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# THE ISOLATED HYPOGASTRIC NERVE-VAS DEFERENS PREPARATION OF THE RAT

BY

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Huković (1961) described the isolated hypogastric nerve-vas deferens preparation from the guinea-pig and its response to nerve stimulation and to drugs. This preparation has often been used for physiological and pharmacological research, but little has been reported about the vas deferens of the rat. It was thought desirable, therefore, to compare the vas of this species with that of the guinea-pig in some detail in order to reveal any important differences in their respective morphology and responses to drugs. Particular attention was directed towards the ability of certain 2-halogenoalkylamine compounds to affect the responses of the vas deferens to added neuro-transmitters and to stimulation of the hypogastric nerve.

## METHODS

### *Isolated preparation*

Wistar rats weighing 300 g and guinea-pigs weighing 250–350 g were used. The nerve and muscle were prepared as described by Graham & Katib (1967) and mounted in a solution of the following composition: NaCl 7.6 g, KCl 0.42 g, $CaCl_2$ 0.24 g, $MgSO_4$7 H2O 0.12 g, $NaHCO_3$ 1 g, glucose 1 g in 1 l. The nerve was stimulated from a SD-5 Grass apparatus with square wave pulses of 0.5 msec duration, frequency range 5–100 shocks/sec every 2 min at a supramaximal voltage in the range 3–5 V. When determining the effect of variation of frequency on any one tissue a constant number of shocks (200) was delivered every 2 min. The two frequencies of 10/sec and 80/sec were used routinely for " slow " and " fast " stimulation respectively. Transmural stimulation was delivered by means of the electrode described by Birmingham & Wilson (1963) with square pulses of 0.1 msec, frequency 25/sec, 80 V for rats, 100 V for guinea-pigs ; 250 shocks every 4 min or as needed. Drugs were added to the bath in some experiments. Some animals were injected intraperitoneally with reserpine 5 mg/kg 48 hr and again 24 hr before removal of the vas deferens. The drugs used were:

*Group 1:* noradrenaline bitartrate (NA); isoprenaline sulphate; dibenamine; N-ethyl-N-1-naphthylmethyl bromoethylamine hydrobromide (SY28); N-dimethyl-phenylethylamine hydrobromide (L2, Graham & James, 1961) and their ethanolamines; pheniprazine hydrochloride; pronethalol.

*Group 2:* acetylcholine chloride (ACh), physostigmine salicylate; 1-hyoscine hydrobromide; atropine sulphate; hemicholinium (HC3); mecamylamine hydrochloride; pempidine tartrate; hexamethonium chloride (C6); procaine hydrochloride.

*Local anaesthetic activity*

This was investigated as reported by Graham & Al Katib (1967). The effects of procaine and of the 2-halogenoalkylamines and their ethanolamines were determined on the responses of vasa from both species to nerve and transmural stimulation.

*Formol-fluorescence*

To locate the presence of adrenergic nerves in cryostat-cut sections of vas deferens the formol-fluorescence method as modified by Spriggs, Lever, Rees & Graham (1966) was used.

*Histology*

The hypogastric nerve was dissected to its point of union with the wall of the vas deferens. This part of the vas was isolated and together with 4 cm of the nerve was fixed in alcoholic Bouin's solution and embedded in wax. Serial sections of 7 $\mu$ were cut, stained with haematoxylin and eosin, and examined microscopically for ganglion-cells.

## RESULTS

### *Characteristics of contraction and reaction to drugs of the rat vas*

Stimulation of the hypogastric nerve produced a fast, strong contraction. The height of contraction for a stimulus of given characteristics is variable so that it is desirable to repeat stimulation regularly for 1 hr in order to induce stability before experimenting. The relaxation at the end of stimulation is immediate. These findings apply to slow and to fast stimuli. Below 10/sec the contractions are small and fail after a time ; above 100/sec contractions are submaximal and also fail after a time. Between 10 and 100/sec they become regular and remain so for several hours, 80/sec giving an optimal response. Transmural stimulation causes contractions with similar characteristics, but variable in force ; usually they are stable at 25/sec and 80 V. Direct stimulation by an agonist or electrically usually produces a stronger contraction.

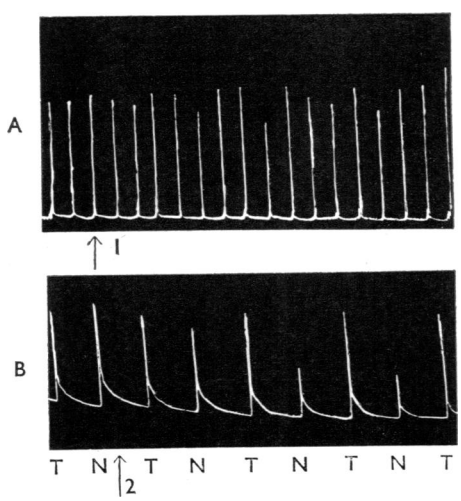


Fig. 1. The effect of ganglion-blocking drugs. Isolated vas deferens of the rat. A: stimulated via hypogastric nerve with electrodes at 2 cm from vas (frequency, 80/sec, 5 V, 0.5 msec, 240 shocks every 2 min). At ↑1 : $C_6$ ($5\times10^{-5}$ g/ml.) was added. B: alternate nerve (N) and transmural (T) stimulation at 2-min intervals ; the former as above, the latter at a frequency of 25/sec, 80 V, 0.1 msec, 240 shocks every 4 min. At ↑ 2: mecamylamine $5\times10^{-5}$ g/ml. was added.

*Evidence for pre- and postganglionic innervation*

*Ganglion-blocking drugs.* Concentrations of hexamethonium ($C_6$) of $10^{-6}$ g/ml. to $5.10^{-5}$ g/ml. in contact for 4 hr have no effect on fast or slow stimulation of the hypogastric nerve (Fig. 1) but $10^{-4}$ g/ml. produces a 50% reduction of response after about 30 min. Transmural stimulation is not affected by $10^{-4}$ g/ml. for 2 hr. Mecamylamine at $5 \times 10^{-5}$ g/ml. quickly and partly blocks hypogastric nerve stimulation but not transmural stimulation (Fig. 1B); pempidine $10^{-6}$ blocks the responses to nerve stimulation more completely in 1 hr and $10^{-5}$ in 30 min whilst having no effect on transmural stimulation.

*Histology.* In the rat the hypogastric nerve contains a small cluster of typical ganglion-cells situated some 2 cm from the vas deferens. A much larger aggregation of ganglion-cells is to be found in the region where the hypogastric nerve approaches the serous coat of the vas deferens (see Pl. 1A). The postganglionic fibres spread widely on and between the muscle layers. In the guinea-pig hypogastric nerve a large collection of ganglion-cells is found 1–1.5 cm from the vas. No ganglion-cells were observed closer than 0.5 cm to the muscle (see Pl. 1B).

*Effect of drugs which act on cholinergic mechanisms*

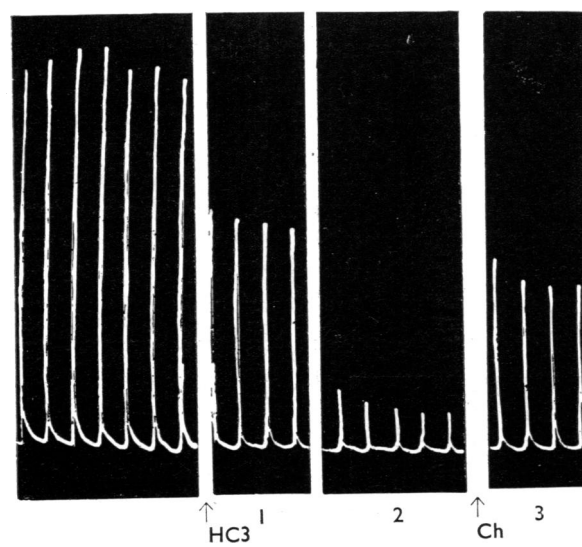


Fig. 2. Isolated hypogastric nerve-vas deferens of rat stimulated at frequency 80/sec, 4 V, 0.5 msec, 240 shocks every 2 min. Addition of HC3 ($5 \times 10^{-5}$ g/ml. at ↑ HC3) produces a decline of response at 1 hr (1) more severe after 2 hr (2). Addition of choline chloride $10^{-3}$ g/ml. (↑ Ch) causes a partial restoration of function after 1 hr (3).

Hemicholinium. At a concentration of $5 \times 10^{-5}$ to $10^{-4}$ g/ml., HC3 produces a slowly progressive failure of the response to "slow" or "fast" stimulation of the hypogastric nerve in rat and in guinea-pig (Chang & Rand, 1960); this effect is reversed very slowly by washing, and more quickly (particularly in the rat) if choline chloride $10^{-3}$ g/ml. is added (see Fig. 2). Noradrenaline $10^{-5}$ g/ml. reduces or abolishes the action of choline in reversing HC3 block in the rat.

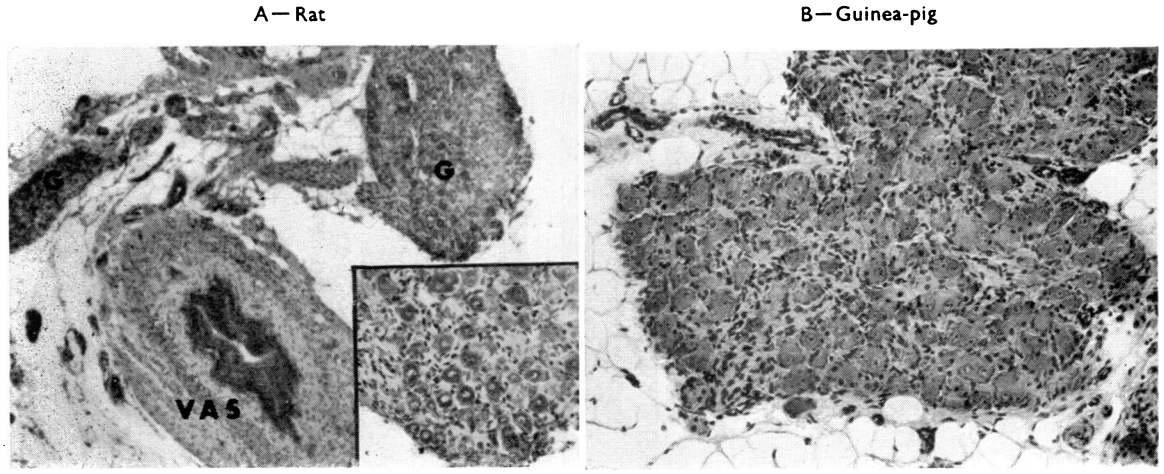


**Plate 1.** **A**: Rat. Transverse section of vas deferens (VAS) showing proximity of ganglion-cells (G) of the hypogastric nerve. × *ca.*22. Inset shows ganglion-cells ×120. **B**: Guinea-pig. Ganglion-cells of hypogastric nerve, located 1.2 cm from the vas deferens. Ganglion-cells were not found closer than 0.5 cm to the vas deferens. ×120. Both histological preparations were stained with eosin and haematoxylin after fixation in alcoholic Bouin's solution.

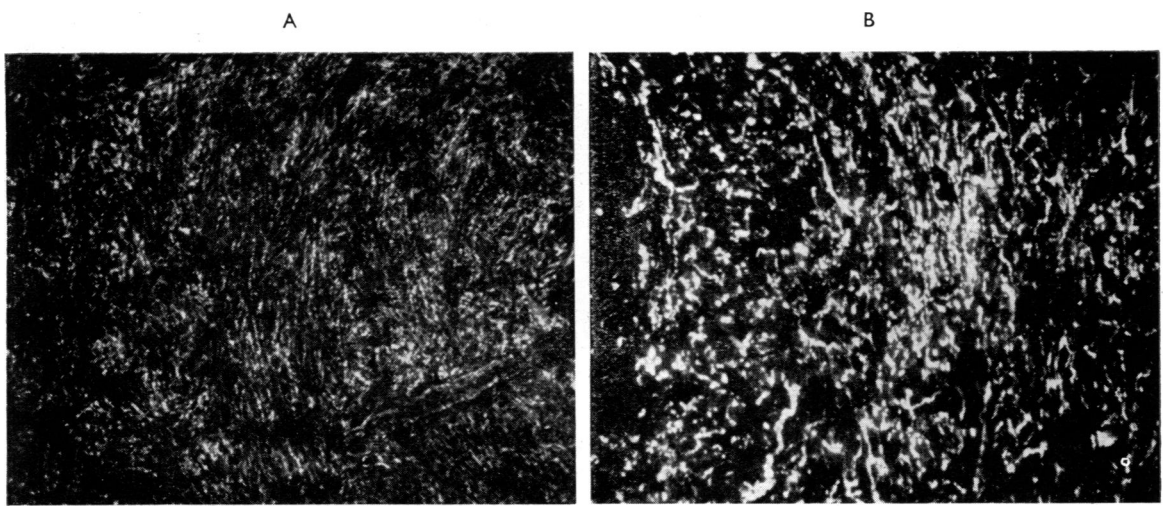


**Plate 2.** Fluorescent catecholamine-containing nerve fibres in transverse sections of vas deferens of (A) rat and (B) guinea-pig. These specimens were prepared simultaneously under identical conditions. ×144.

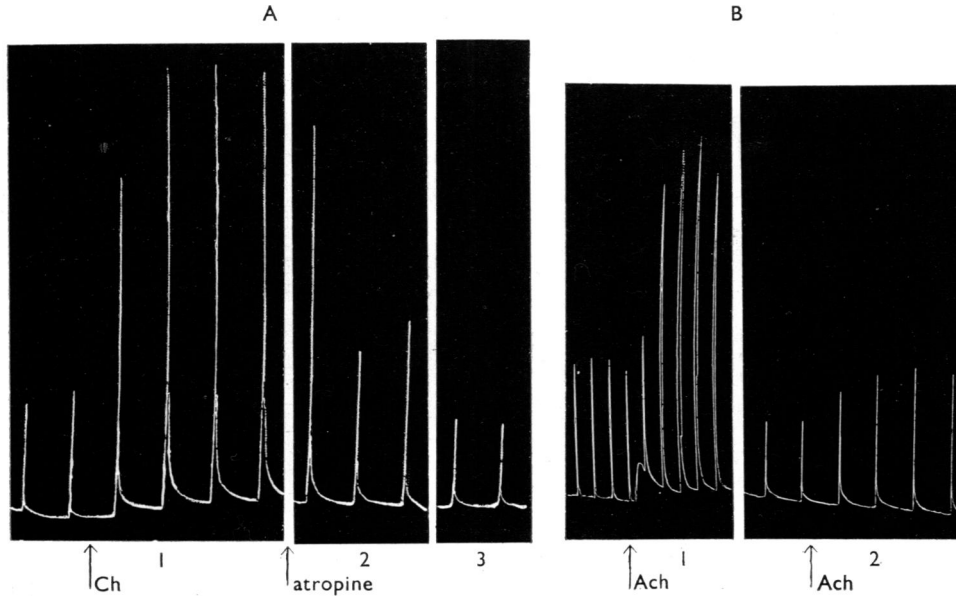


Fig. 3. Isolated rat vas deferens. A: Transmural stimulation at frequency 25/sec, 80 V, 0.1 msec, 250 shocks every 4 min. At ↑ Ch, choline chloride $5\times10^{-5}$ g/ml.; at ↑ atropine, $10^{-4}$ g/ml. between 1 and 2, 12-min interval; between 2 and 3, 16 min. B 1: hypogastric nerve stimulated at frequency 80/sec, 3 V, 0.5 msec, 240 shocks every 2 min. B 2: transmural stimulation as in A. At ↑ ACh, acetylcholine $10^{-7}$ g/ml. added.

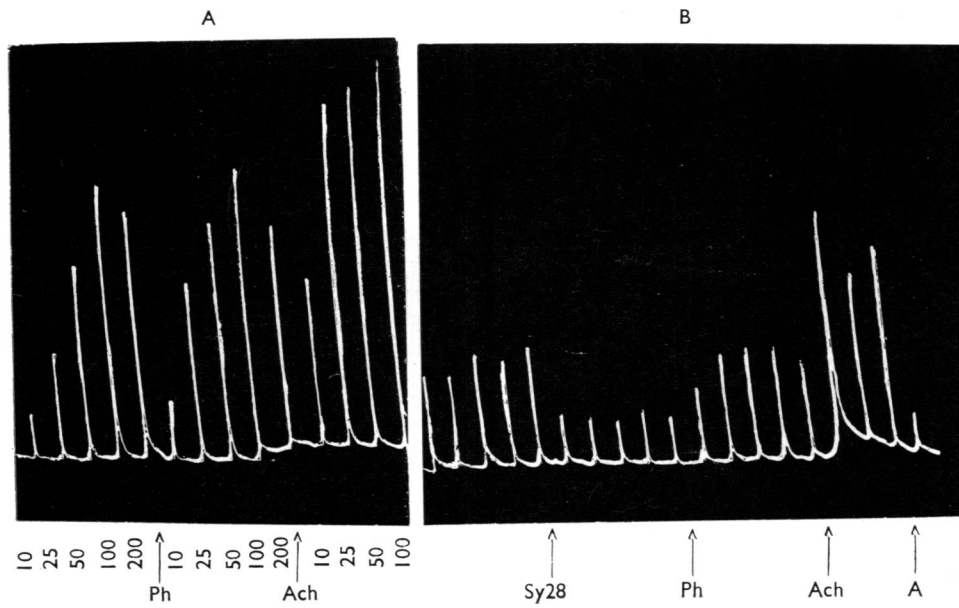


Fig. 4. Isolated hypogastric nerve-vas deferens of rat. A: Stimulated every 2 min, with 200 shocks at frequencies of 10–200/sec, as indicated below each response. Physostigmine (↑ Ph) $10^{-6}$ g/ml. and acetylcholine (↑ ACh) $10^{-7}$ g/ml. potentiate responses to frequencies of 10–100/sec. B: stimulation every 2 min at 10/sec. Compound SY28 $10^{-5}$ g/ml. (↑ SY28) inhibits the responses; physostigmine (↑ Ph) $10^{-6}$ g/ml. and acetylcholine (↑ ACh) $10^{-7}$ g/ml. potentiate the responses in the presence of a concentration ($10^{-5}$ g/ml.) of SY28 which will prevent the effect of added noradrenaline bitartrate. Atropine (↑ A) abolishes this potentiation.

When the rat vas is stimulated transmurally at 25/sec choline chloride at $5 \times 10^{-5}$ g/ml. (see Fig. 3) potentiates the contractions, as also does HC3 in a concentration of $5 \times 10^{-5}$ to $10^{-4}$ g/ml. This effect is atropine-sensitive and is readily abolished by washing the tissue. It is not modified by addition of NA.

*Acetylcholine.* ACh by itself ($10^{-7}$ to $10^{-6}$ g/ml.) causes contraction of the isolated stripped vas deferens. The contraction is potentiated by choline chloride and abolished by atropine or hyoscine $10^{-7}$ g/ml. and also by high concentrations of dibenamine-type compounds, for example SY28 $10^{-5}$ g/ml.

Acetylcholine markedly potentiates the responses to " slow " and " fast " stimulation of the hypogastric nerve (see Fig. 3B) in the normal and in the reserpinized rat and this effect is abolished by atropine or hyoscine $10^{-7}$ g/ml. but not by SY28 at $10^{-5}$ g/ml.

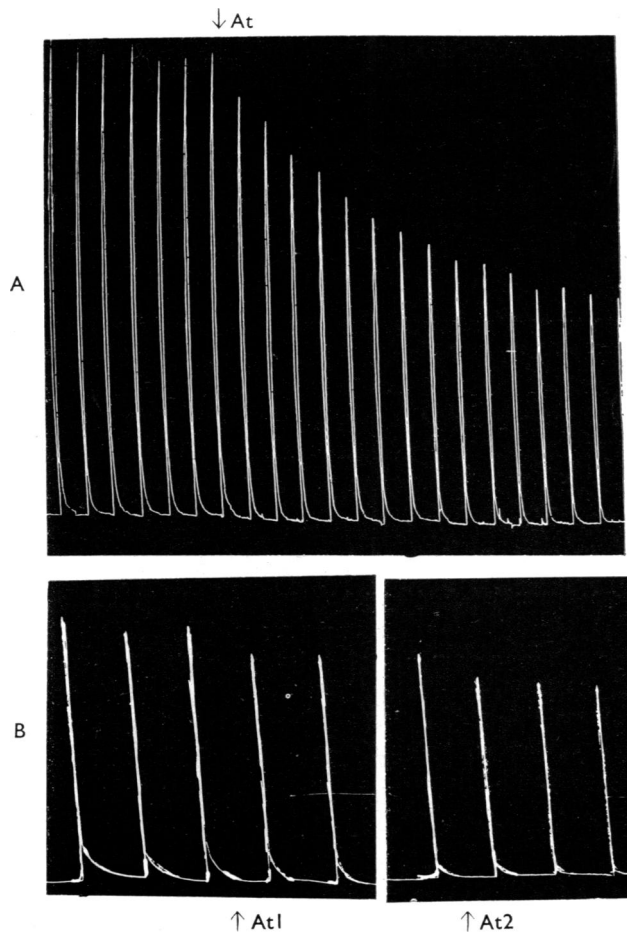


Fig. 5. Isolated vas deferens of rat. A: Hypogastric nerve stimulation: frequency 80/sec, 3 V, 0.5 msec, 240 shocks every 2 min. Atropine sulphate (↓ At) $5 \times 10^{-6}$ g/ml. progressively reduces the response by approximately 50% in 20 min. B: Transmural stimulation: frequency 25/sec, 80 V, 0.1 msec, 250 shocks every 4 min. Atropine sulphate at $5 \times 10^{-6}$ g/ml. (↑ At 1) reduces the response by 17% and at $10^{-5}$ g/ml. (↑ At2) by 23%. Between B1 and B2 a period of 2 hr.

(see Fig. 4B). The potentiation in the reserpinized rat after ACh is much greater than that caused by added NA. A lesser potentiation occurs on transmural stimulation (see Fig. 3B, 2).

*Physostigmine, atropine and hyoscine.* Physostigmine, 1 $\mu$g/ml., augments the response to slow or fast stimulation of the nerve and to transmural stimulation (see Fig. 4A); atropine $10^{-7}$ g/ml. prevents or abolishes this (see Fig. 4B). Atropine sulphate or hyoscine $10^{-6}$ to $5 \times 10^{-6}$ g/ml. causes a marked reduction in the responses to slow and fast stimulation of the nerve (50% within 20 min) but only a 10–25% reduction of the effects of transmural stimulation, which does not increase greatly during 2-hr exposure (see Fig. 5).

*Effect of drugs which act on adrenergic mechanisms*

*Noradrenaline.* Noradrenaline readily causes a contraction of the isolated vas deferens and this is prevented by prior addition of dibenamine-type compounds such as SY28 at $10^{-6}$ g/ml., but not by atropine or pronethalol. Isoprenaline has no effect. The responses of the untreated rat vas deferens to transmural stimulation are potentiated by about 20% in the presence of NA $10^{-7}$ to $10^{-4}$ g/ml., whereas contractions to stimulation of the hypogastric nerve are inhibited by NA $5 \times 10^{-6}$ to $10^{-4}$ g/ml. and are unaffected by NA $10^{-7}$ g/ml. This inhibitory action of NA is readily removed by washing. The potentiation of transmural effects is prevented by SY28 $10^{-6}$ g/ml.

*Mono-amine oxidase inhibitor.* Pheniprazine $10^{-7}$ to $10^{-6}$ g/ml. potentiates the concentrations of the vas to transmural or to slow and fast stimulation of the nerve. This effect is abolished by washing or by adding SY28 $10^{-6}$ g/ml., but not by atropine.

*Histology.* Sections of the vasa deferentia subjected to the formol-fluorescence technique exhibit a dense plexus of beaded fluorescent fibres (see Pl. 2A and B), characteristic of catecholamine-containing adrenergic nerves. In the rat (Pl. 2A) the nerves appear much finer and the nerve plexus fluoresces less strongly and seems to be denser than in the guinea-pig (Pl. 2B).

*Reserpine.* Previous injection of the animals with reserpine 5 mg/kg 48 and 24 hr before death has no apparent effect on responses, whether the isolated preparation is stimulated transmurally or via the hypogastric nerve. Noradrenaline ($10^{-7}$ to $10^{-6}$ g/ml.) potentiates the effect of transmural or of "slow" and "fast" nerve stimulation in the reserpinized preparation, whereas NA $10^{-4}$ g/ml. frequently, but not always inhibits, particularly with nerve stimulation. Noradrenaline causes irregularity of response and may induce spontaneous activity. These effects are abolished on washing out the bath.

*Adrenergic-neurone blocker.* Guanethidine added to the bath in a concentration of $10^{-6}$ g/ml. produces a slowly developing, but ultimately complete, failure of response to "slow" or "fast" hypogastric or transmural stimulation. Addition of dexamphetamine $5 \times 10^{-6}$ g/ml. restores the responses slowly.

*Dibenamine-type compounds.* Compound SY28 $10^{-6}$ 9/ml. has no effect, but $5 \times 10^{-6}$ to $10^{-4}$ g/ml. inhibits the response to "slow" or "fast" nerve stimulation and to transmural stimulation. The block is reversible by washing and there is never any potentiation. There is a marked species difference here, for SY28 $10^{-6}$ to $10^{-5}$ g/ml. potentiates the response to transmural and to "slow" nerve stimulation in the guinea-pig; $10^{-4}$

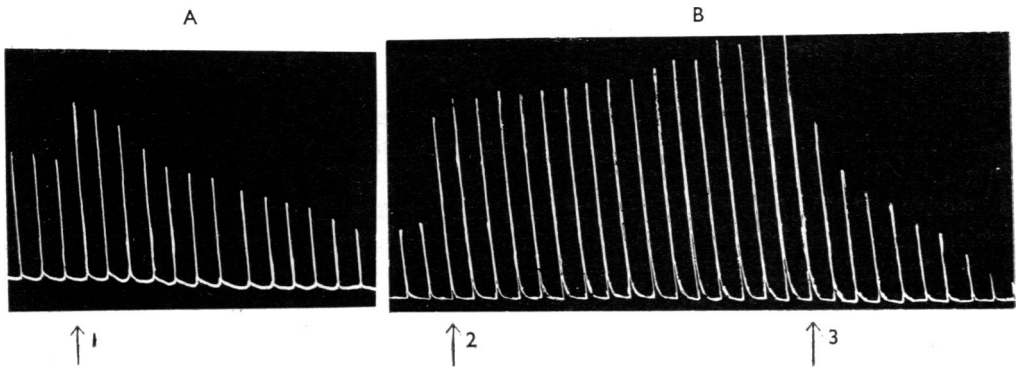


Fig. 6. Isolated hypogastric-nerve vas deferens of rat stimulated at 10/sec, 0.5 msec, 4 V, 200 shocks every 2 min. In A compound L2 (↑ 1) at a concentration of $10^{-5}$ g/ml. potentiates and then blocks. In B the ethanolamine L2-OH potentiates at a concentration of $10^{-7}$ g/ml. (↑ 2) and blocks at $5.10^{-6}$ g/ml. (↑ 3).

g/ml. potentiates and then blocks. This potentiation is not sensitive to atropine nor to hyoscine $10^{-6}$ g/ml., but $10^{-5}$ g/ml. may prevent it in the transmurally stimulated vas deferens of this species; responses to "fast" nerve stimulation in the guinea-pig are blocked more easily ($10^{-6}$ to $10^{-5}$ g/ml.) and again this block is removable by washing. Dibenamine acts like SY28 but a ten times higher concentration of drug is required to exert its effect.

Compound L2 in a concentration of $10^{-6}$ g/ml. always potentiates responses to transmural stimulation or to either frequency of stimulation of the nerve. This potentiation is atropine-sensitive. A concentration of $10^{-5}$ g/ml. potentiates and then blocks; the block is reversible by washing. The species difference is again marked and guinea-pig preparations are always blocked.

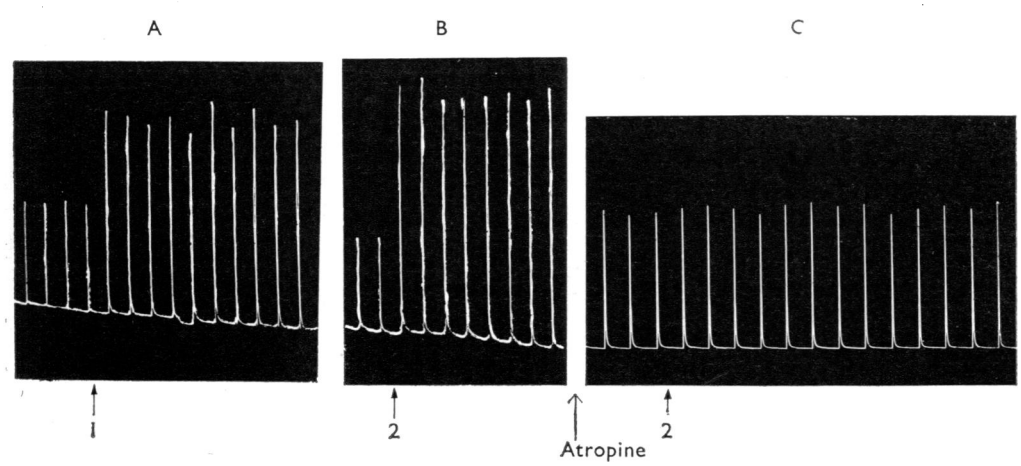


Fig. 7. Isolated rat vas deferens, transmural stimulation 25/sec, 0.1 msec, 80 V, 200 shocks every 4 min. A: The potentiating action of dibenamine—OH (↑ 1) $10^{-6}$ g/ml. B: The greater effect of the same concentration of SY28-OH (↑ 2). C: The absence of potentiation when SY28-OH (↑ 2) is administered in the presence of atropine $10^{-6}$ g/ml.

The alcohol hydrolysis products derived from the halogenoalkylamines differ in action from the parent compounds and quantitatively among themselves. All three potentiate the responses to nerve and transmural stimulation in the rat in concentrations of $10^{-7}$ to $10^{-6}$ g/ml. This potentiation is prevented by atropine (Fig. 7C). Compound L2-OH blocks at a concentration of $5 \times 10^{-6}$ g/ml. (Fig. 6B) but this block is reversible on washing. Again there is a species difference; in the guinea-pig SY28-OH and dibenamine-OH are inactive at $10^{-5}$ g/ml. and higher concentrations produce irregular contractions of the muscle. Compound L2-OH blocks the guinea-pig preparation completely at $10^{-6}$ g/ml. Its activity is about ten times that of the parent halogenoalkylamine; at no time was there evidence of potentiation in the latter species.

## DISCUSSION

There is clear histological evidence (Pl. 1) that the hypogastric nerve in the rat contains abundant ganglion-cells situated close to the serous coat of the vas deferens. This confirms the work of Sjöstrand (1965). An appropriate stimulus to the hypogastric nerve some 2 cm from the vas elicits a contraction and this response is reduced but not abolished by hexamethonium, mecamylamine or pempidine. In the guinea-pig ganglion-cell bodies are also found (Sjöstrand, 1965) but are mostly at a distance of 1–1.5 cm and not nearer than 0.5 cm from the muscle. The guinea-pig also differs from the rat in that the response to nerve stimulation is abolished by concentrations of ganglion-blocking drugs (for example, $C_6$ at $10^{-6}$ g/ml. for 10 min) much smaller than those which produce only a partial blockade in the rat (for example, $C_6$ at $10^{-4}$ g/ml. for 60 min). Transmural stimulation in both species is unaffected by these drugs. Birmingham & Wilson (1963) concluded that stimulation of the nerve is largely pre-ganglionic in the guinea-pig and that only transmural stimulation can be relied on to be postganglionic. This is also true in the rat.

There is evidently a cholinergic mechanism in the hypogastric nerve of the rat, which is facilitated by ACh, blocked by HC3, and restored by choline; but it differs from the similar element in the guinea-pig (Boyd, Chang & Rand, 1960; Bell, 1967; Birmingham & Wilson, 1963; Della Bella, Benelli & Gandini, 1964; Bhargava, Kar & Palmer, 1965) in that HC3 blocks the latter more easily and NA antagonizes this block, whereas in the rat NA in the same concentration has either no effect or increases the block. ACh is more active in the rat than in the guinea-pig in potentiating the response to slow or fast nerve stimulation. These effects are presumably exerted in part at the ganglia, but there is also a postganglionic component because ACh and physostigmine also potentiate responses to transmural stimulation. This postganglionic potentiation is abolished by atropine in both species. The rat is more sensitive to atropine or hyoscine because $5 \times 10^{-6}$ g/ml. produce a definite reduction, of about 20% after 2 hr, but are without effect in the guinea-pig (Birmingham & Wilson, 1963). It is, however, in the effect of atropine or hyoscine on hypogastric nerve stimulation that the more obvious difference is found. The rat ganglia are markedly atropine-sensitive (90% inhibition of responses to nerve stimulation by atropine $5 \times 10^{-6}$ g/ml. for 10 min compared with the guinea-pig 20% after 60 min). The postganglionic cholinergic element can not be of great significance, because hemicholinium does not greatly diminish the response to

transmural stimulation in either species. In the rat, $HC_3$ or choline cause an atropine-sensitive potentiation of the response to transmural stimulation and of the response to ACh and may be attributed to an anticholinesterase activity of these substances and to the presence of cholinergic fibres in the postganglionic innervation. When the $\alpha$-receptors in the vas are blocked by SY28 in the rat, the responses to nerve stimulation are potentiated by ACh or physostigmine. This implies the presence of postganglionic cholinergic fibres but not necessarily a cholinergic link in an adrenergic nerve (Burn & Rand, 1965). These may be true cholinergic postganglionic sympathetic fibres as are alleged to exist in the innervation of the sweat glands in the cat's paw ; alternatively, the hypogastric nerve may contain a mixture of sympathetic and parasympathetic postganglionic fibres.

The dense plexus of catecholamine-containing nerves revealed by the formol-fluorescence technique indicates an extensive adrenergic innervation in both species and confirms earlier studies (Norberg & Hamberger, 1964 ; Falck, Owman & Sjöstrand, 1965). This belief is strengthened by the completeness with which guanethidine $5 \times 10^{-6}$ g/ml. abolishes the responses to both forms of stimulation in the two species. There are, however, differences between the species in the appearance of the fluorescent nerve plexus (Pl. 2) and in the response to added NA. In the rat NA $10^{-7}$ g/ml. has no effect on nerve stimulation unless the animal has been pretreated with reserpine ; whereas NA $10^{-4}$ g/ml. blocks, perhaps at the ganglia. Postganglionic stimulation is potentiated only 20%, whereas in the guinea-pig NA at these concentrations potentiates all forms of stimulation markedly (*ca.* 80%). Addition of NA has long been known to potentiate the response to stimulation of adrenergic nerves (Gillespie & McKenna, 1959), and Huković (1961) reported it in his original description of the hypogastric nerve—vas deferens preparation. Pheniprazine, an inhibitor of monoamine oxidase, also causes less potentiation in the rat than in the guinea-pig. These effects are prevented by prior addition of dibenamine-type compounds—for example, SY28. Further evidence of the difference in adrenergic function is obtained by pre-treatment with reserpine, which, like the analogue syrosingopine (Spriggs, 1966), has no inhibitory effect on adrenergic transmission in the rat. These drugs deplete noradrenaline stores by impairing the uptake and storage of NA in adrenergic nerves (Iversen, 1967a), but it is possible that in the rat the synthesis of NA in these nerves is sufficient to maintain function. In contrast, reserpinization greatly reduces the responses to all forms of stimulation in the guinea-pig, a condition which is reversible by adding NA (Huković, 1961 ; Birmingham & Wilson, 1963 ; Della Bella *et al.*, 1964). In addition sensitization of the muscle in the vas deferens to the direct action of NA after reserpinization is much less marked in the rat. A further indication of the species difference is seen after 2-halogenoalkylamines, especially SY28. This compound closely resembles phenoxybenzamine in structure and actions, and phenoxybenzamine is known to prevent the uptake of NA into adrenergic nerves in addition to the more familiar blockade of $\alpha$-receptors (Iversen, 1967b). Phenoxybenzamine potentiates " slow " nerve stimulation in guinea-pig (Boyd, Chang & Rand, 1960) ; if this potentiation is caused by an anticholinesterase action then Birmingham (1966) and Bell (1967) would localize it to the ganglia and Burn and Rand (1965) to the postganglionic nerve terminal. In the rat SY28 and dibenamine always block responses to stimulation ; in the guinea-pig $10^{-6}$ g/ml. potentiates " slow " nerve and transmural stimulation, and only at $10^{-4}$ g/ml. does it block ; the blockade is reversible by washing. The more potent but shorter-acting compound L2 at $10^{-6}$ g/ml. does potentiate in the rat, whereas

in the guinea-pig it only blocks. These effects are similar to the dual effects of stimulation and block exerted by procaine which have been attributed to anticholinesterase activity (Burn & Rand, 1965) and to local anaesthesia respectively (Graham & James, 1961; Bentley, 1966). The alcohols derived by hydrolysis from the 2-halogenoalkylamine cause marked atropine-sensitive potentiation in the rat and no effect in the guinea-pig. This is further evidence of distinct pre- and postganglionic cholinergic components in the rat. Differences in the action of these drugs are explicable by their differences in potency as $\alpha$-blockers and by their varying rates of hydrolysis. The order of potency of the antagonism to added NA among the compounds used is L2>SY28>dibenamine. One thousand-fold higher concentrations antagonize the responses of the vas deferens to added ACh, the order of potency being SY28>dibenamine>L2. In the rat the potency order in antagonizing transmural stimulation (SY28≏L2>dibenamine) differs from that in the guinea-pig which is of the "NA-order" (L2>SY28>dibenamine).

We conclude that there are quantitative and qualitative differences between the hypogastric nerve mechanism and the transmural mechanism in these two species, which call for care in the interpretation of experimental results. The rat ganglia are atropine-sensitive and the rat postganglionic nerve contains a cholinergic element which is quantitatively more pronounced than that of the guinea-pig. We believe that this is not a cholinergic link in adrenergic nerves but a distinct postganglionic cholinergic innervation like that proposed by others for the guinea-pig vas deferens.

## SUMMARY

1. The isolated vas deferens of the rat and guinea-pig have been stimulated transmurally and at "slow" and "fast" rates via the hypogastric nerve. The effects of drugs on these responses have been compared.

2. Histological examination of the hypogastric nerve confirmed the presence of abundant ganglion-cells close to the vas deferens in both species.

3. Histochemical examination of the vas confirmed the presence of fluorescent catecholamine-containing nerve fibres in both species.

4. Ganglion-blocking drugs depress the response to stimulation via the nerve but not to transmural stimulation. The rat is less sensitive than the guinea-pig.

5. Hemicholinium blocks the response to nerve stimulation and potentiates that to transmural stimulation. The rat is more sensitive than the guinea-pig. Choline reverses the block more quickly in the rat. Atropine prevents the potentiation.

6. Acetylcholine or physostigmine potentiate both forms of stimulation in both species, more markedly in the rat. The potentiation is blocked by atropine.

7. In the rat atropine and hyoscine reduce the response to nerve stimulation by 90%, and to transmural stimulation by 10–20%. In the guinea-pig the reductions are 10–20% and 0%, respectively.

8. In the rat the response to transmural stimulation only is increased by noradrenaline (NA). In the guinea-pig NA potentiates and reserpine inhibits the response to both nerve and transmural stimulation. Pheniprazine potentiates and guanethidine blocks all forms of stimulation in both species.

9. The 2-halogenoalkylamines irreversibly antagonize added NA but reversibly block the response to nerve stimulation. Compound L2 and its ethanolamine, which are strong local anaesthetics are like procaine, reversible nerve blockers.

## REFERENCES


BELL, C. (1967). An electrophysiological study of the effects of atropine and physostigmine on transmission to the guinea-pig vas deferens. *J. Physiol., Lond.*, **189**, 31–42.

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

BHARGAVA, K. P., KAR, K. & PARMAR, SURENDRA S. (1965). Independent cholinergic and adrenergic mechanisms in the guinea-pig isolated nerve vas deferens preparation. *Br. J. Pharmac. Chemother.*, **24**, 641–650.

BIRMINGHAM, A. T. (1966). The potentiation by anticholinesterase drugs of the responses of the guinea-pig isolated vas deferens to alternate preganglionic and postganglionic stimulation. *Br. J. Pharmac. Chemother.*, **27**, 145–156.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **21**, 568–580.

BOYD, H., CHANG, V. & RAND, M. J. (1960). The anticholinesterase activity of some anti-adrenaline agents. *Br. J. Pharmac. Chemother.* **15**, 525–531.

BURN, J. H. & RAND, M. J. (1965). Acetylcholine in adrenergic transmission. *A. Rev. Pharmac.*, **5**, 163–182.

CHANG, V. & Rand, M. J. (1960). Transmission failure in sympathetic nerves produced by hemicholinium. *Br. J. Pharmac. Chemother.*, **15**, 588–600

DELLA BELLA, D., BENELLI, G. & GANDINI, A. (1964). Eserine and autonomic nervous control of guinea-pig vas deferens. *J. Pharm. Pharmac.*, **16**, 779–787.

FALCK, B., OWMAN, C. & SJÖSTRAND, N. O. (1965). Peripherally located adrenergic neurons innervating the vas deferens and the seminal vesicle of the guinea-pig. *Experientia*, **21**, 98.

GILLESPIE, J. S. & MCKENNA, B. R. (1959). The effect of reserpine on the response of the rabbit ileum and colon to stimulation of their extrinsic nerves *in vitro*. *J. Physiol., Lond.*, **147**, 31*P*.

GRAHAM, J. D. P. & JAMES, G. W. I. (1961). The pharmacology of a series of substituted 2-halogenoalkyl amines. *J. med. Pharmac. Chem.*, **3**, 489–504.

GRAHAM, J. D. P. & AL KATIB, H. (1967). Adrenolytic and sympatholytic properties of 2-halogenoalkylamines in the vas deferens of the guinea-pig. *Brit. J. Pharmac. Chemother.*, **31**, 42–55.

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

IVERSEN, L. L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves*. London: Cambridge University Press. (a) pp. 176–181; (b) pp. 154–157.

NORBERG, K. A. & HAMBERGER, B. (1964). The sympathetic adrenergic neuron. *Acta. physiol. scand.*, **63**, Supp. 238

SJÖSTRAND, N. O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta. physiol. scand.*, **65**, Supp. 257.

SPRIGGS, T. L. B., LEVER, J. D., REES, P. M. & GRAHAM, J. D. P. (1966). Controlled formaldehyde-catecholamine condensation in cryostat sections to show adrenergic nerves by fluorescence. *Stain Technol.*, **41**, 323–327,

SPRIGGS, T. L. B. (1966). Peripheral noradrenaline and adrenergic transmission in the rat. *Br. J. Pharmac. Chemother.*, **26**, 271–281.