Br. J. Pharmac. Chemother. (1967), **31**, 42–55.

ADRENOLYTIC AND SYMPATHOLYTIC PROPERTIES OF 2-HALOGENOALKYLAMINES IN THE VAS DEFERENS OF THE GUINEA-PIG

BY

J. D. P. GRAHAM AND H. AL KATIB

From the Department of Pharmacology, The Welsh National School of Medicine, Cardiff

(*Received February* 20, 1967)

Boyd, Chang & Rand (1960) measured the doses of certain noradrenaline (NA) antagonists which are needed to abolish the effect of stimulating the hypogastric nerve vas deferens of the guinea-pig, and determined the ratio of these doses to the amounts needed to abolish the effects of NA added to the bath which contains the isolated preparation (Huković, 1961). The nerve was stimulated with supramaximal pulses of 2 msec, frequency 10/sec for 10 sec in every 2 min. The dose ratios for the compounds investigated were 43 for phenoxybenzamine, 165 for tolazoline and 7 for ergotamine. These compounds, and also yohimbine and piperoxan, potentiate the effect of nerve stimulation in doses which antagonize added NA. This experiment, made on a relatively simple preparation, confirms the long-standing observation (Yonkman, Stilwell & Jeremias, 1944) that "adrenolysis does not imply that sympatholysis prevails." Since no very convincing explanation of this phenomenon has been proffered, it was decided to re-investigate the effects of NA and of stimulation of the hypogastric nerve on the contractions of the isolated vas deferens of the guinea-pig, about which much is now known in relation to the effects of drugs on it, and to use as antagonists a selection of 2-halogenoalkylamines because they also have been the subject of detailed investigation (Graham, 1962). The insurmountable and irreversible nature of their blockade makes 2-halogenoalkylamines peculiarly suited to this type of experiment, in which it is desirable to recognize clearly the characteristics of the block and its duration.

METHODS

1. In vitro

A. Nerve stimulation. Male guinea-pigs of 300 g were stunned, bled, and both vasa deferentia were removed with 2–3 cm of hypogastric nerve attached; the mesentery was stripped, and the preparations were mounted in a pair of baths in Krebs solution at 37° C, gassed with 5% CO_2–95% O_2. The composition of the Krebs solution was: NaCl, 6.60 g; KCl, 0.35 g; $CaCl_2$, 0.28 g; KH_2PO_4, 0.162 g; Mg $SO_4.7H_2O$, 0.296 g; $NaHCO_3$, 2.10 g, in 1 l. distilled water. Both preparations were stimulated directly with a range of concentrations of NA or through the nerve with square pulses of 0.1 msec, 5 V, delivering trains of 200 shocks every 2 min at frequencies of 10–200/sec. The 2-halogenoalkylamine compounds applied were N-2-chloroethyldibenzylamine hydrochloride (dibenamine); 2-(N-benzyl-2-chloroethylamino)-1-phenoxypropane hydrochloride (SY28) and N, N-dimethyl-2-bromo-phenethylamine hydrobromide (L_2) (Graham & James, 1961) and their synthetic

hydrolysis products, the substituted ethanolamines. Each was added to the bath in a range of concentrations for varying times and the stimuli were repeated, before and after washing. In further preparations procaine hydrochloride was added to the bath in concentrations of 1–100 μg/ml. for periods of time from 10–30 min and stimuli were applied in its presence and after washing; also, atropine 10^{-6} g/ml. and physostigmine 10^{-6}–10^{-5} g/ml. In 3 preparations angiotensin 10^{-6} g/ml. was added.

B. Transmural stimulation. A single vas deferens-hypogastric nerve was removed from 10 guinea-pigs and mounted in the combined electrode adapted for alternation of transmural and of nerve stimulation described by Birmingham & Wilson (1963). Stimuli were applied as follows: (1) NA in a range of concentrations from 1–100 μg/ml. (2) Hypogastric nerve stimulation at 2–3 cm distance from the vas with square pulses of 0.1 msec, 30 V, frequency 25/sec; 250 shocks every 4 min or as needed. (3) Transmural stimulation with square pulses 0.1 msec, 80 V, frequency 25/sec; 250 shocks every 4 min or as needed. Physostigmine (eserine), angiotensin and 2-halogenoalkylamines were used as in 1A above.

C. Superfusion. The hypogastric nerve-vas deferens was removed from a guinea-pig of 300–400 g and mounted in a water-jacketed vessel at 37° C. The nerve was stimulated every 5 min with trains of 200 shocks at 10sec 0.5 msec, 5 V. The muscle was stimulated at times by direct application of electrodes to the surface of the vas. The tissue was superfused with gassed Krebs solution at 37° C containing atropine 10^{-7} g/ml. The excess fluid bathed a second isolated nerve-free stripped vas deferens taken from a guinea-pig of 150 g. Noradrenaline 1 μg was injected into the superfusing fluid at times, and compound SY28 or its ethanolamine in a range of doses at others. The contractions of the vasa deferentia were recorded with auxotonic levers. Ten experiments were performed.

Prostaglandins. The vasa deferentia from 4 guinea-pigs of 125–175 g wt were suspended in 8 ml. baths of gassed Krebs solution at 32° C containing atropine sulphate 10^{-7} g/ml. and NA was added for 30 sec in every 5 min in concentrations of $1–5\times10^{-7}$ g/ml. Prostaglandins E_1 and $F_{1\alpha}$ were added in concentrations of 10^{-7} to 5.10^{-7} g/ml. for 5–10 min; the bath fluid was changed and NA again applied. Compound SY28 was added to the reservoir of Krebs solution in a final concentration of 10^{-7} g/ml. and allowed 10 min contact with the tissue before the NA and PGE_1 were again applied, with or without changes of fluid.

2. In vivo

Five male guinea-pigs of 300 g were anaesthetized with 40 mg/kg pentobarbitone sodium intraperitoneally. The left jugular vein was cannulated. The abdomen was opened in the midline, the intestines were displaced, and the left vas deferens was separated from the epididymis between ligatures and connected to an isotonic frontal lever which magnified ×4 at a 0.5 g load, with minimal lifting of the vas from the abdominal cavity. The blood supply of the vas enters the organ mainly from the urethral end and was unimpaired as a result. The hypogastric nerve of the same side was freed for 2–3 cm, tied, cut and mounted on shielded electrodes under liquid paraffin. The β receptor-blocking agent pronethalol hydrochloride (nethalide) was injected in a dose of 5 mg/kg followed by NA 1–10 μg/kg and a dose-response relation was established for the latter. From this a dose which produced a submaximal contraction was selected and injected repeatedly until 3 comparable consecutive responses occurred. The nerve was stimulated with square pulses of 0.5 msec at 5 V and 10–100 shocks per sec, every 4 min. SY28 was injected intravenously in cumulative doses of 5–20 mg/kg (max. tolerated dose). A range of doses of NA was injected and the hypogastric nerve was stimulated at varying times after the SY28.

3. In vivo-in vitro

In 5 male guinea-pigs the above procedure was repeated, but the right vas deferens and hypogastric nerve was removed from the animal before drugs were injected. This preparation was mounted in the usual way in Krebs solution and the effect upon it of a range of concentrations of NA in the bath, and of stimulation of the nerve with square pulses of 0.5 msec, 5 V, 200 shocks every 2 min at frequencies ranging from 10/sec to 100/sec was determined. The anaesthetized guinea-pig was

injected intravenously with 10–20 mg/kg SY28 and the contralateral nerve and vas were removed 1 hr later and mounted similarly in a bath and tested with the same stimuli. The bath fluid was then changed repeatedly and the stimuli were re-applied over a period of time.

4. Local anaesthesia

The activity of the three haloalkylamines and the related ethanolamines as local anaesthetics was determined on infiltration and compared with that of procaine by the method of Bülbring & Wajda (1945). The ethanolamines of SY28 and of L2, which are neutral in solution, were freshly dissolved in water for each test. The parent compounds, which are acidic in solution, and the ethanolamine of dibenamine which is relatively insoluble were freshly prepared from a stock solution in acid alcohol by dilution with buffer to pH 7.4.

5. Anticholinesterase activity

The antiacetylcholinesterase activity of three haloalkylamines and the ethanolamines was determined by the method of Hestrin (1949) as modified by Jensen-Holm, Lausen, Milthers & Moller (1959). Compounds were freshly dissolved in minimal acid alcohol and neutralized with buffer solution before addition to the reaction mixture. Activity is expressed as an ID50 in molar conc.

RESULTS

In vitro

1A. Nerve stimulation. Noradrenaline readily causes a contraction of the vas deferens of the guinea-pig and this contraction is insurmountably abolished by the 2-halogenoalkylamines in concentrations which do not have any obvious effect on the tone of the preparation—for example, SY28 10^{-6} g/ml. in contact for 10–15 min—but not by the ethanolamines. The order of potency of the antagonism to NA and to dopamine (Graham & Al Katib, 1966a) is L2>SY28>dibenamine, as for the pressor response to injected NA in mammals. In the Huković preparation NA 10^{-7} to 10^{-4} g/ml. potentiates the response to "slow" (10/sec) or to "fast" (30–80 sec) stimulation and this potentiation is antagonized by addition of SY28 to the bath—for example, 1 μg/ml.

Angiotensin 1 μg/ml. has by itself no effect on the tone of the vas deferens nor on the response to added NA but potentiates the response to hypogastric and to transmural nerve stimulation at either frequency. This increase is not prevented by SY28 or by atropine.

TABLE 1

THE ACTION OF SELECTED 2-HALOGENOALKYLAMINES AND THEIR HYDROLYSIS PRODUCTS (−OH) ON THE ISOLATED GUINEA-PIG VAS DEFERENS

(a) The concentrations needed to abolish the effect of stimulating the hypogastric nerve, (b) the ED100 to antagonize added NA, (c) ratio of a/b and (d) the local anaesthetic potency relative to procaine 0=inactive. The number of experiments performed is given in brackets.

	(a)		(b)	(c)		(d)
Compound	Freq. 10/sec	Freq. 80/sec		Freq. 10	Freq. 80	
Dibenamine	5×10^{-3} (6)	10^{-4} (5)	$2{\cdot}6\times10^{-8}$	5×10^{4}	10^{4}	0·5
,,-OH	0	0	0	0	0	0
SY28	3×10^{-4} (6)	5×10^{-5} (8)	2×10^{-8}	3×10^{4}	5×10^{3}	0·75
,, -OH	0	0	0	0	0	0
L2	5×10^{-5} (6)	10^{-5} (10)	$2{\cdot}3\times10^{-8}$	5×10^{3}	10^{3}	0·33
,,-OH	2×10^{-6} (5)	2×10^{-6} (5)	0	—	—	27
Procaine	6×10^{-5} (5)	10^{-5} (9)	0	—	—	1

In calculating (c) the ED100 (b) of all three compounds was taken as 10^{-8} g/ml.

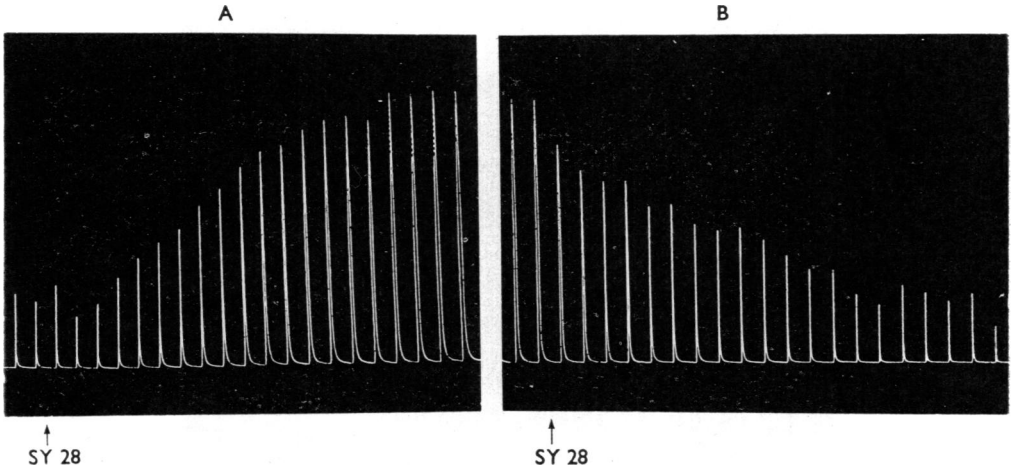

Fig. 1. Hukovic's preparation of guinea-pig vas deferens-hypogastric nerve ; hyoscine HBr, 1 μg/ml., present at all times. Records of the contraction in response to hypogastric nerve stimulation, A at 10/sec, B at 80/sec ; trains of 160 shocks every 2 min at 0.5 msec and 5 V. The 2-halogenoalkylamine SY28 potentiates the response in A and inhibits it in B.

Halogenoalkylamines

The effect of " slow " nerve stimulation (10/sec) is potentiated by compound SY28 at 10^{-6} g/ml. (see Figure 1A) ; higher concentrations—for example, 10^{-4} g/ml.—potentiate and then block. Dibenamine elicits a similar pattern of response but requires at least ten times as high a molar concentration. Compound L2 never potentiates. The order of potency of potentiation of responses to " slow " stimulation is therefore SY28>dibenamine. The potentiation is not affected by atropine or hyoscine, 10^{-6} g/ml. Responses to " fast " nerve stimulation (80/sec) are inhibited by all three compounds (see Figure 1B), the order of potency being L2$\gg$SY28>dibenamine. The concentrations of these agents required to abolish the effect of stimulation of the hypogastric nerve is much higher than the amount needed to abolish irreversibly the response to added NA. In Table 1 an approximate figure for the ratio of nerve blocking to NA-antagonizing concentrations is given. Nerve-blocking potency correlates with local anaesthetic potency, viz. L2-OH>L2>SY28$\backsimeq$dibenamine ; SY28-OH and dibenamine-OH are inactive. Eserine potentiates the effect of " slow " stimulation of the nerve. As others have reported (Birmingham, 1966) this effect is reduced by atropine. The effect of " fast " stimulation is inhibited by eserine in the presence of atropine or hyoscine.

1B. Transmural stimulation at 25 sec, which ensures post-ganglionic stimulation, produces the same pattern. Physostigmine 10^{-6}–10^{-5} g/ml. potentiates, and this is abolished by atropine 10^{-6} g/ml. Noradrenaline potentiates the response ; this is not prevented or abolished by atropine. SY28 10^{-6}–10^{-5} g/ml. also potentiates the response ; this potentiation is not prevented by 10^{-6} g/ml. atropine, but 10^{-5} g/ml. does abolish it. L2 always antagonizes. Higher concentrations of SY28—for example, 10^{-4} g/ml.—also block (see Fig. 4B). The blocked nerve, whether stimulated preganglionically at a distance of 2 cm from the vas or transmurally, recovers readily on washing ; the response

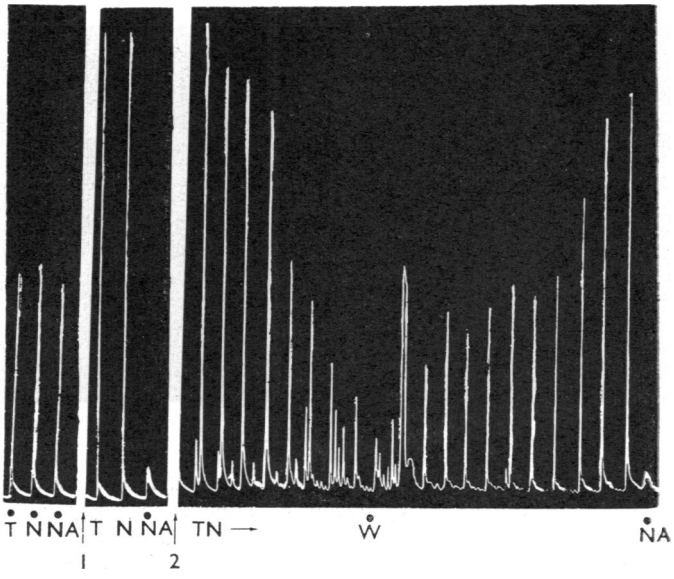

Fig. 2. Isolated vas deferens of guinea-pig stimulated alternately transmurally (T) and via hypogastric nerve (N) at 25/sec. At the first arrow compound SY28 potentiates both T and N at a concentration of $1–5 \times 10^{-5}$ g/ml., while blocking the effect of 10^{-7} g/ml. NA, but blocks both T and N at 10^{-4} g/ml. ($\uparrow_2$). This concentration causes spontaneous activity of the muscle. The block of both types of nerve stimulation but not of added NA is readily removed by washing (W).

to added noradrenaline, dopamine, or, incidentally, histamine and acetylcholine, does not. This difference is shown in Fig. 2 with noradrenaline.

The synthetic ethanolamines, as stated, are ineffective as antagonists of added NA but they are not entirely inactive as blockers of the nerve. Dibenzylethanolamine (dibenamine-OH) and the ethanolamine of SY28 in concentrations of 10^{-6} to 5×10^{-6} g/ml. have no effect on the responses of the vas to transmural or to hypogastic nerve stimulation at any frequency; concentrations greater than 10^{-4} g/ml. produce irregular contraction of the muscle. L2-OH in concentrations of 1–10 μg/ml. causes complete block of both types of indirect stimulation at all frequencies. In this respect this ethanolamine is at least 10 times as potent as its parent compound. The block is entirely and easily reversible by washing (see Fig. 3).

Procaine in a concentration of 10^{-6}–10^{-5} g/ml. reduces and may abolish the response to "fast" or to "slow" stimulation of the nerve or transmural stimulation, as described by Bentley (1966). The inhibition increases with time of exposure or increasing concentration of the local anaesthetic. The block is readily reversible by washing (see Fig. 4A).

1C. Superfusion. When NA is added to the fluid which superfuses the upper vas deferens (from the 300 g guinea-pig) in an amount sufficient to cause a moderate contraction, it causes a marked contraction of the lower vas (from the 150 g guinea-pig). Direct application of the electrode to the muscle causes the upper vas to contract strongly,

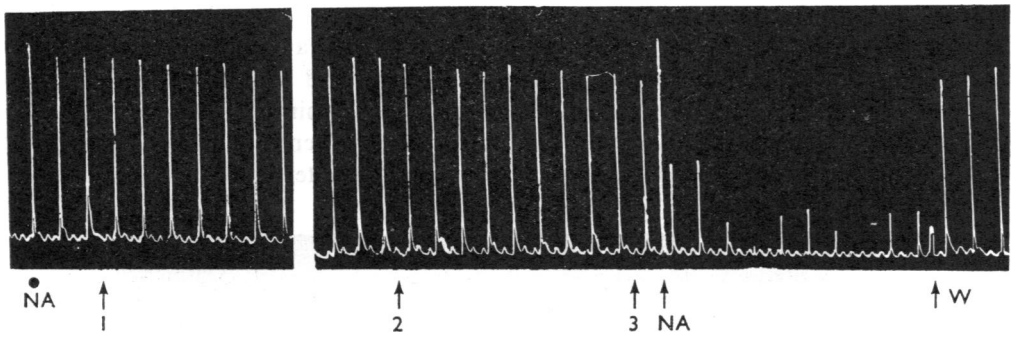

Fig. 3. Huković preparation of guinea-pig vas deferens. Hypogastric nerve stimulated with trains of 200 shocks every 2 min at 10/sec. The synthetic ethanolamines of dibenamine (↑ 1), SY28 (↑ 2), and L2 (↑ 3) were added in concentrations of 10^{-6} g/ml. The latter abolishes the effect of nerve stimulation but not of added NA. The block is easily reversed by washing (W).

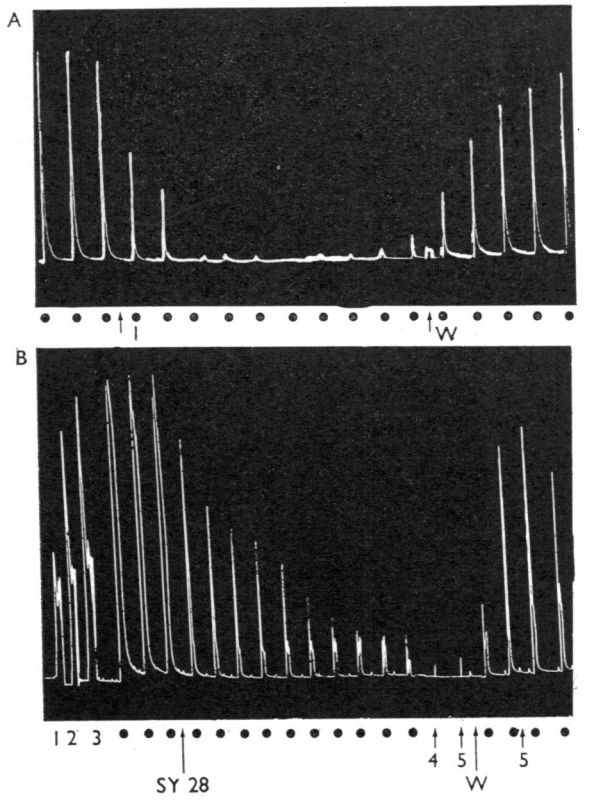

Fig. 4. Huković preparation of guinea-pig vas deferens. Nerve stimulated with trains of 240 shocks every 2 min at 80/sec. In A the reversible block which follows the addition of procaine 2×10^{-5} g/ml. at (↑ 1). In B responses to NA 10^{-7}, 2×10^{-7} and 5×10^{-7} g/ml. at (1, 2, 3), followed by three stimulations of the nerve (●) as in A. Addition of SY28 in a concentration of 5×10^{-5} g/ml. abolishes the response to nerve (●) and to NA ($4=10^{-6}$; $5=10^{-5}$ g/ml.). The nerve block is easily reversible (after W) but not the antagonism to NA (see repeat of 5).

but the lower does not follow suit. Stimulation of the hypogastric nerve causes a contraction of the upper vas which is followed after a delay by a small contraction of the recipient vas. This we attribute to an overspill of released sympathin. When SY28, 10^{-6} g/ml. is put in the superfusion fluid and applied for 20 min to both vasa the effect of added NA is abolished in both, the effect of stimulating the nerve at 10/sec is potentiated on the donor vas and the response of the recipient vas is greater than before (see Fig. 5).

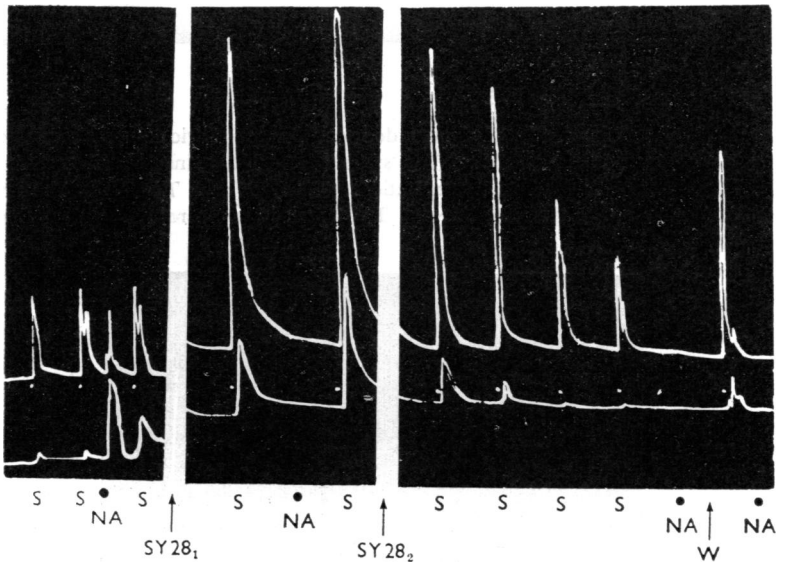

Fig. 5. Two guinea-pig vas deferens preparations with Krebs solution containing atropine 10^{-6} g/ml. The upper tracing is the response of the donor vas from a 350 g animal, stimulated with trains of 200 shocks every 5 min at 10/sec. The lower tracing is a recipient stripped vas from a 150 g animal. Stimulation of the nerve to the donor (S) causes a contraction of the donor followed after a short delay by a smaller contraction of the recipient vas. Injection of NA 10^{-6} g/ml. into the perfusing fluid contracts both and potentiates subsequent responses of the recipient. Addition of SY28 10^{-6} g/ml. to the fluid ($SY28_1$) abolishes the response of both to added NA but increases the response of both to nerve stimulation: transferred transmitter is still active on the recipient when superfused NA is antagonized. An increase in the concentration of SY28 to 10^{-5} g/ml. ($SY28_2$) abolishes the response in both vasa ; it is, however, easily restored by washing (↑ W), while the effect of NA 10^{-6} g/ml. was still abolished.

SY28 ethanolamine 10^{-6} g/ml. does not antagonize superfused NA nor potentiate the response to nerve stimulation of either vas.

1D. Prostaglandins. PGE_1 in concentrations of $2\text{–}5 \times 10^{-7}$ g/ml. causes a strong contraction of the isolated vas deferens after a delay which is longer than that noted after addition of NA. If NA is added after washing the PGE_1 from the bath the action of NA is potentiated by a factor of approximately 5. Compound SY28 in a concentration of 10^{-7} g/ml. abolishes the action of NA 5.10^{-7} g/ml. ; it also abolishes that of PGE_1 at $1\text{–}3 \times 10^{-7}$ and markedly reduces that at 5×10^{-7} g/ml. (see Fig. 6). As soon, however, as the bath fluid is changed (still in the presence of atropine and of SY28) a contraction of

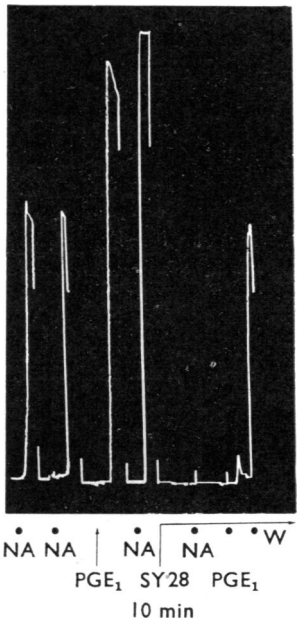

Fig. 6. Isolated vas deferens of the 150 g guinea-pig, in gassed Krebs solution at 32° C containing atropine sulphate 10^{-7} g/ml. Noradrenaline 3.10^{-7} g/ml. (NA) causes a contraction. PGE_1 at 5.10^{-7} g/ml. causes a marked contraction, after a delay. After the PGE_1 is washed out, the subsequent response to NA is potentiated. Addition of SY28 10^{-7} g/ml. to the reservoir of bathing fluid and exposure of the vas to it for 10 min or more abolishes the response to NA and greatly reduces that to PGE_1. As soon as the PGE_1 is washed out a contraction occurs.

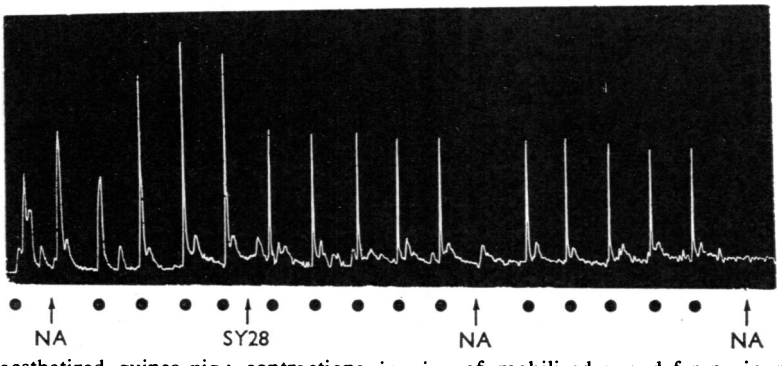

Fig. 7. Anaesthetized guinea-pig; contractions *in vivo* of mobilized vas deferens in response to stimulation of cut hypogastric nerve with trains of 240 shocks every 4 min at 80/sec. (●), Intravenous injection of NA 5 μg/kg potentiates the response to nerve stimulation. Intravenous injection of SY28 15 mg/kg abolishes the direct response of the vas to injected NA and the potentiation by NA of hypogastric nerve stimulation but does not abolish the effect of stimulating that nerve.

the vas takes place. Prostaglandin $F_1\alpha$ is inactive on isolated guinea-pig vas deferens in a concentration of 2×10^{-7} g/ml. for 10 min and has no effect on subsequently added NA.

2–3. *In vivo and in vivo-in vitro experiments*

The results obtained on the isolated vas deferens are confirmed by experiments on the anaesthetized guinea-pig. Intravenous NA causes a contraction of the vas deferens, as does stimulation of the hypogastric nerve. Injection of an active halogenoalkylamine (SY28) abolishes the effect of injected NA but not of nerve stimulation. Injection of NA

potentiates the effect of nerve stimulation and an active halogenoalkylamine such as SY28 prevents this (see Fig. 7). A dose of such a drug which is not lethal to the animal will not block nerve stimulation at frequencies of 10–100/sec.

If the specimens of nerve and vas are removed from the anaesthetized guinea-pig before and after intravenous injection of SY28 the same pattern of response is found. The control vas responds to NA *in vitro*, the vas deferens taken after injection of the animal with SY28 does not; both respond in the bath to transmural or to hypogastric nerve stimulation. In the control, the addition of NA to the bath potentiates responses to "slow" or "fast" stimulation; in the preparation taken from the guinea-pig after injection of SY28 added NA no longer potentiates and may inhibit (see Fig. 8).

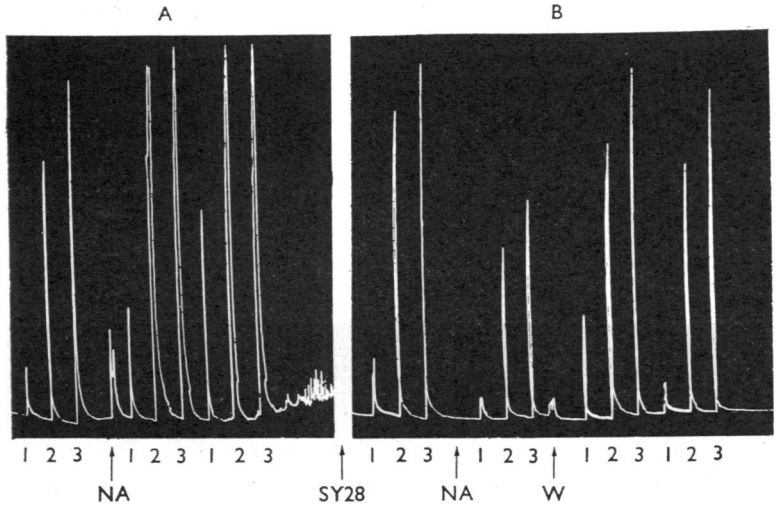

Fig. 8. Vasa deferentia with attached nerve removed from an anaesthetized guinea-pig. In A the control Huković preparation shows the potentiating action of NA 10^{-6} g/ml. added to the bath on the responses to trains of 200 shocks every 2 min at frequencies 10(1), 50(2), and 100(3) per sec applied to the nerve, and the spontaneous activity which is aroused. The responses at all three frequencies are potentiated. In B responses of the other nerve-vas taken from the same animal 1 hr after intravenous injection of SY28 15 mg/kg and treated similarly. The effect of NA is now to inhibit the responses to stimulation, but responses recover readily after washing.

4. *Local anaesthetic potency*

The potencies of the compounds as local anaesthetics tested by infiltration are shown in Table 1 where they are compared with procaine as unity. The great potency of the L2-ethanolamine is to be noted; it has been recorded previously (Graham & James, 1961).

TABLE 2

MOLAR CONCENTRATION OF 2-HALOGENOALKYLAMINES REQUIRED TO REDUCE BY 50% ACETYLCHOLINESTERASE ACTIVITY OF RED BLOOD CELLS FROM RATS, *IN VITRO*

Compound	ID_{50}
SY28	$0{\cdot}2\times10^{-5}$
,,-OH	0
Dibenamine	$3{\cdot}2\times10^{-5}$
,,-OH	0
L2	$6{\cdot}8\times10^{-2}$
,,-OH	0

5. *Anti-acetylcholinesterase activity*

The relative potencies are recorded in Table 2. The order of potency is SY28> dibenamine≫L2. This is not in agreement with antagonism of NA, nor with nerve block, but is in agreement with the relative order for potentiation of " slow " nerve stimulation. The ethanolamines are inactive.

DISCUSSION

Potentiation by noradrenaline of the effects of stimulating the isolated vas deferens *via* the hypogastric nerve has been reported (Huković, 1961), but not for transmural stimulation. It is prevented by compound SY28. Intravenously injected NA potentiates the effect of hypogastric nerve stimulation in anaesthetized guinea-pigs, and pretreatment with SY28 prevents this but does not block the nerve. If the potentiation is a consequence of filling of storage complexes in adrenergic nerves with NA, SY28 (*in vivo* and *in vitro*) must prevent this access while not preventing release of the NA already in the nerve. At this dosage or concentration level of SY28 the receptors for injected or added NA are irreversibly blocked.

Hertting & Suko (1966) described the action of angiotensin by which it potentiates the vasoconstrictor effect on the perfused spleen of stimulating sympathetic nerves and attribute this to a " decrease in flow rate." If this phenomenon is the result of an increase in tone of the innervated smooth muscle, it may be the same as the action of angiotensin on the vas deferens—that is, an additive effect of two stimulants of smooth muscle which have different modes of action. Angiotensin, however, does not potentiate the effect of added NA, which must to this extent differ from the transmitter of the sympathetic nerve to this muscle. Benelli, Della Bella & Gandini (1964) suggest that angiotensin, as a ganglion stimulant, causes an increased neurosecretion of NA after nerve stimulation. This might apply to the Huković preparation in which the nerve is largely preganglionic but not to the transmurally stimulated preparation of the stripped vas, if that is wholly post-ganglionic as we believe.

In the isolated (Huković) preparation dibenamine and SY28, but not L2, potentiate the effects of low frequency indirect stimulation. This confirms the finding of Boyd *et al.* (1960) with phenoxybenzamine, and of others. The potentiation has been correlated with anticholinesterase activity and this parallelism has played a part in the elaboration of the hypothesis of a cholinergic link in adrenergic nerves. Consideration of the anti-AChE activities of these compounds would support this idea, in that compound L2, which is inactive, is a very weak anti-AChE and is extremely labile, while SY28 is the strongest in all respects. Birmingham (1966) found that atropine reduces eserine-induced potentiation of transmural stimulation to a greater extent than the potentiation of hypogastric nerve stimulation. SY28 potentiates transmural stimulation and a concentration of atropine (10^{-6} g/ml.), which in our experience abolishes eserine potentiation of transmural stimulation, does not affect SY28 transmural potentiation. Nevertheless 5×10^{-6} g/ml. reduces it and 10^{-5} may abolish it; this is a high concentration of atropine and one suspects a non-specific mechanism. The 2-halogenoalkylamines, or the products of their hydrolysis, markedly potentiate the effect of bradykinin in contracting the smooth muscle of the vas deferens (Graham & Al Katib, 1966b) and a similar kinin-preserving

effect (Krivoy & Kroeger, 1964) is not impossible as an explanation of a non-specific potentiation. The resistance of SY28 potentiation of transmural stimulation to atropine does not help to distinguish between a pre- or post-ganglionic cholinergic link in adrenergic nerve. Clegg, Hall & Pickles (1966) have shown that prostaglandins E_1 and E_2 enhance the effect of stimulants on guinea-pig uterus. They attribute this action, which we find to be very marked in guinea-pig vas deferens, to an effect at a different site from that involved in the direct stimulant action of PG itself. Davies, Horton & Withrington (1967) have demonstrated the appearance of PGE_2 in the splenic venous blood of the dog following stimulation of the splenic (sympathetic) nerve. Close arterial injection of 5 mg phenoxybenzamine, which is of a similar potency to SY28, blocked the response of the spleen to nerve stimulation and abolished the output of PGE_2. Evidence has been presented recently (Ferreira & Vane, 1967) that intravenous injection of NA in the dog also releases PGE. We have found that SY28 10^{-7} g/ml. antagonizes the direct stimulating effect of PGE_1, $1–3 \times 10^{-7}$ g/ml. and abolishes the enhancement by it of added NA on the isolated vas deferens. The greatly enhanced response of the donor and recipient vasa to nerve stimulation in the superfusion experiment when the effect of added NA was abolished by SY28 cannot, therefore, readily be attributed to release of PGE following nerve stimulation. If this mechanism released sufficient PG in the isolated vas deferens of the guinea-pig to cause marked contraction of that organ despite the presence of SY28, the vas would also be expected to contract from released PGE when superfused NA was added. We conclude that release of prostaglandins do not account for the observed facts.

The concentrations of 2-halogenoalkylamine which block nerve stimulation are in excess of those which abolish the response to added NA, and the dose ratios exceed those for piperoxan or phentolamine, and that for ergotamine. Such doses abolish the stimulant action of histamine, acetylcholine, NA, dopamine and K^+ (Graham & Al Katib, 1966a). The type of blockade of nerve exerted differs in that it is easily reversible by washing, whereas the characteristic of the 2-halogenoalkylamine is that it insurmountably and irreversibly antagonizes NA. Furthermore L2-ethanolamine, which is inactive as an antagonist of NA, is a powerful blocker of hypogastric or transmural nerve-stimulation. It is a local anaesthetic of great potency. The block exerted by these compounds and by procaine is reversed by washing with similar ease. It is suggested that 2-halogenoalkylamines are adrenergic blockers in isolated preparations by virtue of their local anaesthetic properties and do not act by occupation of the specific receptors for transmitter. The customary explanation for the discrepancy between the doses of anti-adrenaline drugs which are needed to abolish the smooth muscle stimulating effects of injected NA and the similar effects of stimulating post-ganglionic sympathetic nerve is that the antagonist is little able to penetrate to the subsynapic receptors, whereas it passes readily from the extracellular fluid to receptors elsewhere on the cell membrane. Examination of the presumptive terminal region of sympathetic nerve in relation to smooth muscle in the vas deferens of the rat (Richardson, 1962) and of guinea-pig arterioles (Lever, Graham, Irvine & Chick, 1965 ; Lever, Graham & Spriggs, 1967) reveals that the synaptic interval varies widely in different muscles (180–250 A° in the rat vas ; 830–4000 A° in arterioles) and that no cell specialization occurs at the point of contact, which is an interval between naked axon on the one hand and undifferentiated muscle membrane on the other. The amount of basement substance present on the opposing surfaces is

variable. Unless NA can pass freely through the axonal Schwann-cell covering it must be through this uncovered presynaptic region that it goes to charge the storage granules and from this membrane must come the NA released when the nerve is stimulated. Since 2-halogenoalkylamine prevents the enhancement of nerve stimulation which normally is a consequence of injecting or adding NA, these compounds must also reach the synaptic space and prevent uptake, as they are known to do in many tissues (Farrant, Harvey & Pennefather, 1964). The adrenergic blocking action of these compounds or of the derived ethanolamine also indicates a capacity to penetrate the synapse to the presynaptic membrane. The postulated barrier must therefore exist on the post-synaptic membrane, but there is no morphological basis for it. When SY28 in the superfusing fluid prevents the action of NA on the donor and recipient vasa the α-receptor on the muscle cells of both organs must be blocked, with the exception of the subsynaptic regions of the cell membranes which may be protected by the hypothetical barrier. The released NA spills over in excess amounts because of the blockade of the scattered α-receptors which would otherwise bind it; since the recipient vas contracts to an enhanced degree despite the α-blockade the transmitter NA must freely penetrate to the unblocked α-receptors in the subsynaptic region of the recipient cells; but injected or added NA produces no response in the recipient vas. This supposition is therefore absurd. The only remaining explanation is that in the hypogastric nerve-vas deferens of the guinea-pig the transmitter substance is not solely NA (and it is not ACh nor PGE), but something which is unaffected by SY28 and atropine.

SUMMARY

1. Many adrenolytic drugs are known to possess feeble sympatholytic properties and the 2-halogenoalkylamines are no exception.

2. In the guinea-pig vas deferens stimulated *via* the hypogastric nerve or transmurally noradrenaline (SY28 sensitive), physostigmine (partly atropine sensitive) and angiotensin (not sensitive to either blocking drug) potentiate responses to nerve stimulation when added to the bath.

3. Dibenamine and 2-(N-benzyl-2-chloroethylamino)-1-phenoxypropane hydrochloride (SY28) potentiate low frequency nerve (10/sec) and transmural (25/sec) stimulation but not higher frequency (80/sec) nerve stimulation. N,N-dimethyl-2-bromo-2-phenethylamine hydrobromide (compound L2) always inhibits.

4. None of the compounds blocks either type of nerve stimulation in concentrations which abolish the effect of added noradrenaline.

5. The substituted ethanolamines of dibenamine and SY28 are inactive; that of compound L2 readily blocks nerve stimulation of either type. These compounds do not antagonize noradrenaline.

6. A similar pattern of responses may be demonstrated *in vivo* or with *in vitro* preparations taken from animals treated *in vivo* with the blocking drugs.

7. The order of potency of the halogenoalkylamines dibenamine and SY28 as potentiators of low frequency nerve and transmural stimulation is the same as the observed anticholinesterase activity.

8. The order of activity of the three halogenoalkylamines as blockers of nerve stimulation is the same as the order of potency as local anaesthetics and unlike noradrenaline-antagonism the effect resembles that of procaine and of the ethanolamine of compound L2 in being readily reversed by washing.

9. The perfusate from a superfused donor vas deferens causes a contraction in a recipient vas deferens when the nerve to the donor is stimulated.

10. Added noradrenaline contracts both vasa. If SY28 is added to the superfusing fluid neither vas responds to noradrenaline but both respond to an increased extent to stimulation of the hypogastric nerve of the donor.

11. Prostaglandin E_1 causes a contraction of isolated guinea-pig vas deferens and potentiates the effects of added NA, but SY28 abolishes or markedly reduces both these prostaglandin effects.

We are indebted to Professor N. B. Chapman, of Hull, for the gift of 2-halogenoalkylamines, ethanolamines and relevant data; to Dr. A. T. Birmingham, of King's College, Strand, London, W.C.2, for the loan of electrodes. The nethalide was a gift from Imperial Chemical Industries Limited, and the prostaglandins from Professor V. R. Pickles, Cardiff.

REFERENCES

BENELLI, G., DELLA BELLA, D. & GANDINI, A. (1964). Angiotensin and peripheral sympathetic nerve activity. *Br. J. Pharmac. Chemother.*, **22**, 211–219.

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.

BIRMINGHAM, A. T. & WILSON, A. B. (1963). Preganglionic and post ganglionic stimulation of the guinea. pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **21**, 569–580.

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

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

BÜLBRING, E. & WAJDA, I. (1945). Biological comparison of local anaesthetics. *J. Pharmac. exp. Ther.*, **85**, 78–84.

CLEGG, P. C., HALL, W. J. & PICKLES, V. R. (1966). The action of ketonic prostaglandins on the guinea-pig myometrium. *J. Physiol., Lond.*, **183**, 123–144.

DAVIES, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1967). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *J. Physiol., Lond.*, **188**, 38P–39P.

FARRANT, J., HARVEY, JUDITH A. & PENNEFATHER, J. N. (1964). The influence of phenoxybenzamine on the storage of noradrenaline in rat and cat tissues. *Br. J. Pharmac. Chemother.*, **22**, 104–112.

FERREIRA, S. H. & VANE, J. R. (1967). The disappearance of prostaglandins from the circulation. *Br. J. Pharmac. Chemother.*, **30**, 1.

GRAHAM, J. D. P. (1962). The 2-halogenoalkylamines, in *Progress in Medicinal Chemistry*, vol. 2, pp. 132–171. Butterworth, London.

GRAHAM, J. D. P. & JAMES, G. W. L. (1961). The pharmacology of a series of substituted 2-halogeno-alkylamines. *J. med. pharm. Chem.*, **3**, 489–504.

GRAHAM, J. D. P. & AL KATIB, H. (1966a). The action of trypsin on blockade by 2-halogenoalkylamines. Speculation on the nature of the alpha receptor for catecholamine. *Br. J. Pharmac. Chemother.*, **28**, 1–14.

GRAHAM, J. D. P. & AL KATIB, H. (1966b). The effect of 2-halogenoalkylamines on the biological activity of some peptides. *Br. J. Pharmac. Chemother.*, **27**, 377–386.

HERTTING, G. & SUKO, J. (1966). Influence of angiotensin, vasopressin or changes in flow rate on vaso-constriction, changes in volume and (^{3}H)-noradrenaline release following post-ganglionic sympathetic nerve stimulation in the isolated cat spleen. *Br. J. Pharmac. Chemother.*, **26**, 686–696.

HESTRIN, S. (1949). The reaction of acetylcholine and other carboxylic acid derivitives with hydroxyl-amine, and its analytical application. *J. biol. Chem.*, **180**, 249–261.

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

JENSEN-HOLM, J., LAUSEN, H. H., MILTHERS, K. & MOLLER, K. O. (1959). Determination of the cholinesterase activity in blood and organs by automatic titration. With some observations on serious errors of the method and remarks on the photometric determination. *Acta pharmac. tox.*, **15**, 384–394.

KRIVOY, W. & KROEGER, D. (1964). The preservation of bradykinin by phenothiazines *in vitro*. *Br. J. Pharmac. Chemother.*, **22**, 329–341.

LEVER, J. D., GRAHAM, J. D. P., IRVINE, G. & CHICK, WENDY J. (1965). The vesiculated axons in relation to arteriolar smooth muscle in the pancreas. A fine structural and quantitative study. *J. Anat.*, **99**, 299–313.

LEVER, J. D., GRAHAM, J. D. P. & SPRIGGS, T. L. B. (1967). Electron microscopy of nerves in relation to the arteriolar wall. (*Symp. electr. Activ. Innerv. Blood Vessels, Cambridge.*) *Bibl. anat.*, **8**, 51–55.

RICHARDSON, K. C. (1962). The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. *J. Anat.*, **96**, 427–442.

YONKMAN, F. F., STILWELL, D. & JEREMIAS, R. (1944). The adrenolytic and sympatholytic actions of yohimbine and ethyl yohimbine. *J. Pharmac. exp. Ther.*, **81**, 111–115.