Brit. J. Pharmacol. (1963), **20**, 299–306.

OBSERVATIONS ON THE ISOLATED VAS DEFERENS

BY

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(*Received December* 3, 1962)

Experiments on the isolated vas deferens of guinea-pig and rat had unexpected results in several ways. The effect of stimulation of the hypogastric nerve was not abolished, but increased, by parasympathetic blocking agents or by sympathetic blocking agents such as dihydroergotamine and phenoxybenzamine. The sensitization is considered not due to anticholinesterase activity of the drugs. Prolonged contact with a stimulating agent evoked rhythmic contractions. Addition as well as removal of a drug from the bath caused a response. The results of experiments involving chronic denervation, addition of hexamethonium and histological examination tally with the assumption of a distribution of ganglionic cells along the nerve just outside the organ.

In recent years the nerve-muscle preparation consisting of the hypogastric nerve and the vas deferens of the guinea-pig has attracted considerable attention (Boyd, Chang & Rand, 1960, 1961; Chang & Rand, 1960; Huković, 1961; Burnstock & Holman, 1961, 1962a, b). The preparation is comparatively cheap, reliable and easy to set up and it was thought suitable for classroom experiments on sympathetic transmission. This idea had, however, soon to be abandoned since on addition of drugs known to affect sympathetic transmission the preparation did not behave according to the classical scheme.

METHODS

The experiments were made with vasa deferentia from guinea-pigs weighing 250 to 500 g and from a small number of rats weighing 300 to 400 g. The animals were killed by a blow on the neck and the abdomen opened; the intestines were moved to one side and the vas deferens on each side was exposed and dissected free. The nerve, easily identified in the mesentery of the colon, was dissected free. The preparation was set up in a 10 ml. bath and connected to an isotonic frontal writing lever which registered its movements on a smoked drum. The bath contained, unless otherwise stated, Tyrode solution (NaCl 8.00 g, KCl 0.20 g, $CaCl_2.6H_2O$ 0.40 g, $MgCl_2.6H_2O$ 0.20 g, $NaHCO_3$ 1.00 g, NaH_2PO_4 0.052 g and glucose 1.00 g in 1 l. of distilled water). The temperature was kept constant at 31° C. A mixture of 95% oxygen and 5% carbon dioxide was constantly bubbled through the bath. Drugs to be tested were dissolved in Tyrode solution and injected into the bath. The Tyrode solution in the bath was exchanged either by overflow or by drainage from the bottom followed by refilling.

The nerve to the vas deferens was stimulated from a Grass stimulator at frequencies varying in different preparations between 0.2 and 50 shocks/sec; rectangular pulses with a duration of 2msec and of supramaximal voltage were used. The electrode was of the type described by Burnstock & Holman (1961).

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Cholinesterase activity was determined manometrically. Vasa deferentia were cleaned, washed, dried between filter paper and weighed; several were pooled and homogenized in glass homogenizers with a few ml. of Krebs' bicarbonate buffer (NaCl 0.90 g, KCl 0.046 g, $CaCl_2$ 0.0366 g, KH_2PO_4 0.0211 g, $MgSO_4.7H_2O$ 0.0382 g and $NaHCO_3$ 0.273 g in 130 ml. of distilled water). The final volume was adjusted to five times the number of grams wet weight of the tissue. Of this solution 1.7 ml. was pipetted into the main compartment of a Warburg flask, in the side arm of which was 0.2 ml. of a 0.6% acetylcholine solution; the final volume was adjusted to 2 ml. by buffer or by the substance (dissolved in buffer) to be tested. Preliminary experiments showed that the acetylcholine concentration used gave the maximal activity. The gas phase was 95% nitrogen and 5% carbon dioxide. The anticholinesterase activities of eserine, $\alpha\alpha$-diphenyl-γ-piperidinobutylamide (Hoechst 9980), tolazoline, yohimbine and dihydroergotamine were expressed as percentage inhibitions. All values were corrected for changes in temperature and pressure in the apparatus, and for the results of "enzyme blanks" and "substrate blanks."

Choline acetylase activity of innervated and chronically denervated preparations was determined by Dr I. Nordenfelt according to the technique earlier described (Nordenfelt, 1963).

The contents of noradrenaline and adrenaline were determined fluorimetrically after the simplified extraction and purification described by Bertler, Carlsson & Rosengren (1958). Three pairs of innervated or chronically denervated preparations were pooled for each determination. Denervation was performed aseptically using ether anaesthesia, two to ten days before the determinations.

Histological examination of the hypogastric nerve was made by Dr B. Falck after staining the nerves with methylene blue and after preparation according to the technique described earlier (Falck, 1962).

RESULTS

Contractions with drugs. A dose/response relationship could be obtained with noradrenaline, acetylcholine and histamine. After a dose larger than that giving a maximal response the height of the contraction usually declined and a series of contractions occurred. The pattern of response to noradrenaline differed from that to acetylcholine. The frequency of the contractions was much higher with acetylcholine and the relaxation between the contractions was less complete. The rhythmic contractions induced by histamine resembled those with acetylcholine. Typical tracings of the rhythmic contractions caused by acetylcholine and by noradrenaline are shown in Figs. 1 and 2.

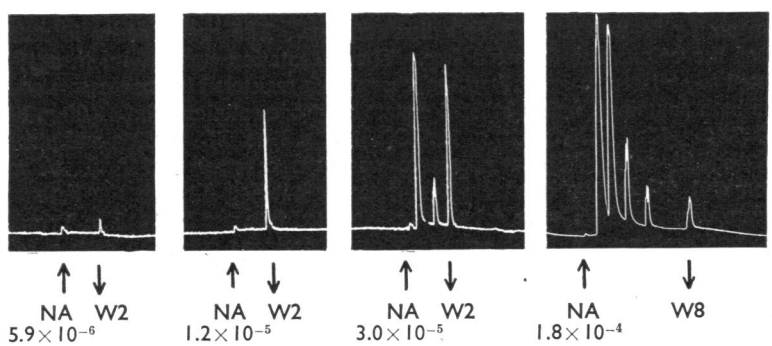

Fig. 1. Contractions of the vas deferens in response to noradrenaline (NA), 5.9×10^{-6} M to 1.8×10^{-4} M. At W, washing was performed (at W2 2 min and at W8 8 min after the addition of noradrenaline).

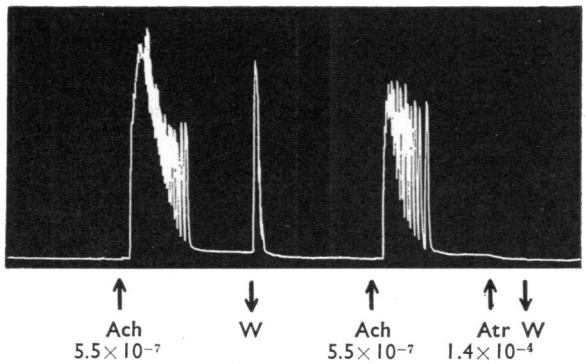

Fig. 2. Contractions of the vas deferens in response to acetylcholine (Ach, 5.5×10^{-7} M) and at W to washing before and after atropine (Atr, 1.4×10^{-4} M).

The response to noradrenaline was abolished by phenoxybenzamine (3.3×10^{-8} M) and by dihydroergotamine (1.7×10^{-6} M), the response to acetylcholine by atropine (1.4×10^{-6} M) and by $\alpha\alpha$-diphenyl-γ-piperidinobutylamide (3.1×10^{-6} M), and the response to histamine by mepyramine (3.5×10^{-7} M). Contractions could also be elicited by potassium chloride (1.3×10^{-2} M). About 2.6×10^{-2} M potassium chloride caused rhythmic contractions similar to those after noradrenaline. Reduction of the calcium content of the Tyrode solution to one-third also caused rhythmic contractions. When isoprenaline was added to preparations already exhibiting rhythmicity the contractions were abolished irrespective of the agent causing the contractions. A concentration of isoprenaline as low as 4.7×10^{-5} M was usually effective.

Not only the addition of noradrenaline, acetylcholine or histamine, but also the removal of these drugs from the bath caused contractions. The " washout effect " was found both when the bath was drained and refilled and when overflow washing was used, but not when no drug had been administered. The effect was abolished by the presence in the bath of the antagonist to the drug tested (Fig. 2). When a series of doses of a drug was tested, starting with low doses, the first effect seen was invariably a contraction when the drug was washed out. The threshold dose for the washout response was of the order of a tenth to a hundredth of the threshold dose for the response obtained on adding the drug. The height of the response to washing was influenced by the time lapse between the addition of the drug and the washing. With acetylcholine, and to a smaller degree with noradrenaline and histamine, the response declined with lapse of time (Fig. 3). If eserine (2.4×10^{-5} M) was present the decline with time was much less pronounced when acetylcholine was tested in this way. Keeping the time interval constant, a dose/response relationship could be obtained for the " washout effect."

Contraction with nerve stimulation. The contraction on electrical stimulation of the hypogastric nerve increased with increasing stimulus frequency up to 20 to 50 shocks/sec. The threshold frequency varied between 0.2 and 5 shocks/sec. With

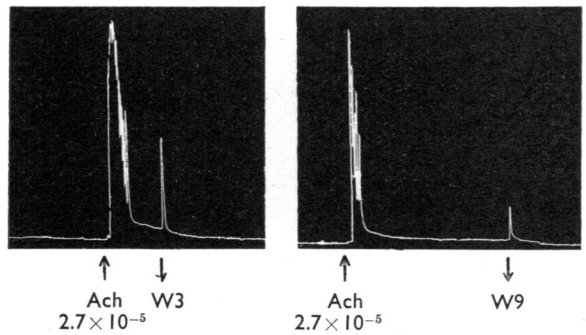

Fig. 3. Change of contraction on washing (W) with time after addition of acetylcholine (Ach, 2.7×10^{-5} M) to the vas deferens. Washings were 3 min (W3) and 9 min (W9) after the additions of acetylcholine.

a frequency of 1 shock/sec or less, 4 to 6 shocks had to be given before the contraction started. Once started the contraction developed very fast. On prolonged stimulation rhythmic contractions similar to those seen after big doses of noradrenaline appeared. The response to nerve stimulation was decreased or abolished by isoprenaline (4.7×10^{-5} M; Fig. 4).

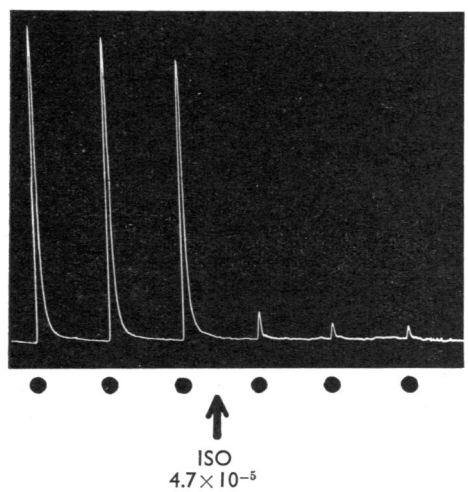

Fig. 4. Contractions of the vas deferens in response to stimulation of the hypogastric nerve (5 shocks/sec in 10 sec with an interval of 2 min; indicated by black dots) before and after the addition of isoprenaline (ISO, 4.7×10^{-5} M).

Sympathetic blocking agents. The hypogastric nerve is considered to contain sympathetic fibres to the vas deferens. The addition of phenoxybenzamine or of dihydroergotamine in concentrations from 10^{-8} M to 10^{-4} M did not, however, decrease the contraction elicited by stimulation of the nerve. On the contrary, the response was increased when the drug concentrations were larger than 3.3×10^{-5} M (phenoxy-

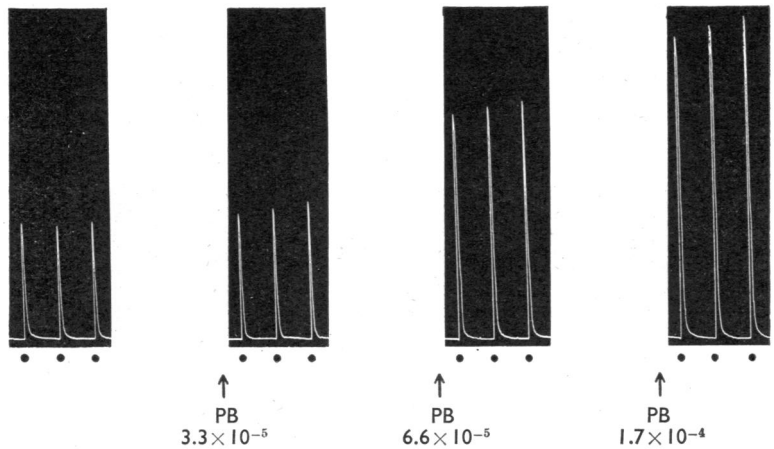

Fig. 5. Contractions of the vas deferens in response to nerve stimulation (10 shocks/sec in 10 sec with an interval of 2 min; indicated by black dots) in the presence of increasing concentrations of phenoxybenzamine (PB).

benzamine ; Fig. 5) and 3.4×10^{-5} M (dihydroergotamine). With further increases in concentration of these agents the response to stimulation increased, and when the concentrations of the drugs exceeded 2.0×10^{-4} M rhythmic contractions started and continued for hours. Tolazoline and yohimbine (2.8×10^{-7} M to 3.1×10^{-3} M) were also tested. In low concentrations these substances increased the response to nerve stimulation. With high concentrations (3.1×10^{-3} M tolazoline and 2.8×10^{-5} M yohimbine) a blocking action was observed. These high concentrations did not, however, influence the rhythmic contractions caused by phenoxybenzamine or dihydroergotamine.

The sympathetic blocking agents did not increase the response to noradrenaline. Phenoxybenzamine (3.3×10^{-10} M and 3.3×10^{-9} M) had no effect on the responses induced by noradrenaline, while 3.3×10^{-8} M reduced them. The response to acetylcholine, on the other hand, was increased by the presence of these drugs, for example, phenoxybenzamine in concentrations above 3.3×10^{-5} M.

Parasympathetic blocking agents. $\alpha\alpha$-Diphenyl-γ-piperidinobutylamide has about the same potency as atropine as a parasympathetic blocking agent, but much less sympathetic and ganglionic blocking effect (Emmelin & Strömblad, 1957). Both this drug and atropine increased the response to nerve stimulation. The lowest effective concentration of $\alpha\alpha$-diphenyl-γ-piperidinobutylamide was 3.1×10^{-5} M; this concentration also increased the response to noradrenaline. Higher concentrations of the drug abolished the effect of acetylcholine added to the bath, whereas lower concentrations never increased the response to acetylcholine. When the concentration of $\alpha\alpha$-diphenyl-γ-piperidinobutylamide exceeded 1.6×10^{-4} M a long-lasting rhythmic response was set up. This was abolished by isoprenaline (4.7×10^{-5} M), but not by tolazoline (3.1×10^{-3} M) or yohimbine (2.8×10^{-5} M).

Hexamethonium. The effect of hexamethonium on the response to nerve stimulation was tried with several preparations. Sometimes the response was abolished

(with concentrations from 3.7×10^{-7} M to 3.7×10^{-4} M); on other occasions it persisted. Stimulation at various sites along the nerve revealed that hexamethonium abolished the response to stimulation of the nerve 3 to 5 cm from the organ, but not if the nerve was stimulated 1 to 5 mm distant (Fig. 6).

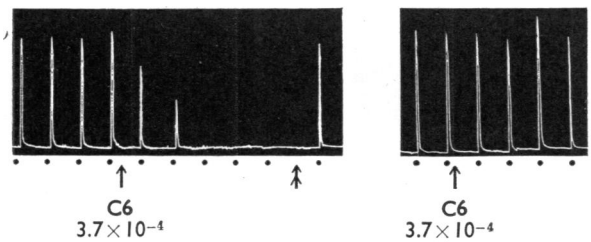

Fig. 6. Contractions of two preparations in response to nerve stimulation (at black dots; 10 shocks/sec in 10 sec with intervals of 2 min) without and in the presence of hexamethonium (C6, 3.7×10^{-4} M). The hypogastric nerve was stimulated 3 to 5 cm (left-hand records) and 1 to 5 mm (right-hand records) from the vas deferens. At the double arrow the electrode was moved as near the vas deferens as possible.

Anticholinesterases. Eserine sensitized the preparation to nerve stimulation as well as to both acetylcholine and noradrenaline. Sensitization to acetylcholine was found when the concentration of eserine was 2.4×10^{-7} M to 2.4×10^{-6} M, whereas sensitization to nerve stimulation or to noradrenaline required 2.4×10^{-5} M to 2.4×10^{-4} M. Occasionally a concentration of 2.4×10^{-3} M caused rhythmic activity. This was not found with 2.4×10^{-5} M even after 1 hr. This last concentration of eserine inhibited by more than 99% the cholinesterase activity *in vitro* (Table 1). Neostigmine (3.3×10^{-7} M to 3.3×10^{-3} M) was used in a few instances, with results similar to those with eserine.

TABLE 1

PERCENTAGE INHIBITION OF CHOLINESTERASE FROM THE VAS DEFERENS *IN VITRO* BY DIFFERENT CONCENTRATIONS OF DRUGS

Hoechst 9980 = αα-diphenyl-γ-piperidinobutylamide

	Concentration (M)			
Drug	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Eserine	76	100	—	—
Hoechst 9980	—	2	2	—
Phenoxybenzamine	0	0	—	—
Dihydroergotamine	—	0	0	—
Tolazoline	—	—	58	85
Yohimbine	0	0	0	—

Choline acetylase. The vas deferens contained easily measurable quantities of choline acetylase. In three preparations 360, 480 and 535 μg of acetylcholine per hour were synthesized by each g of dry tissue (after washing with acetone). This activity corresponds roughly to that found for salivary glands (Nordenfelt, 1963).

After section of cholinergic nerves, the choline acetylase activity of the denervated organ falls rapidly to a few percent of the value of the innervated organ (Hebb &

Waites, 1956). For the vas deferens no such decrease could be found after section of the hypogastric nerve. The choline acetylase activities (expressed as acetylcholine released) in five experiments carried out 2, 3, 4, 7 and 10 days after denervation were 600, 320, 560, 440 and 520 μg/g/hr respectively.

Catechol amines. The vas deferens contained both adrenaline and noradrenaline. The innervated preparations contained 2.34 μg of noradrenaline per g wet weight (s.e. of mean $= \pm 0.30$, $n=5$). Preparations in which the hypogastric nerve had been cut 1 week in advance contained 2.90 μg/g wet weight (s.e. of mean $= \pm 0.63$, $n=5$). The adrenaline content was 0.20 μg/g wet weight (s.e. of mean $= \pm 0.09$, $n=5$) in innervated preparations and 0.41 μg/g wet weight (s.e. of mean $= \pm 0.11$, $n=5$) in denervated ones. Thus there was no decrease in either noradrenaline or adrenaline content 1 week after denervation.

Histology. Histological examination of the hypogastric nerve after staining it with methylene blue showed the presence of ganglionic cells near the vas deferens. These cells fluoresced when examined by the method of Falck (1962) for monoamines.

DISCUSSION

The hypogastric nerve is considered to contain postganglionic sympathetic fibres to the vas deferens (see Gruber, 1933). On stimulation of this nerve the vas deferens contracts. The contraction was bigger when sympathetic or parasympathetic blocking agents were present. The sensitizing action of sympathetic blocking agents on this preparation, first observed by Boyd *et al.* (1960), was considered by these authors to be due to an anticholinesterase activity of the drugs. This, however, seems unlikely since we found no inhibition of cholinesterase activity by phenoxybenzamine, dihydroergotamine, yohimbine or $\alpha\alpha$-diphenyl-γ-piperidinobutylamide in appropriate concentrations. No alternative explanation can at present be offered.

The fact that the response to nerve stimulation could not be abolished by sympathetic or parasympathetic blocking agents does not exclude either acetylcholine or adrenaline and noradrenaline as transmitter substances. To get information about the transmitter substance, innervated and denervated preparations were tested for the presence of choline acetylase and catechol amines. Chronic section of the hypogastric nerve did not, however, significantly alter the contents of these substances. This finding is not in accordance with the belief that the hypogastric nerve contains mainly postganglionic fibres to the vas deferens. It is possible that the main nerve-supply is not in the hypogastric nerve, or more likely that the nerve sections made distal to the hypogastric ganglion were in fact preganglionic denervations. This latter explanation was supported by histological examination which revealed ganglion cells in the nerve just outside the organ. The results with nerve-stimulation in the presence of hexamethonium accord with this finding, as well as results recently reported by Sjöstrand (1962). For anatomical reasons it seems impossible to make an extensive postganglionic denervation of the organ to facilitate studying the transmitter substance of the postganglionic fibres. The experiment of Huković (1961), showing that big doses of reserpine could abolish the response to nerve stimulation and that the response could be restored on addition of noradrenaline, seems to favour the hypothesis of a noradrenergic transmission. Furthermore Falck (1962)

has demonstrated by a fluorescence method the presence of nerve fibres containing catechol amines in the musculature of the vas deferens.

Prolonged intense stimulation of the preparation either via the nerve or by drugs caused rhythmic contractions. It is of interest to note that low concentrations of isoprenaline could extinguish this response as well as single contractions caused by the various modes of stimulation. It seems reasonable to suggest that this is a membrane effect similar to the membrane stabilizing action of adrenaline on the taenia coli suggested by Bülbring (1957).

A contraction could be elicited not only by addition of a drug but also by removal of the drug. Since this response to washing was abolished independently by the appropriate antagonist to acetylcholine, noradrenaline or histamine, it could not be due to mechanical interference with the preparation. Furthermore this "washout effect" was dose-dependent and showed a definite threshold. In line with this is the observation that the "washout effect" with acetylcholine diminished rather rapidly with time of contact of the drug, and that the decrease with time was much less in the presence of eserine.

We are indebted to Dr B. Falck for great help with the histological preparations and to Dr I. Nordenfelt for the determination of the choline acetylase activity.

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