Br. J. Pharmac. Chemother. (1966), **27**, 164–176.

EFFECTS OF ADRENERGIC NEURONE BLOCKING AGENTS ON VOLUNTARY MUSCLE STIMULATED AT DIFFERENT FREQUENCIES

BY

A. F. GREEN AND R. HUGHES

From the Wellcome Research Laboratories, Langley Court, Beckenham, Kent

(*Received February* 28, 1966)

Neuromuscular paralysis of voluntary muscle is caused by high doses of bretylium (Boura & Green, 1959 ; Dixit, Gulati & Gokhale, 1961), guanethidine (Dixit *et al.*, 1961 ; Kroneberg & Stoepel, 1962) and bethanidine (Boura & Green, 1963). In certain studies of the action of these compounds on the activation of smooth muscle caused by different frequencies of adrenergic nerve stimulation, bretylium showed a relatively greater blocking action when the frequency was high (Boura & Green, 1959 ; Green & Robson, 1964), guanethidine caused an almost parallel shift of nerve frequency-response curves (Boura & Green, 1962 ; Green & Robson, 1964) and the effect of bethanidine on the slope of nerve frequency-response curves was usually intermediate between that of the two other compounds (Boura & Green, 1963 ; Green & Robson, 1964). It was therefore of interest to compare the action of these compounds on neuromuscular transmission in voluntary muscle at different rates of nerve stimulation. In these studies we have also examined triethylcholine and (+)-tubocurarine, since the susceptibility to paralysis of voluntary neuromuscular junctions by triethylcholine increased to a greater extent with elevation of the frequency of nerve stimulation than did susceptibility to paralysis by (+)-tubocurarine (Bowman & Rand, 1961 ; Bowman, Hemsworth & Rand, 1962).

A further aspect of this study has been the examination of the action of the adrenergic neurone blocking agents on the responses of voluntary muscle to direct electrical stimulation. Vernikos-Danellis & Zaimis (1960) reported that bretylium and guanethidine, at doses below those causing neuromuscular blockade, reduced the maximum twitch height of the tibialis anterior muscles of anaesthetized cats, and Kroneberg & Stoepel (1962) found that high local concentrations of guanethidine depressed muscle responses to direct stimulation in curarized cats and in isolated rat diaphragm preparations.

METHODS

Phrenic nerve-diaphragm. Preparations from 130 to 150 g male Wistar rats were set up as described by Bülbring (1946), in Krebs solution containing 0.2% dextrose and bubbled with 95% O_2 and 5% CO_2 (Krebs and Henseleit, 1932). Duplicate preparations were run in parallel using two organ baths, each containing 100 ml. Krebs solution at 35° C. The electrode system resembled that described by Garry & Wishart (1951). The phrenic nerve was threaded through a thin rubber

diaphragm into a small glass tube filled with Krebs solution and containing one electrode, while the other electrode was placed in the organ bath. For direct stimulation the wire attached to the writing lever was one electrode, the other being embedded in the Perspex holder except at the point of attachment of the diaphragm. Stimulation was at supramaximal voltage using square wave shocks of 0.2 msec duration for the nerve and 2 msec for the muscle. In some experiments direct muscle stimulation was applied after adding sufficient (+)-tubocurarine, usually 1.0 μg/ml., to the bath to abolish responses to nerve stimulation. The contractions were recorded on smoked paper.

Anaesthetized animals. Rabbits were anaesthetized with urethane (approximately 1.8 g/kg i.v.) and cats with chloralose (80 mg/kg i.v.). The sciatic nerve of both legs were exposed between the hamstring muscles, crushed centrally and placed on shielded bipolar platinum electrodes. The hind limbs were fixed by means of steel drills through the knee joints and securely clamped at the ankle joints to a rigid frame. The tendons of both gastrocnemius muscles were cut at their insertions and attached to Grass FT 10 or FT .03 semi-isometric strain gauges connected to Grass 5P1 carrier preamplifiers. The muscle load at rest was 200 g and the maximum tension developed was 500 to 1,000 g. Both sciatic nerves were stimulated with rectangular pulses of supramaximal voltage and 0.05 to 0.1 msec duration, one through an R.F. isolation unit, the other through a 1:1 isolation transformer, so that there was no common connection between the electrodes. For direct stimulation the active electrodes were small bulldog clips impregnated with electrode jelly fixed to each tendon; the indifferent electrodes were the drills fixed through the knee joints. Direct stimulation was effected by rectangular pulses of supramaximal voltage and 1 to 5 msec duration after intravenous injection of sufficient (+)-tubocurarine to abolish indirect responses. The carotid blood pressure was recorded with a Statham P23Gc pressure transducer and a Grass 5P1 preamplifier. Respiration was registered by a thermistor fixed inside a polythene canula inserted into the trachea. The thermistor was part of an A.C. bridge, the output from which was fed into a Grass 5P5 preamplifier. Recordings were made on a Grass multichannel Polygraph.

Drugs. Solutions of all the drugs used were made up in 0.9% w/v NaCl solution and infused or injected into the ear veins of the anaesthetized rabbits or the jugular veins of the anaesthetized cats. The drugs used were bretylium tosylate, guanethidine sulphate, bethanidine sulphate, tubocurarine chloride, procaine hydrochloride, triethylcholine iodide and choline chloride. The doses stated were in terms of these salts.

RESULTS

Rat Isolated Diaphragm

Indirect stimulation. Figs. 1,*a* and *b* are records of experiments in which bretylium and triethylcholine increased the twitches of rat diaphragms caused by stimulation of the phrenic nerve with 1 shock per 10 sec at supramaximal voltage while paralysing duplicate preparations stimulated at 1 shock per sec. When preparations stimulated at the higher frequency were no longer responding, change to the slower stimulation rate allowed some recovery. Similarly, preparations showing strong responses to low rates of indirect stimulation, despite the presence of bretylium or triethylcholine, rapidly failed when the high rate of nerve stimulation was applied.

Records of similar experiments using guanethidine and (+)-tubocurarine are shown in Fig. 2. Their blocking action was less dependent on the frequency of stimulation but nevertheless was slightly more rapid with the higher frequency. In Fig. 2 the preparation under the influence of (+)-tubocurarine that had failed to respond to the high rate of stimulation showed temporary recovery when the low rate was substituted but such recovery was not common to all experiments of this type. Recovery on changing from high to low frequency was at most slight in experiments using guanethidine.

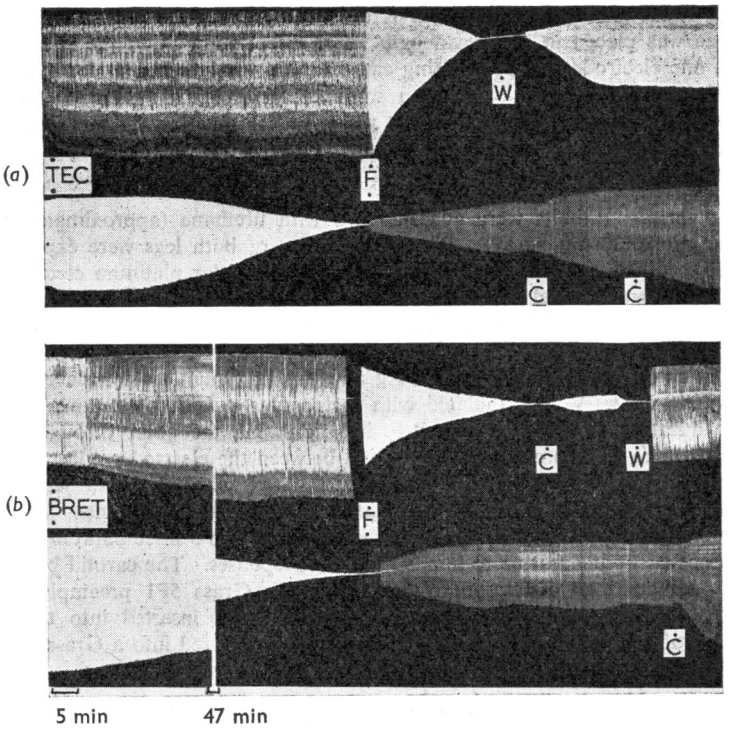

Fig. 1. Twitch responses of isolated rat diaphragms, stimulated indirectly. (*a*) Upper trace: 750 μg/ml. triethylcholine at TEC augmented responses to stimulation at 1 shock/10 sec; when at F the stimulation rate was increased to 1 shock/sec, paralysis rapidly ensued. Partial recovery occurred after washing at W. Lower trace: a duplicate preparation stimulated at 1 shock/sec showed first enhancement of twitch and later paralysis. Partial recovery followed change of stimulation frequency to 1 shock/10 sec at F; full recovery followed 2 additions of 20 μg/ml. choline at C. (*b*) Upper trace: 100 μg/ml. bretylium at BRET enhanced responses while the stimulation rate was 1 shock/10 sec; after increasing the stimulation rate to 1 shock/sec at F paralysis ensued. Recovery was slight on addition of 100 μg/ml. choline (C) but complete after washing (W). Lower trace: in a duplicate preparation the same concentration of bretylium suppressed responses to stimulation at 1 shock/sec. Partial recovery followed reduction of the stimulation rate to 1 shock/10 sec at F and greater recovery was caused by 50 μg/ml. choline at C. At the gap in the record, 47 min. Time, 5 min.

The results of many similar experiments are summarized in Table 1. Here are included only those experiments in which the compounds were added to the bath in single amounts, but these are supported by an almost equal number of tests in which the compounds were added in progressively increasing concentrations. Concentrations of 100 to 150 μg/ml. bretylium or 500 to 1,200 μg/ml. triethylcholine caused 90% paralysis of responses to 1 shock/sec but consistently, and to similar extents, enhanced the responses to 1 shock/10 sec. The effect of 250 μg/ml. bretylium was more rapid. At a time when 90% paralysis of responses to the high rate of nerve stimulation had occurred, responses to the low rate had declined to about half their initial amplitudes. In contrast, the rapidity of the paralysing action of triethylcholine and its frequency

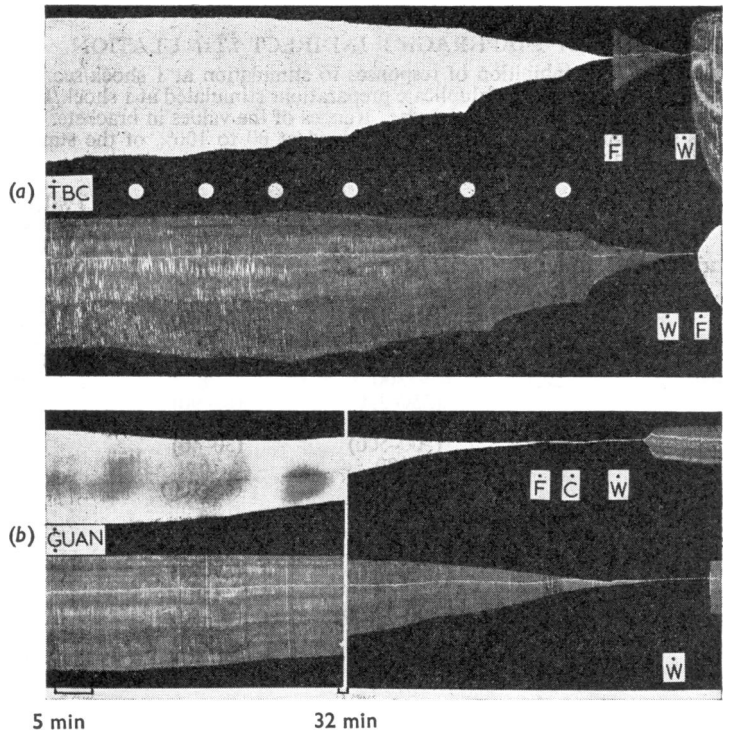

Fig. 2. Twitch responses of isolated rat diaphragms stimulated indirectly at 1 shock/10 sec or 1 shock/sec. (*a*) Tubocurarine (additions of 0.1 μg/ml. at TBC and the white circles) had a slightly greater inhibitory action in the preparation stimulated at 1 shock/sec (upper trace) than on the duplicate preparation exposed to 1 shock/10 sec (lower trace). F, change to the other frequency ; W, wash. (*b*) Guanethidine, 250 μg/ml., paralysed the preparation stimulated at 1 shock/sec (upper trace) a little earlier than it paralysed that exposed to 1 shock/10 sec. In the upper trace, reducing the frequency to 1 shock/10 sec at F allowed only slight return of contractility and 100 μg/ml. choline at C was not antagonistic. Both preparations showed poor recovery after washing at W.

dependence were similar over a two-fold range of bath concentrations. The frequency dependence of blockade by guanethidine, bethanidine, (+)-tubocurarine or procaine was relatively low and similar for each drug.

Under the conditions used in Figs. 1 and 2, choline was antagonistic to paralysis by bretylium and triethylcholine but not to that by guanethidine, bethanidine, (+)-tubocurarine or procaine.

Direct stimulation. In most of the above experiments the diaphragms were stimulated directly when responses to indirect stimulation had been suppressed. The responses were found to be strong except in tests using guanethidine or procaine ; these compounds greatly reduced the muscle response.

Effects on directly stimulated muscle were also tested in preparations which had ceased to respond to nerve stimulation after addition of appropriate amounts of (+)-tubocurarine, usually 1 μg/ml. ; the compounds were tested at the concentrations

TABLE 1

ISOLATED RAT DIAPHRAGM: INDIRECT STIMULATION

Mean concentrations causing 90% inhibition of responses to stimulation at 1 shock/sec, the mean times taken and the simultaneous twitch heights of duplicate preparations stimulated at 1 shock/10 sec, expressed as percentages of those before adding the compounds. Ranges of the values in brackets. Twitch heights in control preparations stimulated with 1 shock/sec remained at 80 to 100% of the starting levels over periods of 90 to 120 min; with 1 shock/10 sec twitch heights were fully maintained

Compound	Number of tests	90% paralysis at 1 shock/sec: Concentration μg/ml.	90% paralysis at 1 shock/sec: Time min	Twitch height at 1 shock/10 sec (initial=100)
Bretylium	4	125 (100–150)	72 (45–100)	127 (120–140)
	2	250 (250–250)	45 (40–50)	100 (100–100)
Guanethidine	2	300 (250–350)	78 (65–90)	15 (10–20)
	4	500 (500–500)	35 (30–40)	20 (0–60)
Bethanidine	4	200 (200–200)	76 (45–100)	40 (20–80)
(+)-Tubocurarine	4	1·1 (1·0–1·5)	22 (12–30)	41 (30–60)
Triethylcholine	4	612 (500–750)	56 (47–70)	133 (127–145)
	2	1,200 (1,200–1,200)	50 (50–50)	130 (130–130)
Procaine	4	150 (150–150)	35 (31–40)	48 (40–50)

TABLE 2

ISOLATED RAT DIAPHRAGM: DIRECT STIMULATION

Effect of concentrations that suppress twitch responses caused by indirect stimulation at 1 shock/sec (see Table 1) on responses to direct muscle stimulation at 1 shock/sec and 1 shock/10 sec after neuromuscular paralysis with 1 μg/ml. (+)-tubocurarine. The twitch heights at the termination of the tests, expressed as percentages of those at the beginning are shown for each compound. Also shown are the values at the times when procaine and guanethidine had caused 95% neuromuscular paralysis and when triethylcholine produced maximal potentiation. Ranges of values in brackets

Compound	Number of tests	Concentration μg/ml.	Time min	Twitch height (initial=100): 1 shock/sec	Twitch height (initial=100): 1 shock/10 sec
Control	4	—	123 (120–130)	67 (50–80)	103 (100–107)
Bretylium	4	125*	124 (110–160)	72 (63–90)	115 (100–133)
Guanethidine	4	500	35 (35–35)	35 (30–40)	33 (25–40)
			79 (66–93)	0	0
Bethanidine	2	200	120 (120–120)	75 (50–100)	100 (80–120)
Triethylcholine	3	750	10 (3–15)	130 (120–140)	118 (110–125)
			120 (115–125)	107 (90–130)	100 (90–110)
Procaine	4	150	35 (35–35)	37 (20–75)	52 (36–65)
			87 (40–120)	9 (0–25)	39 (30–50)

* The concentration was built up additively.

which, in the absence of (+)-tubocurarine, had suppressed the responses to indirect stimulation with 1 shock/sec (Table 2). These concentrations of bretylium or bethanidine did not impair twitch responses caused by direct muscle stimulation at 1 shock/sec; in their presence contractions were as well maintained as in controls. Triethylcholine increased the responses and caused a small transient recovery of responses to indirect stimulation. Comparison of the results in Tables 1 and 2 indicates that, within the times that guanethidine or procaine took to suppress responses to indirect stimulation by 90%, marked impairment of responses to direct stimulation had developed. The effect of both compounds on the directly stimulated muscle increased with the period of exposure; guanethidine caused full suppression within 90 min.

In control preparations responses to direct stimulation with 1 shock/10 sec were better maintained than those where the rate was 1 shock/sec. They were augmented for a short while after addition of triethylcholine and for a longer period during exposure to bretylium, but enhancement appeared to be less than in the experiments using indirect stimulation (*cf.* Table 1). The contractions were unaffected by bethanidine and reduced by guanethidine and procaine. The enhancement caused by triethylcholine could be attributed to an anti-curare action since its addition to the bath was accompanied by a considerable return of responses to indirect stimulation particularly at the lower rate. No anti-curare effect was shown by bretylium.

Rabbit Gastrocnemius Muscle

Indirect stimulation. An experiment in which a slow infusion of bretylium into an anaesthetized rabbit paralysed the twitch response of one gastrocnemius stimulated at

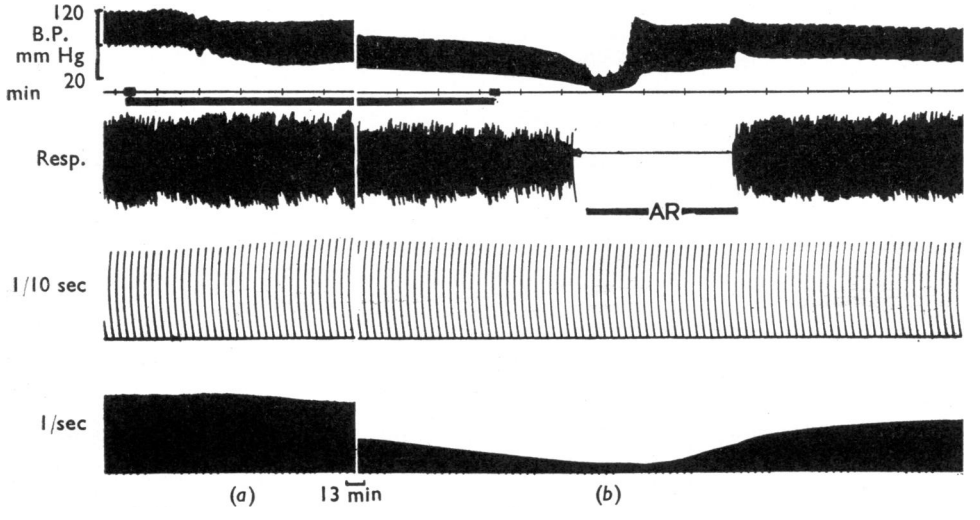

Fig. 3. Rabbit 2.5 kg; urethane anaesthesia. Records from top downwards—carotid blood pressure, time in min, respiration, twitch response of one gastrocnemius muscle stimulated indirectly with 1 shock/10 sec and responses of the other gastrocnemius muscle stimulated with 1 shock/sec. Infusion of 50 mg/kg bretylium over the time indicated by the horizontal bar, lowered blood pressure, arrested breathing, slightly enhanced the muscle twitch responses to 1 shock/10 sec and depressed the muscle responses to 1 shock/sec. AR, artificial respiration. Between *a* and *b*, 13 min.

the rate of 1/sec while slightly increasing the response of the contralateral muscle to indirect stimulation at the rate of 1 shock/10 sec is shown in Fig. 3. Similar effects were observed with triethylcholine but amounts of guanethidine, bethanidine or (+)-tubocurarine causing 90% inhibition of responses to the high rate of nerve stimulation invariably caused some depression of responses to the low rate (Table 3). Such amounts also produced apnoea and usually a substantial fall of blood pressure.

TABLE 3

EFFECTS ON CONTRACTILE RESPONSES OF INDIRECTLY STIMULATED GASTROCNEMIUS MUSCLE IN ANAESTHETIZED RABBITS

Mean doses by slow intravenous infusion causing 90% inhibition of twitch heights with stimulation at 1 shock/sec, the mean times taken, and the simultaneous twitch heights of the contralateral muscles stimulated at 1 shock/10 sec, expressed as percentages of those before giving the compounds. Ranges of values in brackets

Compound	Number of rabbits	90% paralysis at 1 shock/sec		Twitch height at 1 shock/10 sec (initial=100)
		mg/kg	Time min	
Bretylium	5	41 (20–50)	18 (10–24)	111 (103–120)
Guanethidine	4	120 (80–180)	17 (12–22)	80 (50–100)
Bethanidine	5	44 (26–68)	33 (23–45)	83 (60–95)
(+)-Tubocurarine	5	0·29 (0·12–0·50)	8 (5–12)	89 (80–100)
Triethylcholine	4	232 (180–300)	30 (19–40)	120 (105–140)

The same compounds were also tested by rapid intravenous injection in groups of four rabbits. At doses sufficient to cause 90% suppression of responses to the high stimulation rate, responses to the low rate were again increased by triethylcholine (100 to 350 mg/kg caused a mean increase of 30%), unaffected by bretylium (18 to 20 mg/kg) and decreased by guanethidine (70 to 100 mg/kg, 28% inhibition), bethanidine (6.5 to 17 mg/kg, 19% inhibition) and (+)-tubocurarine (0.07 to 0.15 mg/kg, 16% inhibition). Depression of breathing requiring forced ventilation was usual except in the rabbits given bretylium or bethanidine. Their depressant action was often small and usually brief.

In the experiments in which the blocking agents were infused, 5 to 10 mg/kg i.v. of choline sometimes slightly increased muscle responses that had been depressed by triethylcholine or (+)-tubocurarine but not those depressed by bretylium or guanethidine.

Direct stimulation. In a few experiments intravenous doses of 20 to 50 mg/kg bretylium, 100 mg/kg guanethidine and 10 to 50 mg/kg bethanidine, amounts sufficient to fully suppress the response of the gastrocnemius muscle to indirect stimulation with 1 shock/sec, were administered to rabbits in which complete neuromuscular paralysis had been produced with tubocurarine (0.25 to 0.5 mg/kg i.v. and further amounts as needed). Both gastrocnemius muscles were stimulated directly, one at 1 shock/sec, the other at 1 shock/10 sec. The contractions of the muscles stimulated at 1 shock/sec tended to decline slowly during experiments lasting an hour or more, but this decline was more marked after guanethidine and perhaps slightly enhanced by bretylium or bethanidine. Responses to the stimulation rate of 1 shock/10 sec were better sustained; they tended

to decline slightly after injection of guanethidine but were not appreciably changed by bretylium or bethanidine. It is uncertain as to whether the small diminutions in muscle contractions observed were due to a direct action on muscle fibres, since all these treatments, especially bethanidine, lowered blood pressure by 30 to 50 mm Hg.

Cat Gastrocnemius Muscle

Indirect stimulation. Bretylium and bethanidine had less effect than guanethidine on responses of the gastrocnemius muscle of cats to stimulation of the sciatic nerve at a low rate when injected rapidly in amounts that had similar paralysing action of responses to stimulation at a high rate (Fig. 4). The paralysing action of the guanethidine also differed in being much more persistent. The collected results (Table 4) showed that, at doses rapidly causing 90% paralysis of responses to indirect stimulation at 1 shock/sec, bretylium and bethanidine slightly increased or slightly reduced responses to stimulation at 1 shock/10 sec, whereas guanethidine and (+)-tubocurarine consistently decreased the responses at the low stimulation rate. Triethylcholine slowly produced 90% paralysis of responses to the high rate of stimulation while consistently enhancing responses to the low rate. The onset of paralysis was slow even when the dosage was increased

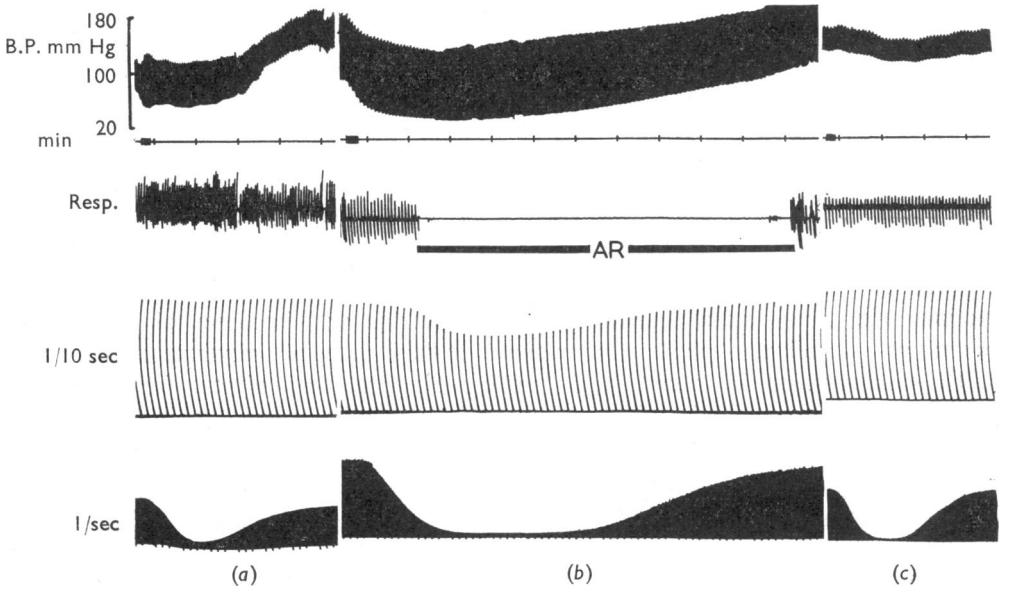

Fig. 4. Records from 3 cats (*a*, *b* and *c*); chloralose anaesthesia. From the top down—carotid blood pressure; time in min and indication of dosing (blocks); respiration; twitches of gastrocnemius muscle stimulated indirectly at 1 shock/10 sec; twitches of gastrocnemius muscle stimulated indirectly at 1 shock/sec. (*a*) Cat, 3.2 kg male; 12 mg/kg bretylium greatly depressed the responses of the muscle stimulated at the high rate but scarcely affected those of the muscle stimulated at the low rate; blood pressure rose slowly. (*b*) Cat, 3.5 kg male; 28 mg/kg guanethidine i.v. lowered the blood pressure, increased the pulse pressure, arrested breathing (AR, artificial respiration) and reduced the gastrocnemius muscle responses to both rates of stimulation. (*c*) Cat, 2.4 kg male; 4 mg/kg bethanidine i.v. lowered blood pressure and paralysed only the response of the muscle stimulated at the high rate.

TABLE 4

EFFECTS ON CONTRACTILE RESPONSES OF INDIRECTLY STIMULATED GASTROCNEMIUS MUSCLE IN ANAESTHETIZED CATS

Mean intravenous doses causing 90% inhibition of twitch heights with stimulation at 1 shock/sec, the mean times taken, and the simultaneous mean twitch heights of the contralateral muscle stimulated at 1 shock/10 sec, expressed as percentages of those before giving the compounds. Ranges of values in brackets

Compound	Number of cats	90% paralysis at 1 shock/sec		Twitch height at 1 shock/10 sec (initial=100)
		mg/kg	Time min	
Bretylium	5	12 (9–21)	2·0 (0·5–3·0)	98 (95–103)
Guanethidine	4	27 (22–30)	2·4 (2·0–3·0)	80 (70–87)
Bethanidine	4	7·3 (4·0–9·0)	2·5 (1·5–3·0)	100 (90–105)
(+)-Tubocurarine	5	0·084 (0·05–0·10)	1·7 (1·0–3·0)	86 (70–100)
Triethylcholine	4	110 (40–150)	35 (14–59)	111 (105–125)

to 300 mg/kg. Breathing was not appreciably changed or only slightly depressed by these doses of bretylium, bethanidine or (+)-tubocurarine, whereas the guanethidine and triethylcholine frequently caused apnoea.

When the compounds were administered by slow intravenous infusion over periods of 4 to 25 min in groups of 4 cats the following were the mean doses causing 90% inhibition of responses to indirect stimulation at 1 shock/sec: 22 mg/kg of bretylium, 30 mg/kg of guanethidine, 16 mg/kg of bethanidine and 0.22 mg/kg of (+)-tubocurarine. Similar paralysis was produced by 325 mg/kg of triethylcholine delivered in 30 to 40 min. Responses of the contralateral muscle to indirect stimulation with 1 shock/10 sec were unaffected by the above amounts of bretylium or bethanidine but showed 5 to 25% reductions after the guanethidine and (+)-tubocurarine and increases of 10 to 40% after the triethylcholine. Apnoea was produced by these doses of bretylium, guanethidine and (+)-tubocurarine in about half the experiments, and almost invariably by triethylcholine. In contrast, breathing was not affected by the bethanidine in the majority of tests and was never more than moderately depressed.

Blood pressure changes caused by the adrenergic neurone blocking agents were complex because of the coexistence of adrenergic neurone blockade, sympathomimetic effects and vasodilator actions at high dose levels (Boura & Green, 1965). At the time of 90% paralysis of contractions of the gastrocnemius muscle to stimulation at 1 shock/sec the blood pressure was lowered in most of the bethanidine and guanethidine experiments and some of the bretylium tests, but once when guanethidine was infused and in several tests in which bretylium was injected rapidly the blood pressure was elevated. Blood pressure was lowered substantially by (+)-tubocurarine and slightly by triethylcholine.

Intravenous doses of 5 to 10 mg/kg choline reduced paralysis caused by triethylcholine in each of six experiments, in two the twitch height being almost fully restored. Choline was weakly antagonistic to bretylium (4 expts) and (+)-tubocurarine (4 expts) but not to guanethidine (3 expts).

Direct stimulation. The effects of the adrenergic neurone blocking agents on contractions of muscles stimulated directly with shocks at the rate of 1/sec or 1/10 sec were studied in artificially respired anaesthetized cats given sufficient (+)-tubocurarine to suppress responses to nerve stimulation. Blood pressure was low largely because of tubocurarine's ganglion blocking action. Table 5 shows that the decline of the direct

TABLE 5

EFFECTS ON CONTRACTILE RESPONSES OF DIRECTLY STIMULATED GASTROCNEMIUS MUSCLE IN CURARIZED ANAESTHETIZED CATS

The mean twitch height of the muscles stimulated at 1 shock/sec or 1 shock/10 sec, at the times stated, expressed as percentages of those before giving the compounds. Means and ranges of values. * and ** difference from control value significant at the 5% and 1% levels respectively

Compound	Number of cats	mg/kg i.v.	Time min	Twitch height (initial=100)	
				1 shock/10 sec	1 shock/sec
Controls	10	—	50 (40–80)	97 (90–100)	95 (85–100)
Bretylium	5	10	36 (20–48)	100 (100–100)	97 (85–100)
	4	20	50 (30–72)	96 (85–100)	90 (70–100)
	4	50	56 (35–90)	88 (75–100)	70** (50–90)
Guanethidine	4	10	61 (40–102)	100 (100–100)	93 (85–100)
	4	20	64 (40–96)	100 (100–100)	89 (80–100)
	4	30	49 (40–71)	84* (75–90)	72** (65–80)
Bethanidine	5	10	26 (20–33)	96 (90–100)	78* (60–100)

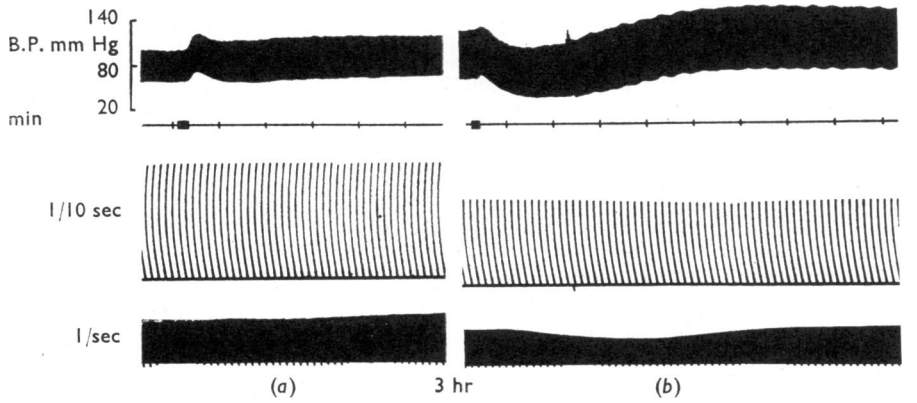

Fig. 5. Cat, 3.5 kg male, chloralose anaesthesia ; gastrocnemius muscle responses to indirect stimulation were abolished with tubocurarine (first dose 0.75 mg/kg i.v.). Records from top downwards —carotid blood pressure, time in min and indication of doses (blocks), twitches of gastrocnemius muscle stimulated directly at 1 shock/10 sec, twitches of gastrocnemius muscle stimulated directly at 1 shock/sec. (*a*) Intravenous injection of 5 mg/kg guanethidine increased the blood pressure and the twitch responses to stimulation at 1 shock/sec. (*b*) After 3 hr, when a total of 30 mg/kg guanethidine had been administered ; another 20 mg/kg guanethidine lowered blood pressure and further reduced the muscle twitch responses.

muscle response in control experiments lasting about an hour was small and not significantly changed by 10 or 20 mg/kg of either bretylium or guathethidine or 10 mg/kg of bethanidine. Responses only to the high rate of stimulation were depressed significantly by 50 mg/kg of bretylium and marginally by 10 mg/kg of bethanidine, whereas 30 mg/kg of guanethidine also depressed responses to the low rate. This dose of bretylium greatly exceeds, and that of bethanidine slightly exceeds, the dose causing full neuromuscular paralysis but 30 mg/kg of guanethidine is just within the range of the neuromuscular paralysing doses of this compound (Table 4). There is doubt, however, as to whether the observed small reductions in the response of the muscle to direct stimulation are due to a direct action on the contractile mechanism, because of the complexity of cardiovascular changes. When blood pressure rose in consequence of the sympathomimetic action of guanethidine the gastrocnemius muscle twitches were usually enhanced but when large intravenous doses lowered blood pressure they diminished (Fig. 5). Enhancement of gastrocnemius muscle contractions was also produced by intravenous adrenaline.

DISCUSSION

These experiments confirm that neuromuscular blocking actions are common to bretylium (Boura & Green, 1959; Dixit *et al.*, 1961), guanethidine (Dixit *et al.*, 1961; Kroneberg & Stoepel, 1962) and bethanidine (Boura & Green, 1963). They show also that whereas concentrations of guanethidine causing neuromuscular paralysis in rat diaphragms markedly reduce the twitch response of the diaphragm to direct stimulation, as reported by Kroneberg & Stoepel (1962), bretylium and bethanidine do not appreciably affect the directly stimulated muscle. This distinction was confirmed in studies using curarized diaphragms. Similarly, tested by intravenous injection in anaesthetized rabbits and cats given (+)-tubocurarine to suppress neuromuscular transmission, guanethidine contrasted with bretylium and bethanidine by reducing slightly the response of the gastrocnemius muscles to direct stimulation, when given at doses causing neuromuscular paralysis in non-curarized preparations. A similar effect was produced by intra-arterial guanethidine in cats (Kroneberg & Stoepel, 1962). While these inhibitions of responses to direct muscle stimulation in anaesthetized animals may be analogous to those in the isolated diaphragm preparations, this is uncertain because of the dependence of the contractility of muscle on its vascular supply and the great complexity of the vascular changes produced by large intravenous amounts of guanethidine. Our results using the gastrocnemius muscles of curarized anaesthetized animals are to some extent at variance with the report of Vernikos-Danellis & Zaimis (1960) that not only guanethidine but also bretylium, at doses below those causing neuromuscular blockade, reduced the maximum twitch height of the tibialis anterior muscles of cats; however, our animals, in contrast apparently to those used by these authors, had been curarized.

The above experiments support the conclusions of Bowman & Rand (1961), Bowman *et al.* (1962) and Bowman & Hemsworth (1965) that the neuromuscular paralysing action of triethylcholine intensifies more sharply with increasing frequency of nerve stimulation than does the corresponding action of (+)-tubocurarine and is antagonized by choline, and that triethylcholine weakly antagonizes (+)-tubocurarine. They show also that in diaphragms exposed to triethylcholine some restoration of responses to a low rate of nerve simulation rapidly follows when the preparation has ceased to respond to a high frequency of stimulation.

In experiments using rat diaphragm and rabbit or cat gastrocnemius muscle preparations the neuromuscular blocking action of bretylium was almost as dependent upon the frequency of nerve stimulation as that of triethylcholine, but the nerve frequency dependence of blockade by guanethidine was similar to that for (+)-tubocurarine. Moreover, in rat diaphragm, but not gastrocnemius muscle preparations, choline was as effective an antagonist of bretylium as of triethylcholine but did not appreciably affect paralysis caused by guanethidine or (+)-tubocurarine. These results, and the finding that neuromuscular blocking concentrations of guanethidine, but not of bretylium, reduce the response of frog rectus muscle to acetylcholine (Gokhale, Gulati, Kelkar & Joshi, 1963), suggest that the action of bretylium is predominantly presynaptic and that of guanethidine largely postsynaptic. Bretylium, guanethidine and bethanidine all possess local anaesthetic actions (Boura & Green, 1959, 1963, 1965) but the actions of guanethidine alone resemble those of procaine on the rat diaphragm preparation. Procaine showed little discrimination between responses to high and low rates of indirect nerve stimulation in the presence of (+)-tubocurarine. Presynaptic and postsynaptic effects have been ascribed to procaine (Straughan, 1961).

Analogies may also be drawn between the present results for the relative dependences on nerve frequency of the blocking action of bretylium, guanethidine and bethanidine on the neuromuscular junction of voluntary muscle and earlier results for adrenergic nerve–smooth muscle junctions. Three showed that bretylium exerted its greatest effect against the highest rate of nerve stimulation, that guanethidine caused a parallel shift of nerve frequency–muscle responses curves and that the frequency dependence for bethanidine was intermediate (Boura & Green, 1959, 1962 and 1963; Green & Robson, 1964). These observations, in contrast to some others (Boura & Green, 1965), could easily be fitted to the hypothesis of Burn and Rand (1960 & 1965) that the adrenergic neurone blocking agents interfere with a supposed intermediary function of acetylcholine in adrenergic nerves; it could be postulated that bretylium acts largely by suppressing the release of acetylcholine and that guanethidine competes with the acetylcholine.

SUMMARY

1. The effects of bretylium, guanethidine, bethanidine, (+)-tubocurarine and triethylcholine on muscle contractions caused by direct and indirect stimulation at high and low frequencies have been compared, using rat isolated diaphragm preparations and the gastrocnemius muscles of anaesthetized rabbits and cats.

2. Bretylium, like triethylcholine, caused much greater inhibition of the contractions of all three muscle preparations when the rate of nerve stimulation was high; in rat diaphragms and rabbit gastrocnemius muscles responses to stimulation at 1 shock/10 sec were enhanced at a time when responses to 1 shock/sec were greatly inhibited.

3. In isolated rat diaphragms the blocking actions of guanethidine, bethanidine, (+)-tubocurarine and procaine were much less dependent upon the frequency of nerve stimulation than those of bretylium and triethylcholine. Somewhat similar but smaller differences between the effects of the compounds on high and low rates of stimulation were found in gastrocnemius muscle preparations, except that in the cat there was no distinction between the relative actions of bretylium and bethanidine.

4. In the isolated diaphragm preparation the neuromuscular paralysing action of bretylium, like that of triethylcholine and in contrast to that of guanethidine, bethanidine,

(+)-tubocurarine or procaine, was antagonized by choline. Neuromuscular paralysis by bretylium was weakly antagonized by choline in cats but not in rabbits.

5. At concentrations causing neuromuscular paralysis in isolated rat diaphragm preparations, guanethidine and procaine, in contrast to bretylium and bethanidine, markedly reduced responses to direct stimulation. This distinction was confirmed by testing in diaphragms exposed to (+)-tubocurarine.

6. Similarly, neuromuscular paralysing doses of guanethidine, but not of bretylium or bethanidine, reduced the contractions of directly stimulated gastrocnemius muscles in curarized rabbits and cats.

We gratefully acknowledge Messrs D. C. Cardinal, D. J. Chapple, E. D. Farrow and Mrs B. A. Kennedy for skilled technical assistance, also Ciba Laboratories Ltd. (Horsham) for generous gifts of guanethidine.

REFERENCES

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Br. J. Pharmac. Chemother.*, **14**, 536–548.

BOURA, A. L. A. & GREEN, A. F. (1962). Comparison of bretylium and guanethidine: tolerance, and effects on adrenergic nerve function and responses to sympathomimetic amines. *Br. J. Pharmac. Chemother.*, **19**, 13–41.

BOURA, A. L. A. & GREEN, A. F. (1963). Adrenergic neurone blockade and other acute effects caused by *N*-benzyl-*N'N''*-dimethylguanidine and its ortho chloro derivative. *Br. J. Pharmac. Chemother.*, **20**, 36–55.

BOURA, A. L. A. & GREEN, A. F. (1965). Adrenergic neurone blocking agents. *A. Rev. Pharmac.*, **5**, 183–212.

BOWMAN, W. C. & HEMSWORTH, B. A. (1965). Effects of triethylcholine on the output of acetylcholine from the isolated diaphragm of the rat. *Br. J. Pharmac. Chemother.*, **24**, 110–118.

BOWMAN, W. C. & RAND, M. J. (1961). Actions of triethylcholine on neuromuscular transmission. *Br. J. Pharmac. Chemother.*, **17**, 176–195.

BOWMAN, W. C., HEMSWORTH, B. A. & RAND, M. J. (1962). Triethylcholine compared with other substances affecting neuromuscular transmission. *Br. J. Pharmac. Chemother.*, **19**, 198–218.

BÜLBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Br. J. Pharmac. Chemother.*, **15**, 56–66.

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

DIXIT, B. N., GULATI, O. D. & GOKHALE, S. D. (1961). Action of bretylium and guanethidine at the neuromuscular junction. *Br. J. Pharmac. Chemother.*, **17**, 372–379.

GARRY, R. C. & WISHART, M. (1951). Fluid electrodes with a rubber diaphragm. *J. Physiol. (Lond.)*, **115**, 61–62P.

GOKHALE, S. D., GULATI, O. D., KELKAR, V. V. & JOSHI, N. Y. (1963). Effect of bretylium and guanethidine on some cholinergic effectors. *Arch. int. Pharmacodyn. Ther.*, **145**, 243–253.

GREEN, A. F. & ROBSON, R. D. (1964). Comparison of the effects of bretylium, guanethidine and bethanidine on smooth muscle responses to different rates of sympathetic nerve stimulation. *Br. J. Pharmac. Chemother.*, **22**, 349–355.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. physiol. Chem.*, **110**, 33–66.

KRONEBERG, G. & STOEPEL, K. (1962). Über eine Wirkung von Guanethidin auf Skeletmuskulatur und Rückenmarkreflexe. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **243**, 36–43.

STRAUGHAN, D. W. (1961). The action of procaine at the neuromuscular junction. *J. Pharm. Pharmac.*, **13**, 49–52.

VERNIKOS-DANELLIS, J. & ZAIMIS, E. (1960). Some pharmacological actions of bretylium and guanethidine. *Lancet*, **ii**, 787–788.