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SUBSTANCES BLOCKING SYMPATHETIC POST-GANGLIONIC FIBRES AND THE NEUROMUSCULAR JUNCTION

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In an earlier paper Burn & Froede (1963) described the action exerted on post-ganglionic sympathetic fibres by phenyltrimethylammonium. They showed that this substance, like bretylium, blocked the response to stimulation according to the frequency used, and that stimulation at high frequencies was blocked earlier than stimulation at low frequencies. This indicated that the action was not that of a local anaesthetic. They chose phenyltrimethylammonium because Riker (1953) had shown that it had a curare-like action at the neuromuscular junction, and it was therefore well suited to test the view that substances which blocked the action of acetylcholine at the neuromuscular junction would also block the release of noradrenaline by impulses passing along the sympathetic post-ganglionic fibres. This view was based on the hypothesis of Burn & Rand (1959) that the sympathetic impulse first released acetylcholine, and this in turn released noradrenaline.

Dixit, Gulati & Gokhale (1961) showed that both bretylium and guanethidine blocked the response of the rat diaphragm to stimulation of the phrenic nerve without affecting the response to direct stimulation of the diaphragm. They compared these substances with decamethonium and D-tubocurarine. The significance of their results has been criticized on the ground that the concentrations of bretylium and of guanethidine needed to block the phrenic nerve–diaphragm preparation were high, and that a feeble action was of no significance. We have therefore repeated the observations of Dixit *et al.* on bretylium and guanethidine, and we have also examined the action of mecamylamine on the phrenic nerve-diaphragm. Recently Burn & Gibbons (1964*a*) showed that mecamylamine exerted a blocking action in the sympathetic post-ganglionic fibre. We have also tested the action of D-tubocurarine, dihydro-β-erythroidine, tetraethylammonium and decamethonium on the sympathetic post-ganglionic fibre.

METHODS

We used the phrenic nerve–diaphragm preparation of the rat as described by Bülbring (1946). The bath contained 100 ml. of Tyrode's solution and was supplied with oxygen and 5 % CO_2 in fine bubbles through sintered glass. We also used the Finkleman (1930) preparation of the rabbit ileum and stimulated the periarterial nerves in the mesentery with supramaximal shocks at known frequencies. The bath contained 50 ml. Locke's solution at 32° C and was bubbled with oxygen and 5 % CO_2. The substances used were bretylium tosylate, guanethidine sulphate, mecamylamine hydrochloride, D-tubocurarine chloride, decamethonium iodide, dihydro-β-erythroidine hydrobromide and tetraethylammonium bromide. Doses are given in terms of these salts.

RESULTS

Phrenic nerve–diaphragm preparation. Figure 1 shows the effect of guanethidine in comparison with that of different concentrations of decamethonium. The phrenic nerve was stimulated at the rate of 1/sec, the bath temperature being 32° C. Guanethidine in a concentration of 3×10^{-4} caused a neuromuscular block more rapidly than concentrations of decamethonium $2{\cdot}2 \times 10^{-5}$ and $3{\cdot}1 \times 10^{-5}$. The equivalent concentration of decamethonium was approximately $3{\cdot}6 \times 10^{-5}$. Thus decamethonium was about 8·3 times more active than guanethidine. A similar result was obtained in two other preparations.

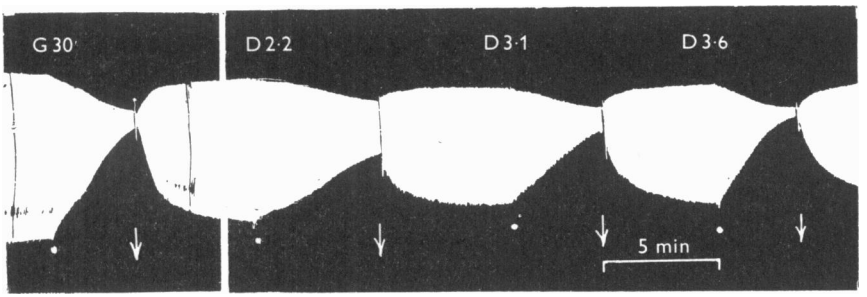

Fig. 1. Rat phrenic nerve–diaphragm preparation. Bath 32° C. Shocks 1/sec. At the dot below G 30 guanethidine was added so that the concentration was 3×10^{-4}. At the arrow the bath was changed. In the right-hand panel decamethonium was added, first to make concentration $2{\cdot}2 \times 10^{-5}$, then $3{\cdot}1 \times 10^{-5}$ and finally $3{\cdot}6 \times 10^{-5}$.

Figure 2 shows the effect of bretylium and of mecamylamine in comparison with decamethonium. Bretylium in a concentration $1{\cdot}5 \times 10^{-4}$ had an effect intermediate between that of decamethonium 2×10^{-5} and 3×10^{-5}, so that decamethonium was about 6 times more active than bretylium. Similarly decamethonium was about 2 times more active than mecamylamine.

Rabbit ileum. We tested four substances for power to block the sympathetic post-ganglionic fibres to the rabbit ileum. One of these was D-tubocurarine, the action of which is shown in Fig. 3. In this experiment a concentration of 5×10^{-5} greatly reduced the inhibitory response to stimulation at the end of 90 min. The results in four experiments are given in Table 1, and these show that the concentration of 5×10^{-5} had little effect in the first hour, but caused a fair degree of block in 1·5–2 hr.

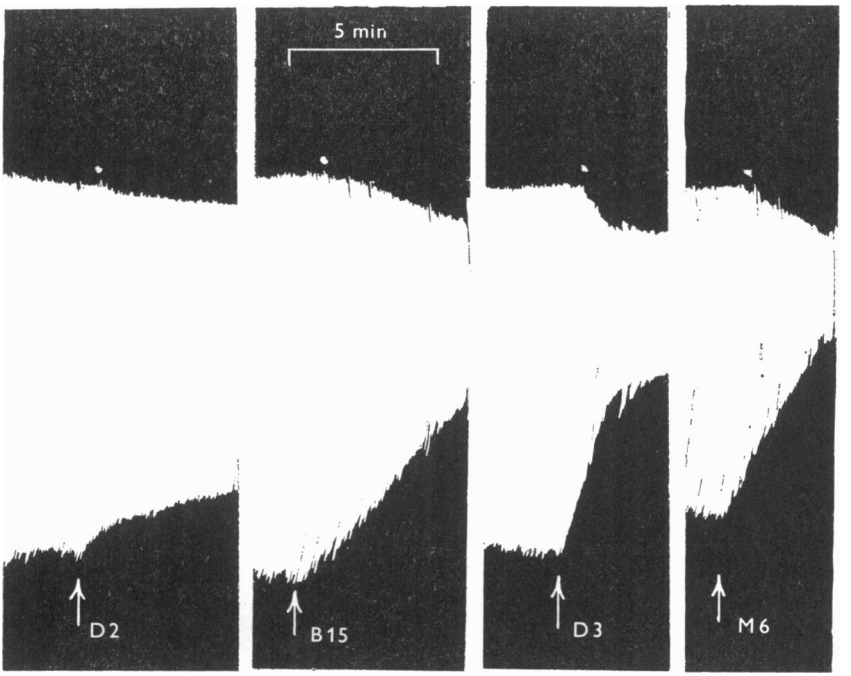

Fig. 2. Rat phrenic nerve–diaphragm preparation. Bath 32° C. Shocks 1/sec. At the arrow D2, decamethonium was added to make concentration 2×10^{-5}, then bretylium to make $1{\cdot}5 \times 10^{-4}$, then decamethonium to make 3×10^{-5} and then mecamylamine to make 6×10^{-5}.

The substance dihydro-β-erythroidine was also tested and a result is shown in Fig. 4 in which 7×10^{-5} caused much block after 2 hr. Other experiments with this substance are shown in Table 1. The concentration of 4×10^{-5} produced slight block after 3 hr, but in another experiment considerable block after 4 hr. The variability in the response to this substance was evident since a concentration of $1{\cdot}4 \times 10^{-4}$ in one experiment had only a small effect in 2 hr.

In two experiments with decamethonium there was a 50 % block when a concentration of 10^{-4} was used and allowed to act for 4 hr.

Finally, observations were made with tetraethylammonium, which has no neuromuscular blocking action as Kensler (1950) observed, but was shown by Burn & Dale (1915) to block sympathetic ganglia. In the concentration 10^{-4}, this substance caused a high degree of block in one experiment in 1 hr, and in a second experiment in 1·5 hr.

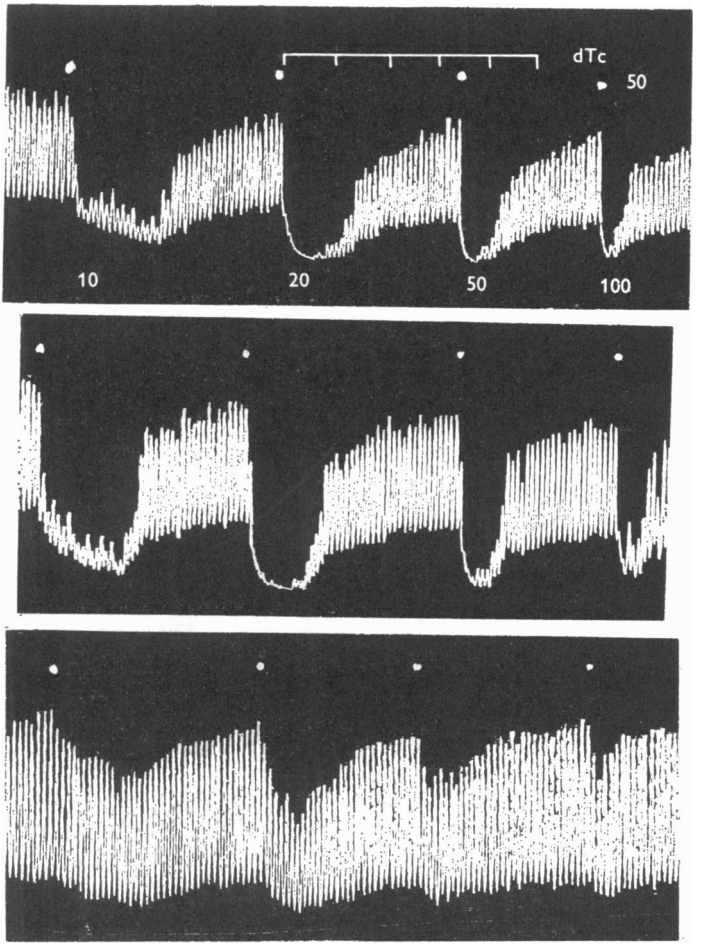

Fig. 3. Rabbit ileum; stimulation of periarterial nerves with 1000 supramaximal shocks at frequencies 10/sec, 20/sec, 50/sec and 100/sec. Upper panel, control observations. Middle panel 2 min after addition of D-tubocurarine 5×10^{-5}. Lower panel 90 min after addition of D-tubocurarine. In middle and lower panels, the stimulations were in the same order as in the top panel.

To show that the block produced by these four substances was not due to failure of stimulation at the electrodes, the Ca^{2+} concentration of the Locke's solution was increased to 4·4 mM at the end of each experiment.

It was observed that the inhibitory response to stimulation was then much greater (Burn & Gibbons, 1964*b*).

DISCUSSION

The results obtained for the action of bretylium and of guanethidine in the phrenic nerve–diaphragm preparation in the present experiments are

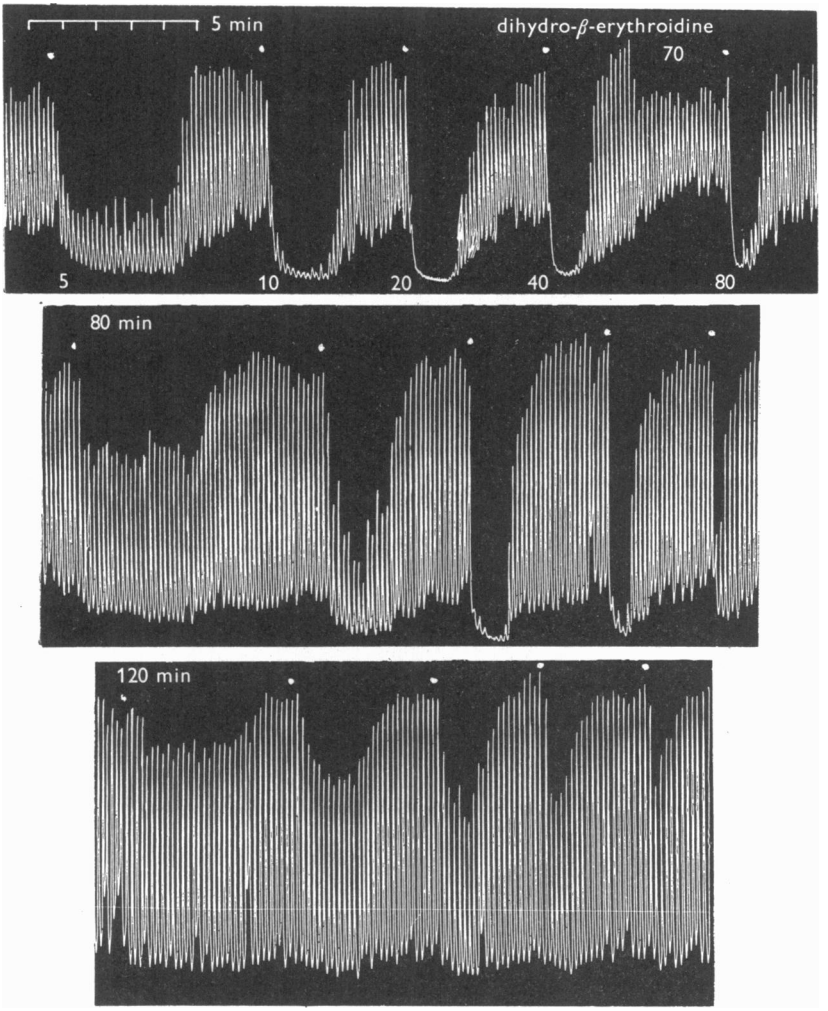

Fig. 4. Rabbit ileum. Stimulation of periarterial nerves with 1000 supramaximal shocks at frequencies 5/sec, 10/sec, 20/sec, 40/sec and 80/sec. Upper panel shows the responses 3 min after addition of dihydro-β-erythroidine to the bath to make the concentration 7×10^{-5}. The middle panel shows the responses 80 min after the addition, and the lower panel shows the responses 120 min after the addition. The stimulations were always made in the order shown in the upper panel.

similar to those obtained by Dixit *et al.* (1961) as shown in Table 2. The results therefore indicate that the action of bretylium and of guanethidine in the phrenic nerve-diaphragm is not very weak as has been suggested. Taking decamethonium as 1, the equivalent amount of bretylium is 6, and of guanethidine is 8·3. The equivalent amount of mecamylamine is 2.

TABLE 1. Action of different substances in blocking the inhibition produced by sympathetic stimulation of the pendular movements of the ileum

Expt.	Substance	Concentration	Time (min)	Degree of block
1	D-tubocurarine	5×10^{-5}	19	Slight
2			46	Slight
3			94	90 %
4			143	80 %
5	Dihydro-β-erythroidine	4×10^{-5}	178	Slight
6		4×10^{-5}	236	90 %
7		7×10^{-5}	115	90 %
8		$1{\cdot}4 \times 10^{-4}$	120	Slight
9	Decamethonium	10^{-4}	238	50 %
10			240	50 %
11	Tetraethylammonium	5×10^{-5}	60	Trace
12			74	Trace
13			120	50 %
14		10^{-4}	57	80 %
15			94	80 %

TABLE 2. Equivalent concentrations producing block in the phrenic nerve diaphragm

	Dixit *et al.*	Present observations
Decamethonium	$3{\cdot}3 \times 10^{-5}$	$3{\cdot}0 \times 10^{-5}$
Bretylium	$2{\cdot}3 \times 10^{-4}$	$1{\cdot}8 \times 10^{-4}$
Guanethidine	$3{\cdot}3 \times 10^{-4}$	$2{\cdot}5 \times 10^{-4}$

Of the four substances, decamethonium is a substance commonly regarded as blocking the neuromuscular junction while mecamylamine is a substance blocking sympathetic ganglia. The observations therefore support the view that the action of bretylium and of guanethidine at the termination of the sympathetic post-ganglionic fibre may be to block the action of acetylcholine there also. This view is strengthened by the evidence given in Table 1, which shows that the three substances, D-tubocurarine, dihydro-β-erythroidine and decamethonium have some action at the termination of the sympathetic post-ganglionic fibre, an action which is most marked for D-tubocurarine. These substances block the release of noradrenaline. It has previously been shown that the ganglion-blocking agents mecamylamine and pempidine (Burn & Gibbons, 1964*a*) block the sympathetic post-ganglionic fibre in concentrations from 7×10^{-6} to 3×10^{-5}. To these may now be added tetraethylammonium which will block the sympathetic post-ganglionic fibre in a concentration of 10^{-4}.

If the dose of bretylium or of guanethidine which blocks the release of noradrenaline is taken as 1, then the equivalent dose of mecamylamine, pempidine and of D-tubocurarine is about 10, though the last compound especially is slow in its action. Thus the main difference between bretylium and guanethidine on the one hand, and D-tubocurarine and decamethonium on the other is not so much the concentration at which they act, as the speed and certainty of the action. The action of dihydro-β-erythroidine, for example, varies greatly in different preparations.

Burn & Froede (1963) thought that bisquaternary compounds did not block the release of noradrenaline. In the light of the results now described this view must be qualified, since D-tubocurarine and decamethonium are bisquaternary compounds, but as already said they act slowly.

Finally, it may be pointed out that Brücke (1935) showed that, whereas the intracutaneous injection of a small dose of acetylcholine caused a pilomotor response in the cat's tail, a large dose blocked the response to sympathetic stimulation; this observation supports the idea that there is a cholinergic link in the release of noradrenaline, and adds to the likelihood that bretylium and guanethidine are substances which block this link.

SUMMARY

1. Bretylium and guanethidine block the release of noradrenaline from the sympathetic post-ganglionic fibre. They also block the neuromuscular junction in the rat phrenic nerve–diaphragm preparation. The concentration in which they do this is of the same order as the concentration in which decamethonium has this effect. Decamethonium is about 6 times more potent than bretylium and 8 times more potent than guanethidine. Mecamylamine which blocks sympathetic ganglia has about half the potency of decamethonium in blocking the neuromuscular junction.

2. Substances which block the release of noradrenaline from the sympathetic post-ganglionic fibre include D-tubocurarine, dihydro-β-erythroidine, tetraethylammonium, mecamylamine, pempidine and decamethonium. Bretylium and guanethidine have about 10 times the activity of mecamylamine, pempidine and D-tubocurarine and act more quickly.

3. The evidence suggests that substances which block the sympathetic post-ganglionic fibre are substances which block the action of acetylcholine just as are substances which block the neuromuscular junction and the sympathetic ganglion.

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