increases of 12 to 250% were seen in six out of nine cases. This effect never lasted longer than 4 minutes. After partial blockade with hexamethonium 10 μg/ml, methacholine again caused a brief increase in three out of four cases, the effect being greater on the low frequency stimulation. The transitory nature of this effect made quantitative estimates difficult.

Histamine also produced little stimulation of the perfused ganglion. Doses of 100 μg caused small contractions of the vas deferens in only two out of ten experiments (8 and 23% of the nerve-induced response). Histamine caused only a brief increase in the response to the 20 Hz stimulation in four out of seven cases (25–100%), but to the 50 Hz stimulation in only two of these. After partial block with hexamethonium, histamine similarly caused only small, brief increases in the contractions to 20 Hz stimulation in two out of three cases and to 50 Hz stimulation in one case.

5-Hydroxytryptamine (100 μg), angiotensin (100 μg) and bradykinin (50 μg) all failed to cause the vas deferens to contract.

The perfused preparation from the rat also differed from the guinea-pig, in that McN-A-343 (200 μg) caused small contractions in five out of eight cases where the

TABLE 3. *Rat perfused hypogastric ganglion, with or without ligature at base of vas deferens. Effects of non-nicotinic drugs injected into perfusion stream*

Stimulant drugs	No. of preparations responding	Mean response (% of hg. stim.)
Nicotinic stimulants		
DMPP (20-40 μg)	0/6	—
TMA (20-40 μg)	2/8	18·5
Non-nicotinic stimulants		
Methacholine (200 μg)*	2/9	8·5
McN-A-343 (200 μg)		
(i) Ganglion only perfused	5/8	12·4
(ii) Vas deferens also perfused	5/6	21·0

Responses expressed as percentage of maximal contraction in response to electrical stimulation of the hypogastric nerve (hg). * Ganglion only perfused.

vas deferens was ligated (mean response, 12·4% of maximal electrically induced response) and where the vas deferens was not ligated, in five out of six cases (mean, 21% of electrically-induced maximum) (Fig. 4). With methacholine, however, only two responses were seen in nine experiments. Other non-nicotinic stimulant drugs were not tested on the rat ganglion (see Table 3).

Effect of physostigmine

The perfused preparation from the guinea-pig remained completely insensitive to McN-A-343 (200 μg) after perfusing with physostigmine (2 μg/ml) in ten experiments, in five of which the base of the vas deferens had been ligated. The effects of methacholine, however, were usually increased by physostigmine. In eleven experiments with this drug, the contractions caused by methacholine were increased up to 5-fold in eight cases, were decreased in one case, and were unchanged in the remaining two experiments. Physostigmine had insignificant effects on the responses evoked by TMA or electrical stimulation of the hypogastric nerve.

With the rat preparation the effect of methacholine was potentiated by physostigmine (2 μg/ml) only once in seven experiments, and McN-A-343 was depressed in two out of four, and not changed in the remaining two experiments.

Effect of chronic decentralization

When tested 2–5 weeks after cutting the hypogastric nerve, the guinea-pig vas deferens would not contract in response to electrical stimulation central to the nerve section, although large responses were obtained if the electrodes were applied to the region of the hypogastric ganglion. Decentralization did not increase the sensitivity to McN-A-343, as no response was obtained to injections of 500 μg to 1 mg in three experiments. Similarly, the sensitivity to methacholine was not increased by decentralization, as this drug caused small contractions in only two out of ten cases.

Nicotinic stimulants also were less effective on decentralized than on normal ganglia; for instance TMA at doses of 20–40 μg caused contractions in only five out of ten preparations, and in all but one of these complete autodesensitization occurred after two or three doses. Nicotine (20 μg) also caused contractions in only one of three cases.

Using rat perfused hypogastric ganglia, denervated 2–5 weeks previously, again no response was seen when electrical stimulation was applied central to the nerve section although the vas deferens contracted when the stimulus was applied to the region of the ganglion. As with the guinea-pig, decentralization caused little increased sensitivity to muscarinic stimulants; both methacholine 100–200 μg and McN-A-343, 200 μg produced contractions in only three out of seven experiments, and with the exception of one methacholine response, these were all very small. Nicotinic stimulants, as in the normal ganglion, were also ineffective. TMA (20–40 μg) caused only one small contraction in six experiments, and DMPP was effective in one of two experiments.

Responses after tachyphylaxis to nicotinic stimulants

After repeated doses of DMPP, even before any marked tachyphylaxis had developed, the guinea-pig hypogastric ganglion became much more sensitive to methacholine (Fig. 5). A greater proportion of preparations responded (ten out of

thirteen) and the contractions of the vas deferens were almost always larger than in preparations not pretreated with DMPP. The mean response of the ten cases that contracted with methacholine was 64·2% of the maximal response to nerve stimulation. However, none of the other nicotinic drugs caused any significant increase in sensitivity. The number of responses to methacholine and their mean size after other nicotinic drugs was as follows: after carbamyl choline, 0/2, nicotine 2/8 (mean 52%) and TMA, 1/5 (5%). Histamine also became more effective after DMPP and the responses to this drug were as follows: after carbamyl choline, 1/5 (8%), carbamyl choline plus atropine, 5/12 (mean 22%), nicotine, 7/12 (mean 5·6%), TMA, 0/4, TMA plus atropine, 0/4, DMPP 5/7 (mean 22%) and DMPP plus atropine, 10/12 (mean 60·2%). Blockade by hexamethonium did not alter the sensitivity of the guinea-pig preparation to either histamine or methacholine.

The rat preparation was not tested after nicotinic tachyphylaxis.

Effect of removing potassium

Only the guinea-pig preparation was used in these experiments. Preliminary responses were obtained to stimulation of the hypogastric nerve at a frequency of 50 Hz for a period of 5 s with supramaximal voltage, and to doses of acetylcholine,

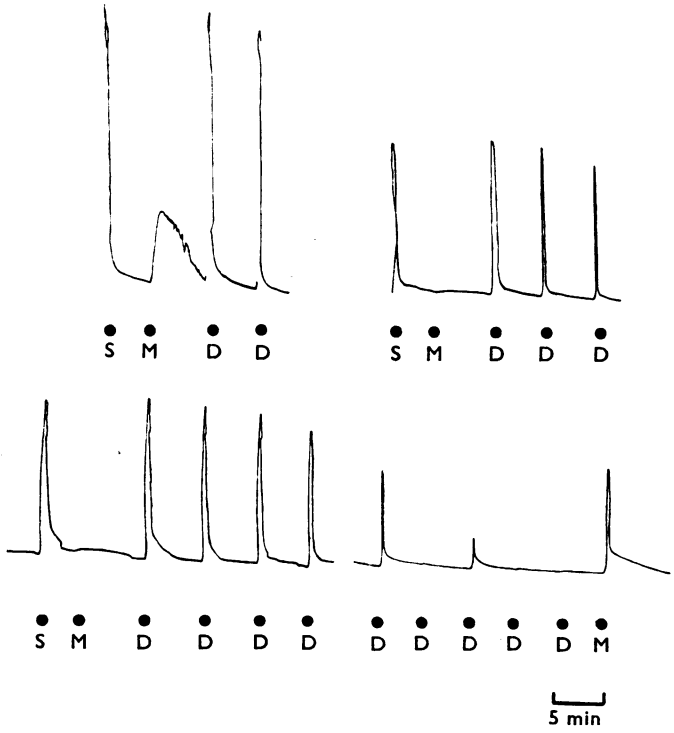

FIG. 5. Guinea-pig perfused hypogastric nerve-vas deferens preparation. Upper left panel, no ligature on vas deferens, upper right panel and lower panels, with ligature. At S, electrical stimulation of hypogastric nerve at 50 Hz, with supramaximal voltage for 5 s. At M, methacholine 100 μg injected into perfusion stream, and at D, DMPP 20 μg injected. Lower right-hand panel is a continuation of left-hand panel, with repeated doses of DMPP. Upper panels show lack of ganglionic stimulation with methacholine, but small, prolonged contraction when the drug acts directly on the smooth muscle. The lower panels show the sensitizing action of DMPP (total of twenty-one doses) on methacholine.

100 μg. These stimuli were applied alternately at 2 min intervals. When the responses were steady, a single dose of 100 μg hexamethonium was given and the stimuli were repeated. When the depressant effects of hexamethonium had worn off, the preparation was perfused with this drug at a concentration of 100 μg/ml until its effect had reached a plateau, when it was removed and the stimuli continued until complete recovery had occurred. The perfusion was then replaced with physiological saline completely lacking in potassium, and the same solution was placed in the bath containing the preparation. The above procedure was repeated in the potassium-free solution, with both the acetylcholine and hexamethonium also dissolved in the same solution. Because this programme took several hours, there was a risk of fatigue complicating the results, so shorter experiments were also done, in which hexamethonium was not perfused until the preparation was in the potassium-free solution.

When the concentration of potassium was normal, it was found that a single dose of hexamethonium briefly depressed both the response to hypogastric nerve stimulation, and to acetylcholine, the latter usually being the more affected. Recovery was complete in 10–15 minutes. When hexamethonium was perfused at a concentration of 100 μg/ml, complete blockade of both stimuli always occurred within 4 minutes. Where the vas deferens was also being perfused, a small slower direct response to acetylcholine remained. Again, recovery was fairly rapid when the perfusing fluid no longer contained hexamethonium.

When perfused with potassium-free saline the preparation continued to respond both to nerve stimulation and to acetylcholine, with negligible alteration in size or

TABLE 4. *Comparison of rat and guinea-pig hypogastric ganglion and cat superior cervical ganglion*

Treatment	Isolated hypogastric ganglion		Perfused hypogastric ganglion		Cat superior cervical ganglion
	Rat	Guinea-pig	Rat	Guinea-pig	
Nicotinic stimulants	No effect	Stimulate, but very rapid tachyphylaxis	Seldom stimulate then one response only	Stimulate with slow tachyphylaxis	Stimulate
Methacholine	No effect	No effect	Usually no effect	Small response in 13/38 cases	Stimulate (Pappano & Volle, 1965)
McN-A-343	No effect	No effect	Small response in 5/8 cases	No effect	Stimulate (Roszkowski, 1961)
Histamine	Not tested	Not tested	Not tested	Small response in 2/10 cases	Stimulate (Trendelenburg, 1954)
Physostigmine	No potentiation of McN-A-343	No potentiation of methacholine	Methacholine potentiated 1/7 McN-A-343 depresses 2/4	Potentiates methacholine, not McN-A-343	Potentiates methacholine, depresses McN-A-343 (Smith, 1966)
No potassium	Not tested	Not tested	Not tested	Drug responses not changed	Hexamethonium stimulates, blocks nervous conduction but not ACh (Perry & Reinert, 1954)
Chronic decentralization	Not tested	Not tested	No increased sensitivity to McN-A-343	No increased sensitivity to McN-A-343 or methacholine. Decreased sensitivity to TMA	Increased sensitivity to acetylcholine, resistance to hexamethonium (Perry & Reinert, 1954)
Perfusion with red blood cells	—	—	No increased response to McN-A-343 or TMA	Increases sensitivity to methacholine, not to McN-A-343 or histamine	Increased sensitivity to non-nicotinic stimulants (Perry & Reinert, 1954)

duration of contractions. In seven experiments, there was no sign whatsoever that hexamethonium (100 μg) caused any stimulation of the ganglion, or that its ability to depress either the response to nerve stimulation or to acetylcholine was less than in the presence of potassium. Furthermore when hexamethonium (100 μg/ml) in potassium-free solution was perfused, there was a complete blockade of both the contractions caused by acetylcholine and by stimulation of the hypogastric nerve. This block occurred as rapidly as in normal physiological saline.

Effect of perfusing with washed cattle erythrocyte suspensions

In preparations perfused with washed cattle erythrocytes, methacholine (200 μg) caused contractions of the vas deferens in five out of six cases (mean response, 52·4% of the maximum response to stimulation of the hypogastric nerve) and this response was augmented in the presence of physostigmine 2 μg/ml in two out of three cases. McN-A-343, however, was still without effect, before or after physostigmine in four experiments, as was histamine in another four cases. Perfusion with erythrocytes did not alter the response to stimulation of the hypogastric nerve, although the contractions in response to TMA fluctuated unpredictably from zero to large responses throughout most experiments. The effect of physostigmine also was unpredictable, augmenting the response to TMA in two out of three experiments, and causing both increases and decreases in the electrically induced response in four other cases.

The rat preparation perfused with erythrocyte suspensions responded to 200 μg of McN-A-343 in only two of five experiments with the vas deferens ligated, and in one of two cases where the vas deferens was perfused. With methacholine, contractions were recorded only in one experiment out of four. TMA still had little activity, causing contractions in only two out of five ligated preparations, and one out of two without the tie on the vas deferens. In every case, only one response was obtained. Electrical stimulation of the hypogastric nerve produced large, reproducible contractions in every case.

Discussion

The perfused hypogastric ganglion of the guinea-pig with or without the vas deferens also in the perfusion circuit, is not difficult to set up, although with the rat it is not always easy to avoid leaks from some very small arteries in the pelvic region. Also, the sympathetic pathways within the walls of the vas deferens in this species appear to be rather susceptible to damage from the ligature that is placed at the base of this organ to exclude the perfusion fluid. Thus there are more frequent failures of the ligated rat vas deferens to respond to electrical stimulation of the hypogastric nerve. For this reason, most experiments reported in this paper were done using the guinea-pig.

Both the fully isolated and the perfused preparations can be used to study the effects of drugs on the hypogastric ganglion. The direct effects of the agonist drugs on the smooth muscle of the vas deferens can be prevented by using the small inner bath with the isolated preparation, or by placing a ligature around the base of the perfused vas deferens. In both cases, the contractions are recorded only from that portion of the vas deferens that is not in direct contact with the drugs under study. With the perfused preparations, the contralateral, unligated vas deferens may be used as a control to demonstrate any direct actions of drugs on the smooth muscle. A

further check is provided by the injection of ink into the perfusion fluid, which confirms that the region containing the ganglion is being perfused, and that the drugs are not leaking through the ligature.

Perfused preparations are almost certainly better oxygenated that the fully isolated ones, especially when the suspension of washed erythrocytes is used. It was noted that the red cells leaving the preparations were always much darker in colour than those being pumped in, which indicated that considerable amounts of oxygen were being removed from the perfusion fluid. The possibility thus exists that when plain saline is used for the perfusion, the oxygen supply, while better than with isolated preparations, is still less than optimal. This may be a major factor in explaining the differences in behaviour between the fully isolated preparations, and those perfused with erythrocytes. Other advantages of a preparation which is perfused, with or without the addition of red blood cells to the fluid are (i) drugs may be presented either briefly as a single dose, which is rapidly washed out, or they may be perfused continuously; (ii) injected drugs are carried more closely to their site of action, and do not have to diffuse from outside the tissue; (iii) the preparation is more sensitive to drugs.

The perfused guinea-pig hypogastric ganglion differed from the isolated one in that it was much more resistant to the autodesensitizing action of nicotinic stimulant drugs, probably because drugs injected into the perfusing stream are in contact with the ganglion cells much more briefly than when added to the isolated organ in a bath.

Several differences have been demonstrated between the guinea-pig and rat perfused preparations. The preparation from the rat is surprisingly insensitive to nicotinic stimulant drugs. Whereas the guinea-pig vas deferens will almost always contract in response to repeated doses of acetylcholine, carbamyl choline, DMPP, nicotine or TMA injected into the fluid perfusing the hypogastric ganglion, the rat preparation usually fails to respond at all, and then contracts only with the first dose and thereafter remains completely insensitive to these drugs. The guinea-pig preparation does ultimately become insensitive, but this occurs gradually and in many experiments contractions were still elicited after twenty or more doses. TMA, as might be expected from a small molecule (Paton, 1961), was noticeably less active in causing tachyphylaxis. This autodesensitization is quite distinct from conventional ganglionic blockade, for example, by hexamethonium. With this drug, the effects of nicotinic stimulants and of nerve stimulation are both blocked, while after autodesensitization, nerve stimulation is still fully effective. This suggests that the injected drugs do not act on the same receptors as does the transmitter released from the nerve endings. A second difference between the rat and guinea-pig perfused preparations is that the rat will respond to McN-A-343, while the guinea-pig appears to be completely insensitive, even after procedures that have been shown to sensitize the cat superior cervical ganglion to muscarinic stimulants. These include preganglionic tetanization, chronic decentralization, and treatment with anticholinesterase drugs. The rat ganglion, on the other hand, is surprisingly, rather insensitive to methacholine, while the guinea-pig vas deferens frequently contracted in response to this drug, although these responses were usually small. They were considerably larger, however, in preparations treated with anticholinesterase drugs. The rat differed from the guinea-pig in a third respect, as it was much less sensitive to the blocking actions of hexamethonium. This is consistent with the findings of Graham

et al. (1968) and with an earlier report of Whyte (1964) that hexamethonium is very ineffective in blocking the carotid occlusion reflex in the rat. With the guinea-pig, both methacholine and histamine sometimes caused an increase in the response to electrical stimulation of the hypogastric nerve. This effect never lasted more than 4 min and was always greater with the low frequency stimulation. McN-A-343 was never observed to cause this facilitation. Preparations from the rat were not tested in this manner.

After chronic decentralization of the guinea-pig hypogastric ganglion, the vas deferens would still contract in response to nicotinic stimulant drugs, although these contractions were smaller than normal, and a number of failures were encountered. Also, tachyphylaxis always developed very rapidly. Decentralization did not increase the sensitivity of either the rat or guinea-pig ganglion to muscarinic stimulants.

The effect of removing potassium from both the perfusing fluid and the saline in the outer bath showed that the guinea-pig hypogastric ganglion behaved differently from the cat superior cervical ganglion. With the latter, Perry & Reinert (1954) reported that in the absence of potassium methonium compounds had a stimulant action of their own, and no longer blocked the effect of acetylcholine, though they did block the response to preganglionic nerve stimulation. With the guinea-pig, however, hexamethonium caused no stimulation whatsoever in the absence of potassium, and it blocked the response to both injected acetylcholine and to stimulation of the hypogastric nerve as effectively as in normal physiological saline. It is possible, of course, that even after perfusing the guinea-pig preparation with potassium-free saline for several hours, some of this ion might still be leaking from intracellular stores. Nevertheless, since the procedure used in the present study is identical with that of Perry & Reinert the differences in the behaviour of the cat and guinea-pig ganglia would seem to be real.

When washed cattle erythrocytes plus dextran were included in the perfusion fluid, the guinea-pig preparations became more responsive to methacholine. Responses to this drug were obtained in all five cases tested, and they were larger on the average than when plain saline was used. This is in agreement with Trendelenburg's finding with the cat superior cervical ganglion. However, the guinea-pig was still completely insensitive to McN-A-343 in the erythrocyte suspension even with doses up to 1 mg. This is in agreement with Smith (1966) who showed that McN-A-343 had little pressor effect in this species.

The increased sensitivity of the guinea-pig ganglion to methacholine and histamine after tachyphylaxis had developed to DMPP is surprising, especially as none of the other nicotinic stimulants, or hexamethonium caused this sensitization. Perry & Reinert (1954) believed that the increased responsiveness of the decentralized cat superior cervical ganglion to the muscarinic effects of acetylcholine involved a change in the metabolism of the ganglion cells after denervation. This in turn affected their ability to concentrate potassium intracellularly. Since it has been shown in the present paper that the guinea-pig hypogastric ganglion does not show the ' effect of denervation ' in the absence of potassium, it is unlikely that the sensitizing effect of DMPP involves an action on the disposition of this ion. DMPP has another action not shared by the other stimulants tested, namely its ability to block post-ganglionic sympathetic nerves, and to potentiate noradrenaline (Bentley, 1962). In this respect it resembles quanethidine, and it seems possible that its ability to

sensitize the ganglion to methacholine may involve the removal of an adrenergic inhibitory effect. Experiments are under way to investigate this further.

The results reported here are consistent with some of the findings reported by Holman *et al.* (1971), who were unable to detect any stimulant action of McN-A-343 on individual cells of the guinea-pig hypogastric ganglion, even after a preganglionic tetanus. They were also unable to show any response to methacholine. Even more surprising was their finding that atropine blocked the response to nicotinic stimulant drugs and to electrical stimulation of the hypogastric nerve. In the present study, as well as in an earlier paper (Bentley, 1966) it was shown that atropine caused only a very slight reduction in the contractions of the vas deferens in response to either nerve stimulation or nicotinic drugs.

The results from the perfused ganglion preparations indicate that it may be unwise to extrapolate findings in fully isolated ganglion preparations to *in vivo* conditions and from one species to another.

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REFERENCES

BENTLEY, G. A. (1962). Studies on sympathetic mechanisms in isolated intestinal and vas deferens preparations. *Br. J. Pharmac. Chemother.*, **19**, 85–98.

BENTLEY, G. A. (1966). The effect of local anaesthetic and anti-adrenaline drugs on the response of sympathetically innervated smooth muscle preparations to electrical stimulation at different frequencies. *Br. J. Pharmac. Chemother.*, **27**, 64–80.

BENTLEY, G. A. (1968). The pharmacology of the hypogastric ganglion. *Aust. J. exp. Biol. med. Sci.* **46**, 21.

BENTLEY, G. A. & SMITH, GILLIAN (1967). Effects of alpha-adrenergic receptor blocking drugs on the response of vas deferens and arterial muscle to sympathetic drugs and stimulation. *Circulation Res. Suppt.* III. Vols. **20** and **21**, 101–109.

FLACKE, W. & GILLIS, R. A. (1968). Impulse transmission via nicotinic and muscarinic pathways in the stellate ganglion of the dog. *J. Pharmac. exp. Ther.*, **163**, 266–276.

GRAHAM, J. D. P., AL KATIB, H. & SPRIGGS, T. L. (1968). The isolated hypogastric nerve–vas deferens preparation of the rat. *Br. J. Pharmac. Chemother.*, **32**, 34–45.

HOLMAN, M. E. MUIR, T. C., SZURSZEWSKI, J. H. & YONEMURA, K. (1971). Effect of iontophoretic application of cholinergic agonists and their antagonists to guinea-pig pelvic ganglia. *Br. J. Pharmac.*, **41**, 26–40.

HUKOVIC, S. (1961). Responses of the isolated sympathetic nerve–ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

JONES, A. (1963). Ganglionic actions of muscarinic substances. *J. Pharmac. exp. Ther.*, **141**, 195–205.

KOMALAHIRINYA, A. & VOLLE, R. L. (1962). Action of inorganic ions and veratrine on asynchronous postganglionic discharge in sympathetic ganglia treated with di-isopropylphosphoro fluoridate (DFP). *J. Pharmac. exp. Ther.*, **138**, 57–65.

PAPPANO, A. J. & VOLLE, R. L. (1965). A comparison of the responses of sympathetic ganglia and chemoreceptors to cholinomimetic agents and sodium cyanide. *J. Pharmac. exp. Ther.*, **147**, 324–329.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug receptor combination. *Proc. Roy. Soc. B.*, **154**, 21–69.

PERRY, W. L. M. & REINERT, H. (1954). The effects of preganglionic denervation on the reactions of ganglionic cells. *J. Physiol., Lond.*, **126**, 101–115.

ROSZKOWSKI, A. P. (1961). An unusual type of sympathetic ganglion stimulant. *J. Pharmac. exp. Ther.*, **132**, 156–170.

SMITH, J. C. (1966). Pharmacologic interactions with 4-(m-chlorophenyl-carbamoyloxy)-2-butynyl-trimethyl ammonium chloride, a sympathetic ganglion stimulant. *J. Pharmac. exp. Ther.*, **153**, 276–284.

TAKASHIGE, C. & VOLLE, R. L. (1962). Bimodal responses of sympathetic ganglia to acetyl choline following eserine or repetitive preganglionic stimulation. *J. Pharmac. exp. Ther.*, **138**, 66–73.

TAKASHIGE, C. & VOLLE, R. L. (1963). Asynchronous postganglionic firing from the cat superior cervical sympathetic ganglion treated with neostigmine. *Br. J. Pharmac. Chemother.*, **20**, 214–220.

TRENDELENBURG, U. (1954). The action of histamine and pilocarpine on the superior cervical ganglion and the adrenal glands of cats. *Br. J. Pharmac. Chemother.*, **9**, 481–487.

TRENDELENBURG, U. (1956). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Br. J. Pharmac. Chemother.*, **11**, 74–80.

TRENDELENBURG, U. & JONES, A. (1965). Facilitation of ganglionic responses after a period of preganglionic stimulation. *J. Pharmac. exp. Ther.*, **147**, 330–335.

WHYTE, J. M. (1964). Anomalous reactions of the rat to ganglion blocking agents. *Nature, Lond.*, **203**, 307.

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