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THE ACTION OF PEMPIDINE AND ANTIADRENALINE SUBSTANCES AT THE SYMPATHETIC POSTGANGLIONIC TERMINATION

BY

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Substances such as bretylium block the terminations of sympathetic postganglionic fibres, so that impulses passing along the fibres are unable to release noradrenaline. The action of these substances has been considered by Burn & Froede (1963) who pointed out that all of them had some blocking action at the neuromuscular junction or at the sympathetic ganglion. To emphasize the relation they examined trimethylphenylammonium which was shown by Riker (1953) to block the neuromuscular junction. They found that it would also block the sympathetic postganglionic fibre. Recently, Burn & Gibbons (1964a) have observed that mecamylamine and pempidine, which block transmission at the sympathetic ganglion, will also block the postganglionic fibre. We have now made further observations on this action.

Various substances which have an antiadrenaline action, chiefly phenoxybenzamine, phentolamine and Hydergine, have been used by Brown & Gillespie (1957) and by Blakeley, Brown & Ferry (1963) in their studies of the release of noradrenaline from the spleen in response to stimulation of the splenic nerves. Boyd, Chang & Rand (1960) showed that tolazoline, piperoxan, yohimbine, ergotamine and phenoxybenzamine had an anticholinesterase action, and they pointed out that such an action might be involved in effects produced by these substances at the sympathetic postganglionic terminations. We have now carried out experiments with piperoxan, yohimbine, phentolamine, Hydergine and tolazoline to investigate this suggestion further. Observations have been made on the toad rectus abdominis, the guinea-pig ileum, the isolated preparation of the hypogastric nerve and vas deferens of the guinea-pig, and the Finkleman preparation of the rabbit ileum. Our results have a bearing on the mechanism of the release of noradrenaline at the sympathetic postganglionic terminations.

METHODS

Rectus abdominis of the toad. Observations were made using the isolated rectus abdominis muscle suspended in Ringer solution of the following composition per litre. Sodium chloride 7.5 g, potassium chloride 0.15 g, calcium chloride 0.2 g, sodium dihydrogen phosphate 0.01 g, and dextrose 1.0 g. The *p*H was adjusted to 7.8 with sodium bicarbonate. The bath volume was 5 ml., and the solution was bubbled with 95% oxygen and 5% carbon dioxide. Experiments were done at room temperature (23° C). Acetylcholine, in 5 ml. of Ringer solution, was added at intervals of 4 min, being left in contact with the muscle for 1 min. When a uniform response had been obtained, the substance to be tested was added to

the bath for 5 min, and the fluid was changed to one containing either acetylcholine alone or acetylcholine together with the substance being tested.

Guinea-pig isolated ileum. Observations were made in Tyrode solution at 32° C bubbled with 95% oxygen and 5% carbon dioxide. Acetylcholine was added at intervals of 3.5 min and left in contact with the muscle for 30 sec. The isotonic contraction was recorded by a frontal lever magnifying five-times. When a uniform response had been obtained, the substance being tested was left in the bath for 5 min, and its effect on the response to acetylcholine was determined. Following each successive bath change the ileum was exposed to the drug for 3.5 min. After a series of three contractions in the presence of the drug, the ileum was washed thoroughly and exposed to acetylcholine only.

Guinea-pig vas deferens. Observations were made in McEwen's (1956) solution at 32° C bubbled with 95% oxygen and 5% carbon dioxide. Preparations were made from guinea-pigs weighing 300 to 500 g. The animals were killed by a blow on the head and the abdomen opened; the intestines were moved to one side, and the vas deferens on each side was dissected free. The hypogastric nerves were identified in the mesentery of the colon, dissected free, and removed with the vas deferens attached. The preparation was mounted in a 170-ml. organ-bath and connected to an isotonic frontal lever with a fivefold magnification. The nerve was held in a submerged electrode of a pattern described by Burn & Rand (1960). Supramaximal stimuli of 1 or 0.5 msec duration and constant voltage were delivered from an electronic rectangular-wave stimulator (C. F. Palmer Ltd.). Trains of 200 shocks at various frequencies were given at 2-min intervals. Hyoscine hydrobromide was added so that the final concentration was 1×10^{-7}.

Finkleman preparation of the rabbit isolated ileum. Observations were made in Tyrode solution at 32° C bubbled with 95% oxygen and 5% carbon dioxide. With the animal freshly killed and the abdomen opened, pieces of ileum 1.5 to 2 in. long were removed together with the mesentery containing the arteries. They were placed in a beaker containing Tyrode solution through which a stream of 95% oxygen and 5% carbon dioxide was passed. A piece was then transferred to a Petri dish, where a thread was tied round the artery. By means of this thread, the mesentery was pulled through a hole of the stimulating electrode described by Burn & Rand (1960). The preparation was placed in a 50-ml. organ-bath and connected to an isotonic lever writing on a smoked drum. Supramaximal stimuli of 1 msec duration were given from the electronic rectangular-wave stimulator at various frequencies using a fixed number of shocks. Stimulation was applied at regular intervals which were usually 3 min.

The substances used were pempidine tartrate, piperoxan hydrochloride, yohimbine hydrochloride, Hydergine (the methanesulphonate of the dihydro derivative of what used to be called ergotoxine), tolazoline hydrochloride, phentolamine hydrochloride, hyoscine hydrobromide, physostigmine salicylate and acetylcholine chloride. Doses of these drugs are stated in terms of the salts.

RESULTS

The action of pempidine. Mecamylamine and pempidine diminish the response of the rabbit isolated ileum to sympathetic stimulation (Burn & Gibbons, 1964a). The action of these substances might have been due to a block of conduction along the postganglionic fibres. Experiments were therefore made at different frequencies of stimulation, since bretylium (Boura & Green, 1959) and trimethylphenylammonium (Burn & Froede, 1963), when acting at the postganglionic terminations, cause a block to stimulation of high frequency before causing a block to stimulation of low frequency.

In three experiments it was found that pempidine (3×10^{-5}) diminished the response to stimulation of high frequency when the response to that at low frequency was unaffected. Fig. 1 shows that pempidine did not modify the response to stimulation at 10 shocks/sec at the time when it diminished the response to 20 shocks/sec, greatly diminished the response to 50 shocks/sec and almost abolished the response to 100 shocks/sec.

Antiadrenaline substances on toad rectus. Piperoxan, yohimbine and Hydergine increased the contraction of the toad rectus caused by acetylcholine. The percentage increase in contraction is given in Table 1. In Fig. 2 the effect of 5×10^{-7} acetylcholine is shown, and

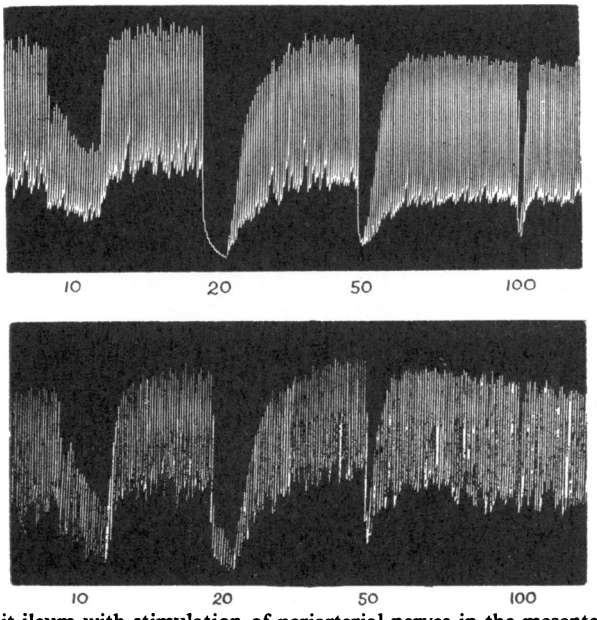

Fig. 1. Isolated rabbit ileum with stimulation of periarterial nerves in the mesentery. The upper panel shows control observations when 1,000 shocks (supramaximal) were applied at frequencies of 10, 20, 50 and 100 per sec. The lower panel shows observations after pempidine (3×10^{-5}) was present in the organ-bath for 25 min. The response to 10 shocks/sec was unchanged; that to 20 shocks/sec was reduced; that to 50 shocks/sec was greatly reduced; and that to 100 shocks/sec was practically abolished.

TABLE 1

PERCENTAGE INCREASE IN ACTION OF ACETYLCHOLINE (0·5 μG/ML.) ON TOAD RECTUS IN PRESENCE OF PIPEROXAN, YOHIMBINE AND HYDERGINE

	Concentrations of piperoxan			
	1×10^{-5}	2×10^{-5}	3×10^{-5}	4×10^{-5}
Expt. 1	14%	20%	23%	—
Expt. 2	—	—	—	45%
	Concentrations of yohimbine			
	1×10^{-5}	2×10^{-5}	3×10^{-5}	2×10^{-4}
Expt. 1	8%	13%	12%	—
Expt. 2	—	—	—	71%
	Concentrations of Hydergine			
	2×10^{-6}	5×10^{-6}	1×10^{-5}	$1{\cdot}5\times10^{-5}$
Expt. 1	62%	—	—	—
Expt. 2	—	20%	24%	41%

also the potentiation of this contraction by concentrations of piperoxan of 1, 2 and 3×10^{-5}. The activity of yohimbine appeared to be about the same as that of piperoxan, and these substances had approximately one-quarter of the activity of physostigmine. Their action was easily removed by washing the muscle, whereas the action of physostigmine persisted.

Hydergine was from two to four times more active than piperoxan and yohimbine. Concentrations of piperoxan and of yohimbine greater than 8×10^{-5} often depressed the

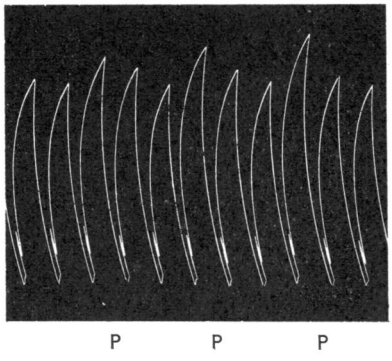

Fig. 2. Contractions of rectus abdominis of the toad in response to acetylcholine (5×10^{-7}), showing the potentiation by piperoxan (P). The third contraction was recorded in the presence of piperoxan, 1×10^{-5}, the sixth was in the presence of piperoxan, 2×10^{-5}, and the ninth was in the presence of piperoxan, 3×10^{-5}.

response to acetylcholine, and in one experiment a concentration of 2×10^{-6} Hydergine also caused depression, the contraction after washing out the Hydergine being increased by 80%. The next contraction was of the initial height.

Guinea-pig ileum. Piperoxan and yohimbine increased the action of acetylcholine on the guinea-pig ileum in the low concentration of 5×10^{-9}, in which concentration physostigmine was equally effective. The action of yohimbine diminished on washing out, but that of piperoxan, like that of physostigmine, was more persistent. Hydergine also potentiated the action of acetylcholine, but it was weaker; the effects of concentrations of 1.5 and of 4.5×10^{-8} are shown in Fig. 3. Piperoxan and yohimbine in higher concentrations (1.3 to 3.3×10^{-6}) and Hydergine (6×10^{-6}) depressed the contraction caused by acetylcholine.

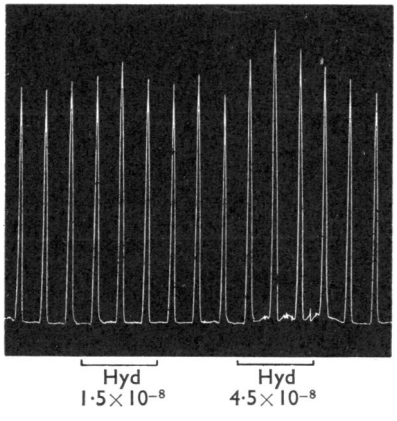

Fig. 3. Contractions of guinea-pig ileum in response to acetylcholine (1×10^{-6}), showing the potentiation by Hydergine (Hyd). Hydergine was present in the bath during the fourth, fifth and sixth contractions in a concentration of 1.5×10^{-8}, and during the tenth, eleventh and twelfth contractions in a concentration of 4.5×10^{-8}.

Vas deferens of the guinea-pig. When the contractions of the vas deferens were elicited by stimulation of the hypogastric nerve, Burn & Weetman (1963) observed that in the presence of hyoscine (1×10^{-7}) the height of the contraction in response to a fixed number of shocks increased as the frequency of stimulation increased up to 20 per sec. Above this frequency the response did not increase and, indeed, became rather less. In our observations the response increased further as the frequency rose to 50 shocks/sec, and then declined at 100 shocks/sec. Burn & Weetman (1963) observed that, when physostigmine or neostigmine were added to the bath, the response to stimulation of low frequency increased and the response to the optimal frequency diminished. Thus both physostigmine and neostigmine modified the response of the vas deferens to hypogastric stimulation as they modified the response of the rat diaphragm to phrenic nerve stimulation (Bülbring, 1946).

Piperoxan (1.2×10^{-5}), acting in the presence of hyoscine, increased the response to stimulation of the hypogastric nerve at frequencies of 5 and 10 shocks/sec, did not affect the response at a frequency of 20 shocks/sec, and decreased the response at frequencies of 50 and 100 shocks/sec (Fig. 4). Similar observations were made with yohimbine in a concentration of 1×10^{-5} (Fig. 5). While the effect of piperoxan in Fig. 4 was observed 80 min after piperoxan had been added to the bath, the effect of yohimbine in Fig. 5 was observed immediately. The immediate effect of piperoxan was maintained for a long time

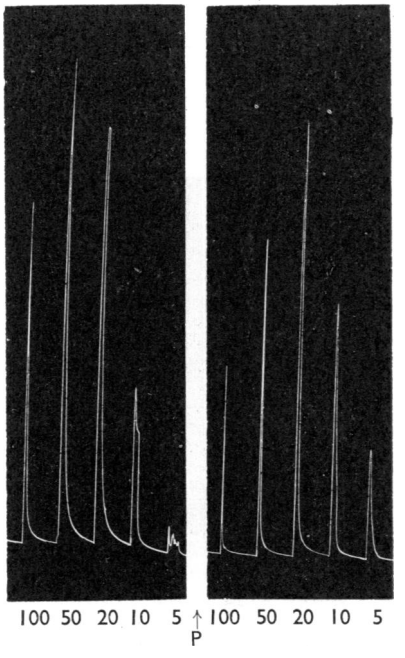

Fig. 4. Contractions of the guinea-pig vas deferens in response to stimulation by 200 supramaximal shocks at the frequencies shown. Hyoscine (1×10^{-7}) was present throughout. The left-hand panel shows control observations. Stimulation was applied at intervals of 2 min. The right-hand panel shows observations in the presence of piperoxan (1.2×10^{-5}) made 80 min after the addition of piperoxan (P) to the bath. Note that the responses to 100 and to 50 shocks/sec were decreased, that to 20 shocks/sec was unchanged, and those to 10 and 5 shocks/sec were increased.

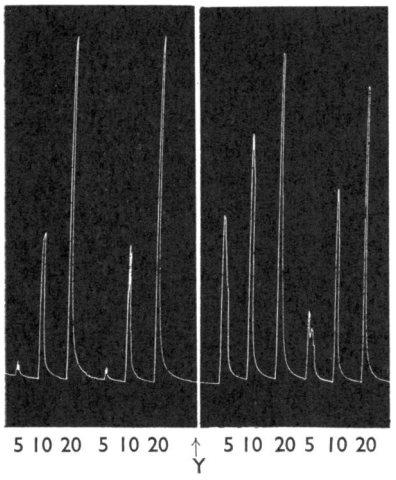

Fig. 5. Contractions of the guinea-pig vas deferens in response to hypogastric stimulation in the presence of hyoscine (1×10^{-7}) to show the action of yohimbine (1×10^{-5}). The left-hand panel shows responses to 200 shocks at 5, 10 and 20 shocks/sec, recorded twice. The right-hand panel shows the change in the responses immediately after adding yohimbine (Y). The response at 5 shocks/sec was greatly increased, that to 10 shocks/sec was increased, though less than at 5 shocks/sec, while the response to 20 shocks/sec was diminished. The next three responses were all reduced, probably by the anti-adrenaline action of yohimbine.

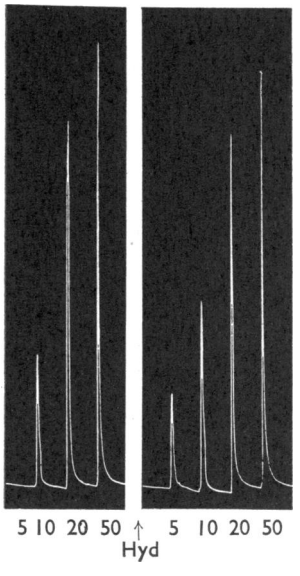

Fig. 6. Similar records to those in Fig. 4, showing the effect of Hydergine (Hyd, 9×10^{-6}). Here also the response to stimulation at 5 and at 10 shocks/sec was increased. The response to 20 shocks/sec was very slightly diminished, and that to 50 shocks/sec was diminished a little more.

because the antiadrenaline action in the concentration used was too small to modify the result, but the antiadrenaline action of yohimbine was exerted very soon.

Hydergine (9×10^{-6}) increased the responses to stimulation at 5 and 10 shocks/sec, but decreased the responses to stimulation at 20 and 50 shocks/sec (Fig. 6).

Other experiments with all three compounds are recorded in Tables 2, 3 and 4.

Observations with tolazoline showed that, in the presence of hyoscine, concentrations from 1 to 6×10^{-5} always caused an increase in the response to stimulation at low frequencies, but the response to stimulation at high frequencies was unchanged or diminished only slightly. At still higher concentrations the antiadrenaline action of tolazoline diminished the responses to all frequencies.

TABLE 2

EFFECT OF PIPEROXAN ON THE CONTRACTION OF THE VAS DEFERENS OF THE GUINEA-PIG IN RESPONSE TO HYPOGASTRIC STIMULATION AT DIFFERENT FREQUENCIES

Hyoscine (1×10^{-7}) was present in the organ-bath. Figures are heights of contraction in mm on the drum, and percentage changes

Conc. of piperoxan	Control at Frequency (shocks/sec)					Piperoxan at Frequency (shocks/sec)					Percentage change at Frequency (shocks/sec)				
	5	10	20	50	100	5	10	20	50	100	5	10	20	50	100
3×10^{-6}	1·5	9	88	114		6	32	73	86		+300	+256	−17	−25	
6×10^{-6}	0·5	18	96	134		9	34	86	108		+1,700	+89	−1	−19	
1×10^{-5}	0·5	7·5	68	127		9·5	39	93	111		+1,800	+420	+37	−13	
$1·2\times10^{-5}$	6	35	92	108	75	24	56	96	70	41	+300	+60	+4	−35	−45

TABLE 3

EFFECT OF YOHIMBINE ON THE CONTRACTION OF THE VAS DEFERENS OF THE GUINEA-PIG IN RESPONSE TO HYPOGASTRIC STIMULATION AT DIFFERENT FREQUENCIES

Hyoscine (1×10^{-7}) was present in the organ-bath. Figures are heights of contraction in mm on the drum, and percentage changes

Conc. of yohimbine	Control at Frequency (shocks/sec)					Yohimbine at Frequency (shocks/sec)					Percentage change at Frequency (shocks/sec)				
	5	10	20	50	100	5	10	20	50	100	5	10	20	50	100
3×10^{-6}	1·5	20	61·5	111	—	10	27	76	97	—	+566	+35	+24	−13	—
6×10^{-6}	1·5	17	60	108	—	5	32	89	99	—	+233	+88	+48	−8	—
1×10^{-5}	4	41	98	—	—	47	69	92	—	—	+1,075	+68	−6	—	—

TABLE 4

EFFECT OF HYDERGINE ON THE CONTRACTION OF THE VAS DEFERENS OF THE GUINEA-PIG IN RESPONSE TO HYPOGASTRIC STIMULATION AT DIFFERENT FREQUENCIES

Hyoscine (1×10^{-7}) was present in the organ-bath. Figures are heights of contraction in mm on the drum, and percentage changes

Conc. of Hydergine	Control at Frequency (shocks/sec)					Hydergine at Frequency (shocks/sec)					Percentage change at Frequency (shocks/sec				
	5	10	20	50	100	5	10	20	50	100	5	10	20	50	100
3×10^{-6}	0	28	89	117	103	4	42	97	108	98	+800	+50	+9	−8	−5
3×10^{-6}	0	19	90	132	119	6	34	93	129	115	+1,200	+79	+33	−2	−3
9×10^{-6}	0	35	98	118	101	25	52	96	111	98	+5,000	+48	−2	−6	−3

The action of phentolamine was to some extent the reverse of the action of tolazoline, in the sense that with phentolamine the reduction in the response to high-frequency stimulation was readily observed, but the increase in the response to low-frequency stimulation was not large. The smallness of the increase was probably explained by the overlap of the anti-adrenaline action with the anticholinesterase action. Had more concentrations of phentolamine been tested the two actions might have been more successfully separated, and a greater increase in the response to stimulation of low frequency obtained.

The rabbit ileum. In the preparation of the rabbit ileum first used by Finkleman (1930), stimulation of the periarterial nerves in the mesentery inhibits the pendular movement. The stimulation is therefore applied to sympathetic fibres, and, since the stimulation is completely unaffected by hexamethonium, the fibres stimulated are postganglionic. We have stimulated at different frequencies as when working with the vas deferens, and Figs. 7

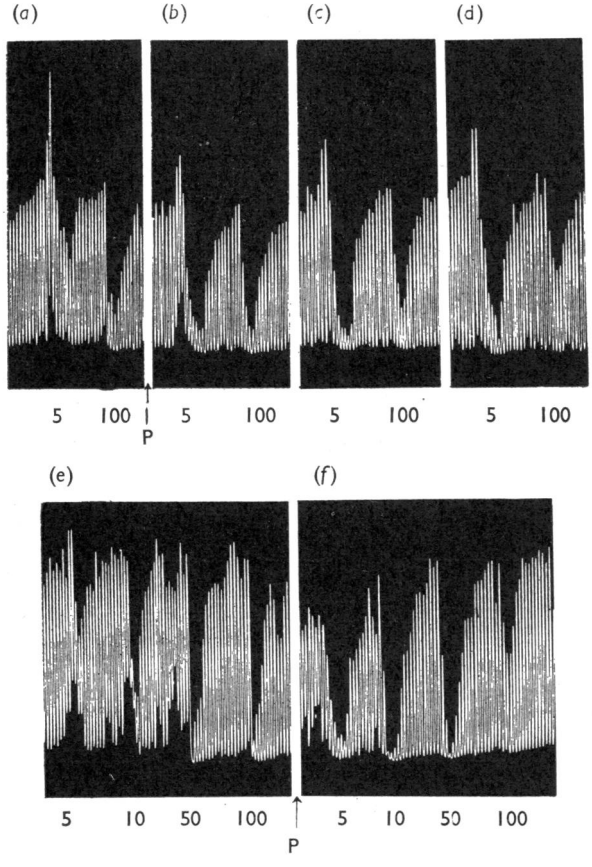

Fig. 7. Rabbit ileum stimulated through periarterial nerves. Upper row: (*a*) control observations, 200 shocks at 5 and at 100 shocks/sec; between (*a*) and (*b*) piperoxan (P, 2 μg/ml.) was added; (*b*) after 2 min, (*c*) after 8 min, (*d*) after 12 min. Note increase in response to 5 shocks/sec and decrease in response to 100 shocks/sec. Lower row: (*e*) control observations at 5, 10, 50 and 100 shocks/sec; (*f*) after addition of piperoxan (8×10^{-6}); note increase in response to 5 and 10 shocks/sec, response to 50 shocks/sec not much changed and response to 100 shocks/sec reduced.

and 8 show the effect of piperoxan and of yohimbine. Whereas in Fig. 7 the initial effect of stimulating at 5 shocks/sec was much smaller than that of stimulating at 100 shocks/sec, this relation was progressively reversed (Fig. 7,*c* and *d*). Thus piperoxan increased the response to stimulation at low frequency and decreased the response to stimulation at high frequency. A similar result was seen when stimulation was applied at four frequencies (Fig. 7,*e* and *f*).

Fig. 8 shows similar results with yohimbine. Yohimbine (2×10^{-6}) increased the response to stimulation at 5 shocks/sec but decreased the response to 100 shocks/sec (Fig. 8,*a*). The effect of yohimbine was next tested on four frequencies. The response to 5 shocks/sec was much increased, to 10 shocks/sec was somewhat increased, to 50 shocks/sec was slightly decreased and to 100 shocks/sec was clearly decreased (Fig. 8,*b* and *c*).

Fig. 9 shows similar results with Hydergine. The low concentration of 5×10^{-8} increased the response to stimulation at 5 shocks/sec, and decreased the response to stimulation at

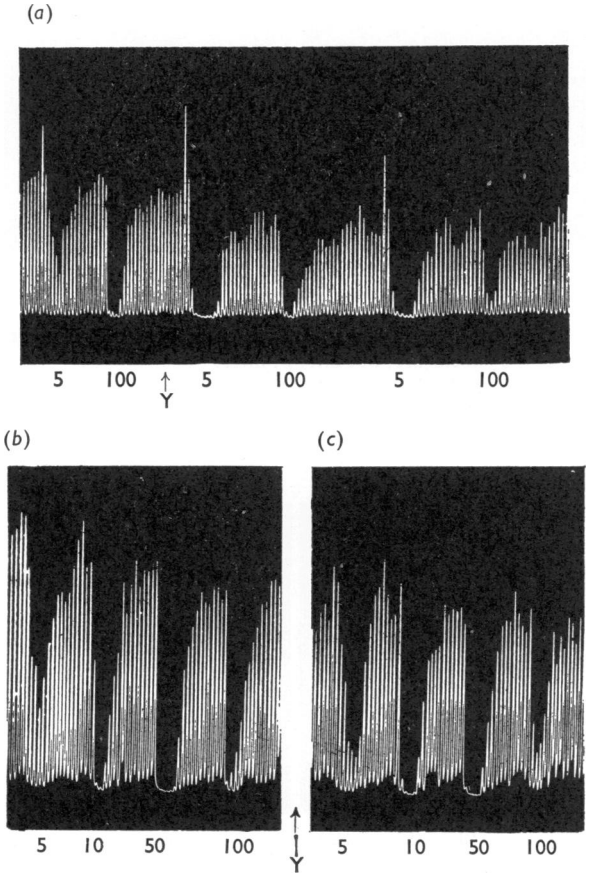

Fig. 8. Similar to Fig. 7, showing the effect of yohimbine (2×10^{-6}) in upper and lower row. In the upper row yohimbine (Y) at arrow caused increased response to 5 shocks/sec and decreased response to 100 shocks/sec. In the lower row, yohimbine increased the response to 5 and 10 shocks/sec, but decreased the response to 50 (slightly) and 100 shocks/sec.

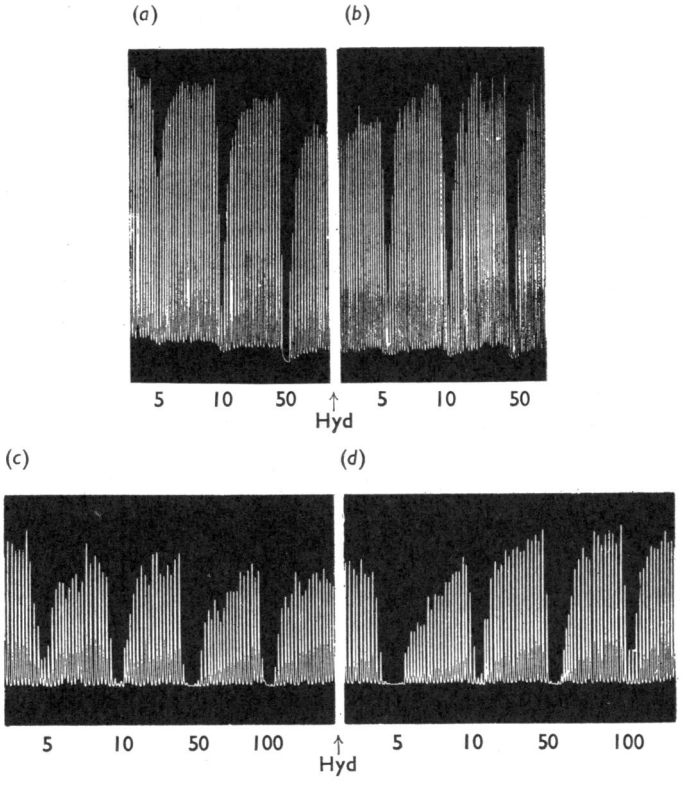

Fig. 9. Similar to Figs. 7 and 8, showing in upper row the effect of Hydergine (5×10^{-8}). Hydergine increased the response to 5 shocks/sec, but did not affect the response to 10 shocks/sec. It decreased the response to 50 shocks/sec. In the lower row, Hydergine (6×10^{-8}) greatly increased the response to 5 shocks/sec; it slightly diminished the response to 50 shocks/sec; and greatly diminished the response to 100 shocks/sec. In the right-hand panel there is doubt about the stimulation given at 10 shocks/sec.

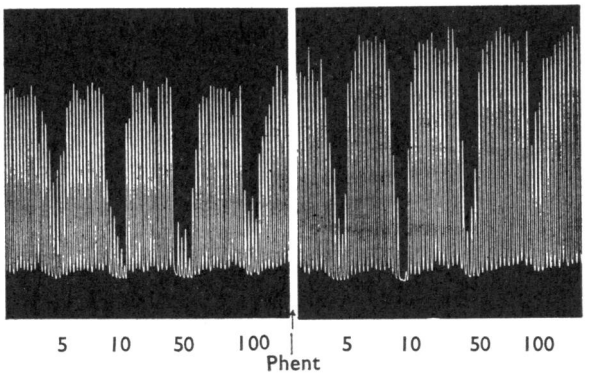

Fig. 10. Similar to Figs. 7, 8 and 9. It shows the effect of phentolamine (5×10^{-6}). Phentolamine (Phent) increased the response to 5 and to 10 shocks/sec, but decreased the response to 50 and to 100 shocks/sec.

50 shocks/sec, but had little effect on stimulation at 10 shocks/sec (Fig. 9,*a* and *b*). Fig. 9,*c* and *d* records changes caused by Hydergine, 6×10^{-8}. The response to stimulation at 5 shocks/sec was greatly increased, that to 50 shocks/sec was slightly decreased, and that to 100 shocks/sec was much decreased.

Fig. 10 shows that phentolamine (5×10^{-6}) increased the response to 5 and to 10 shocks/sec, but decreased the response to 50 and 100 shocks/sec.

DISCUSSION

The substances which block the sympathetic postganglionic fibre at its termination include in the first place acetylcholine, nicotine, xylocholine (choline 2,6-xylyl ether), bretylium and guanethidine; of which the first two and the last two have been examined most completely. All four of them can release noradrenaline from sympathetic nerve endings, and all four can block the effect of postganglionic stimulation. For example, in the presence of atropine, all four increase the rate and force of beat in rabbit isolated atria, but have no such action in atria from rabbits treated with reserpine. Thus the difference between them is quantitative rather than qualitative. Acetylcholine and nicotine exert the blocking action only in high concentration; normally they exert the stimulant action. Bretylium and guanethidine show the stimulant action only when presented for the first time in high concentration; normally they exert the blocking action.

Burn & Froede (1963) have shown that there are several substances related to acetylcholine which block the sympathetic postganglionic fibre. Xylocholine is, of course, one. Others are 2-furfurylmethyltrimethylammonium, trimethylphenylammonium and edrophonium. These are quaternary ammonium compounds and, like bretylium and guanethidine, cause block at the neuromuscular junction.

Another substance causing block of the sympathetic postganglionic fibre is 1,1-dimethyl-4-phenylpiperazinium iodide, which is again a quaternary ammonium compound, but resembles nicotine in its action. There are two other substances which in structure have a similarity to nicotine and which are ganglion-blocking agents, mecamylamine and pempidine; these also block the sympathetic postganglionic fibre. They are respectively a secondary amine and a tertiary amine, and are not bisquaternary compounds like hexamethonium. The block produced by pempidine at the postganglionic termination resembles the block caused by hexamethonium at the ganglion. It is a block which is effective earlier for high rates of stimulation than for low.

Pempidine, therefore, behaves at the postganglionic termination like a substance which blocks acetylcholine. Including the nine substances discussed by Burn & Froede (1963) there are in all eleven substances which have been shown to block this termination and all have some blocking action at the neuromuscular junction. It would appear that any substance which has a blocking action at that junction is likely to block the postganglionic termination also.

The results support the evidence of Boyd *et al.* (1960) that both piperoxan and yohimbine have an anticholinesterase action. They also provide evidence that Hydergine has an anticholinesterase action. We have observed that they potentiate the action of acetylcholine on the toad rectus and on the guinea-pig ileum. We have also seen that they act like physostigmine and neostigmine on the isolated preparation of the hypogastric nerve and vas

deferens. In this preparation physostigmine and neostigmine behave as they do in the phrenic nerve-diaphragm preparation, increasing the response to stimulation of low frequency and decreasing the response to stimulation of high frequency. Thus it appears that piperoxan and yohimbine exert an anticholinesterase action in the hypogastric nerve-vas deferens preparation. However, in the hypogastric nerve ganglia are present and it is conceivable that these may be the site of the action.

No sympathetic ganglia are present in the Finkleman preparation, and in this preparation piperoxan, yohimbine, Hydergine and phentolamine have been shown to increase the effect of stimulating at low frequency and to decrease the effect of stimulating at a high frequency. The action of piperoxan and of yohimbine must then be exerted at the termination of the postganglionic fibres, and the action indicates that there is a cholinergic link in the transmission at that point. At low frequency of stimulation these substances increase the concentration of acetylcholine and therefore they increase the amount of noradrenaline liberated. At high frequency of stimulation, so great a concentration of acetylcholine accumulates in their presence that the transmission is partly blocked and less noradrenaline is released.

The observations on piperoxan are of interest in relation to the experiments of Willey (1962). He found that the intravenous injection of piperoxan caused an increase in the amount of noradrenaline in the blood of cats anaesthetized with chloralose, though not in spinal cats. He showed that this rise was due to an effect of piperoxan in the periphery. He perfused the spleen of a cat under chloralose with blood from a spinal cat. He showed that, when piperoxan was injected into the blood flowing through the spleen, there was a rise in the concentration of noradrenaline in the splenic vein from 8.6 to 24.4 ng/ml., these being mean figures from three experiments. Since the effect of piperoxan was not seen in spinal cats it clearly depended on the stream of impulses passing along the sympathetic postganglionic fibres. In the light of the present results the conclusion may be drawn that the injection of piperoxan, by exerting an anticholinesterase effect, increased the amount of noradrenaline released by this stream of impulses.

Willey (1962) also observed a rise in the amount of noradrenaline in the splenic vein after the injection of phenoxybenzamine. Burn & Gibbons (1964b) have shown that phenoxybenzamine also has an action in the hypogastric nerve-vas deferens preparation and in the Finkleman preparation of the rabbit ileum such that the response to stimulation is increased at low frequency and is decreased at high frequency. Hobbiger (cited in Burn & Gibbons, 1964b) has found that phenoxybenzamine has an anticholinesterase action on homogenates from guinea-pig brain and intestine. Thus the action of phenoxybenzamine in increasing the noradrenaline in the blood can be explained in the same way as the action of piperoxan.

Jang (1941), working on the preparation of the rabbit ear described by Gaddum & Kwiatkowski (1938), showed that piperoxan increased the constriction caused by postganglionic stimulation at a time when the constriction caused by adrenaline was diminished. He also observed in the spinal cat that yohimbine increased the effect of sympathetic stimulation on the nictitating membrane but greatly diminished the effect of adrenaline. It seems likely that in both cases piperoxan and yohimbine increased the amount of noradrenaline liberated by stimulation so much that, although the effect of a given amount of adrenaline was diminished, the effect of stimulation was increased. Recently, Blakeley, Brown & Geffen (1964) have repeated the view that the action of phenoxybenzamine in

increasing the amount of noradrenaline which appears in the splenic vein when the splenic nerves are stimulated at a frequency of 10 shocks/sec is due to the effect of phenoxybenzamine in blocking uptake of noradrenaline into the postganglionic axons. This view cannot account for the main part of the increase. Gillespie & Kirpekar (1963) have infused noradrenaline into the spleen and determined the proportion taken up. In the normal spleen about 70% is taken up. But in the presence of cocaine or in the presence of phenoxybenzamine less than 20% is taken up. They found that cocaine was as effective as phenoxybenzamine in blocking uptake. But Blakeley *et al.* (1963) found that cocaine did not increase the amount of noradrenaline liberated in the splenic vein when the splenic nerves were stimulated at 10 shocks/sec. Therefore it seems clear that the action of phenoxybenzamine cannot in the main be due to block of uptake. It is rather to be explained in terms of the cholinergic link and the anticholinesterase action of phenoxybenzamine.

Finally, attention should be drawn to the findings that all antiadrenaline compounds examined have been shown to have an anticholinesterase action. This is a completely new point in the pharmacology of these substances. The first evidence came from the observations of Loewi & Navratil (1926) on ergotamine, but it is thanks to Boyd *et al.* (1960) that the anticholinesterase action has been shown to be possessed not only by ergot alkaloids, but also by all the antiadrenaline compounds they examined. Some workers were not inclined to accept their findings but, since Hobbiger (cited in Burn & Gibbons, 1964b) has confirmed them for tolazoline and phenoxybenzamine, they need no longer be doubted. This anticholinesterase action presents an unusual feature. In the vas deferens all these substances behave like physostigmine and neostigmine, in causing an increase in the response to stimulation of low frequency and a decrease in the response to that of high frequency. This resemblance is very clear for phenoxybenzamine, piperoxan and yohimbine. With Hydergine, and especially tolazoline, depression of the response to high-frequency stimulation is less well marked, and with phentolamine the increase of the response to stimulation of low frequency is also less well marked. But, on the whole, the resemblance is there.

However, in experiments on the Finkleman preparation of the rabbit ileum the situation is different. Here again, all the antiadrenaline compounds without exception increase the response to stimulation of low frequency and decrease it to stimulation of high frequency. But this has not yet been demonstrated for physostigmine and neostigmine. These substances have so much action on the acetylcholine which is continually formed in the intestine, and perhaps on the acetylcholine liberated by sympathetic stimulation, that the behaviour of the ileum becomes very irregular. Thus a problem is presented. The problem is to see if the antiadrenaline substances have an anticholinesterase action which is particularly evident at sympathetic postganglionic terminations, and which can be exerted there more selectively than by physostigmine and neostigmine.

SUMMARY

1. Pempidine is a ganglion-blocking agent like mecamylamine. It is a tertiary amine and not a bisquaternary compound, and therefore penetrates cells more easily. It blocks the postganglionic sympathetic fibre. The block appears at the highest frequency of stimulation first, and at the lowest frequency of stimulation last. This is characteristic of the block of acetylcholine at the ganglion.

2. The antiadrenaline substances piperoxan, yohimbine and Hydergine increase the contraction caused by acetylcholine in the toad rectus and in the guinea-pig ileum. This supports the view that they have an anticholinesterase action.

3. In the hypogastric nerve-vas deferens preparation piperoxan, yohimbine, Hydergine and phentolamine act like physostigmine and neostigmine. They increase the response to stimulation of low frequency and diminish the response to that of high frequency.

4. In the Finkleman preparation of the rabbit ileum, piperoxan, yohimbine, Hydergine and phentolamine increase the inhibitory response to stimulation of low frequency and diminish the response to that of high frequency.

5. The anticholinesterase action of the antiadrenaline compounds may prove to be selective for the sympathetic nerve endings.

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