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THE ACTIONS OF BRETYLIUM: ADRENERGIC NEURONE BLOCKING AND OTHER EFFECTS

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Bretylium caused a specific and lasting depression of many excitatory and inhibitory responses evoked by electrical stimulation of the peripheral sympathetic nervous system, probably by impairing conduction of impulses in adrenergic neurones with consequent failure of noradrenaline and adrenaline release. This effect, which will be referred to as the adrenergic neurone blocking action, was preceded by weak sympathomimetic effects. In the presence of bretylium the effects of adrenaline and noradrenaline were increased, as after sympathectomy. Concentrations producing blocking of adrenergic neurones did not prevent the release of adrenaline and noradrenaline from the adrenal medulla by splanchnic nerve stimulation or by the injection of dimethylphenylpiperazinium iodide, nor did they cause antiparasympathetic or parasympathomimetic effects. No action on the central nervous system has been detected. Curare-like neuromuscular block occurred with 10 to 30 times the amount required to block the response to adrenergic nerve stimulation alone and was accompanied by signs of temporary synaptic block in autonomic ganglia. Adrenergic nerve trunks and sensory nerves in the skin were readily blocked for long periods by topical application of bretylium, whereas the phrenic nerve of the rat was not. Bretylium had little effect on gastrointestinal propulsion or on the sensitivity of smooth muscle to acetylcholine, 5-hydroxytryptamine, adrenaline, or noradrenaline, but moderate amounts depressed the peristaltic reflex and the sensitivity of the guinea-pig ileum to histamine. Bretylium caused postural hypotension in the cat in doses which had little effect on the supine blood pressure. Experiments on the nictitating membrane indicated that compensation for the effects of bretylium on low rates of stimulation of postganglionic sympathetic nerves could be attained by a small increase in the rate of stimulation, whereas compensation for its effects on high rates required an increase in the rate of stimulation beyond physiological limits.

The properties of choline 2,6-xylyl ether bromide, TM 10 (Hey and Willey, 1954; Exley, 1957; Bain and Fielden, 1957), suggested that compounds might be found which would selectively impair the function of adrenergic nerves without antagonizing noradrenaline and adrenaline. A search for such compounds revealed a number of quaternary benzylammonium salts that specifically blocked the effects of stimulating adrenergic nerves, but lacked the parasympathomimetic actions associated with choline 2,6-xylyl ether bromide (Boura, Copp, and Green, 1959). One of the most active and selective of these compounds was the *N*-*o*-bromobenzyl-*N*-ethyl-*NN*-dimethylammonium ion known as bretylium. Trials have shown that bretylium lowers the blood pressure in normotensive and hypertensive men, and that its effects are confined to the peripheral sympathetic nervous system (Boura, Green, McCoubrey, Laurence, Moulton, and Rosenheim, 1959).

This paper contains a description of the acute effects of bretylium, because only brief reports of the pharmacological properties of the drug have been published.

METHODS

Experiments in Vitro.—The preparation described by Finkleman (1930) was used to study the effects of the drug on the depressant action of postganglionic sympathetic nerve stimulation on the rabbit intestine. The nerve was stimulated through platinum electrodes, with supramaximal shocks at 50/sec. A similar procedure was used to examine the action of the drug on contractions of isolated rabbit uterus caused by stimulation of the hypogastric nerve. Usually drugs were added to the bath fluid, but in some experiments they were applied topically to the nerve by introducing the drug dissolved in Tyrode solution into a small

chamber, sealed with rubber dams at each end, through which the nerve had been drawn. Isolated preparations were also used to examine the effect of the drug on the actions of various local hormones. Tyrode solution was used for guinea-pig and rabbit intestine, Locke solution for guinea-pig uterus, a modified Tyrode solution containing 0.02% $MgCl_2$ for rabbit uterus, and the solution described by Gaddum, Peart, and Vogt (1949) for rat uterus. These solutions were oxygenated and maintained at 37°.

Rabbit ears were perfused with Tyrode solution at 37°, and a Thorp impulse counter measured venous outflow. Vasoconstriction was produced by adding drugs to the perfusion fluid or by stimulating the greater auricular nerve adjacent to the medial artery.

Actions at the neuromuscular junction were tested on the rat diaphragm-phrenic nerve preparation (Bülbring, 1946). Single rectangular pulses of 0.7 msec. duration were applied to the nerve through fluid electrodes at the rate of 5/min.

Experiments on Anaesthetized Cats.—Anaesthesia was induced with ether and maintained with chloralose (about 60 mg./kg., intravenously). In dogs, pentobarbitone sodium (30 to 40 mg./kg.) was injected intravenously. Blood pressure was recorded from a carotid artery and injections were usually made into a femoral vein. Contractions of the nictitating membrane were recorded using an isotonic frontal-writing lever. Contractions of the heart were recorded with a Cushny myocardiograph attached to an isometric writing lever which, by closing a mercury switch, operated a Thorp impulse counter, so recording the heart rate.

Various nerves were cut and drawn into fluid electrodes of the type described by Porter and Allamon (1936). They were usually stimulated at 10 to 20 pulses/sec. However in experiments on the nictitating membrane (Fig. 5) various other frequencies were applied to the postganglionic nerve; each of five stimulation rates was applied for 2 min. to the nerves supplying each of the two membranes, five times in random order, and the mean height of contraction determined for each frequency. This was done at the beginning of the experiment, again 10 min. after each of a series of progressively increasing doses of bretylium and finally after the injection of cocaine.

The superior cervical ganglion of the cat was perfused in the manner employed by Perry (1953).

The effect of bretylium on the noradrenaline content of the venous blood from the spleen during stimulation of the splenic nerve was studied by the method of Brown and Gillespie (1957). The nerve was stimulated with 25 pulses/sec. The pressor activity of the plasma was assayed in terms of noradrenaline in the pithed rat.

The position of the cats was supine, except in postural hypotension experiments in which the animals were tilted through approximately 75° until the blood pressure fall was maximal.

Experiments in Unanaesthetized Animals.—Changes in sympathetic tone in the cat were observed by measuring the portion of the nictitating membrane exposed at the lower lid margin. Pupil diameters in mice were measured using a ×10 magnifying lens fitted with a scale. Effects on gastrointestinal propulsion in the rat were examined by the charcoal meal test (Green, 1959).

Local anaesthesia was tested by pricking the skin with a fine needle after intradermal injection in man and guinea-pig. In the latter species, the method of Bülbring and Wajda (1945) was used.

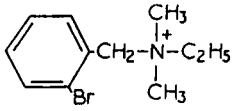
Activity of mice was recorded in individual jiggle cages which were sensitive to limb movements. The mice were placed in the jiggle cage for counting periods of 10 min., starting 15 min. after 50 mg./kg. of bretylium in the first test, and starting 2, 4, and 6 hr. after 2.5, 5, 10, 20, and 40 mg./kg. intraperitoneally in a second test. In each experiment six cages were used in each of six runs, and treatments were randomized between them. In the second test, five groups of six mice received the various doses of bretylium and a control group saline. The mean control counts were 148, 112, and 110/min. at 2, 4, and 6 hr. respectively.

An analgesic action was sought by comparing the pain thresholds of a group of ten rats given 50 mg./kg. subcutaneously with those of a control group injected with saline. The thresholds were measured after ½, 1, 2, 4, and 6 hr. by the pressure and heat methods described by Green and Young (1951).

The intravenous, subcutaneous and oral LD50 values in mice were approximately 81 to 125, 81 to 123, and 53 to 190% of the means respectively ($P = 0.05$).

Before the administration of drugs by stomach tube, mice and rats were allowed water *ad libitum* but had received no solid food for 12 to 18 hr. Cats were restricted to milk and water for 24 hr. and were lightly anaesthetized with ether for the drug administration.

Atropine was given as the sulphate, *N'*,*N'*-dimethyl-N^3-phenylpiperazinium as the iodide, and cocaine as the hydrochloride. The amounts of bretylium refer to the bromide unless the tosylate (Darenthin) is specifically mentioned. The molecular weights of bretylium bromide and bretylium tosylate are 341 and 414 respectively. The structure of bretylium is:



RESULTS

Effects on the Peripheral Sympathetic Nervous System

Rabbit Ileum. — The inhibitory effect of stimulating the visceral nerve on the pendular movements of isolated rabbit ileum was abolished after adding bretylium in concentrations of 1 to

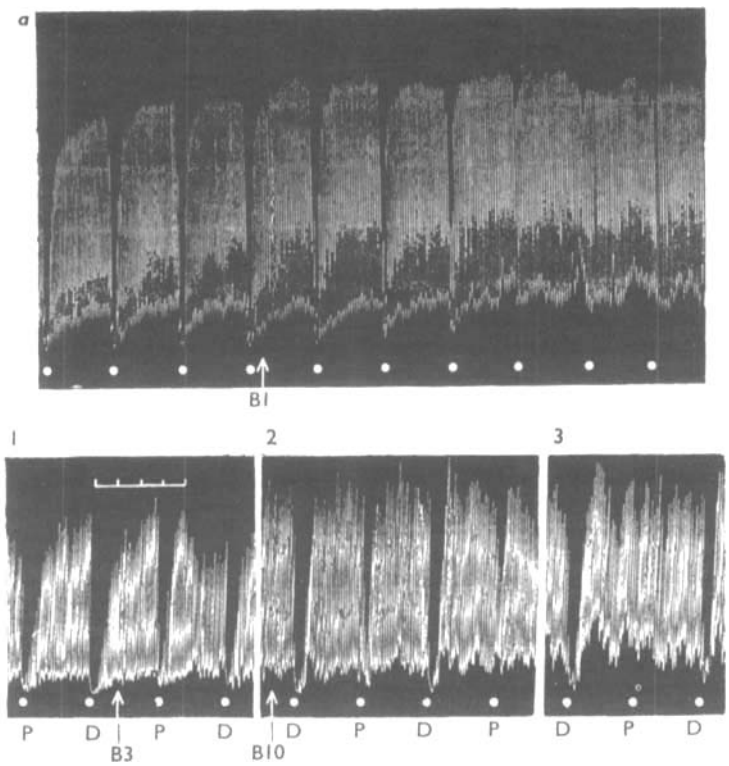


FIG. 1.—The effect of adrenergic nerve stimulation decreasing motility of isolated rabbit ileum. Supramaximal stimulation at white dots (50 pulses/sec. for 20 sec. every 3 min.). *a*, Bretylium was added to the organ bath in a concentration of 1 μg./ml. (at **B1**). *b*, Bretylium was applied to the nerve only in concentrations of 3 and 10 μg./ml. (**B3** and **B10** respectively). *P*, Stimulation proximal to and **D** distal to the nerve bath. Between records *b*1 and *b*2, 15 min., and between *b*2 and *b*3, 30 min.

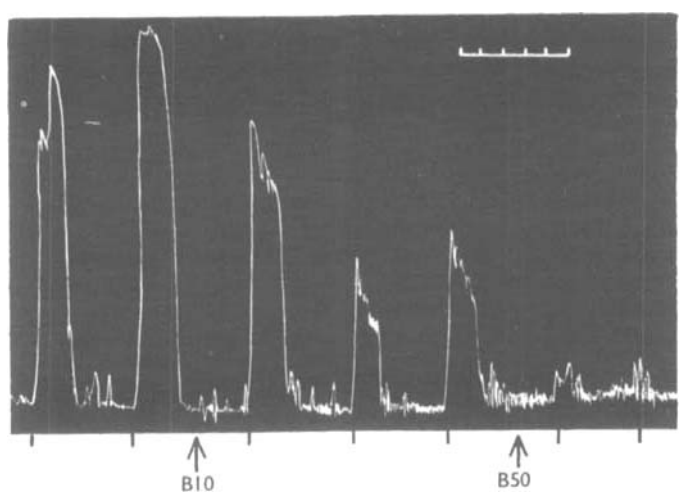


FIG. 2.—Contractions of isolated rabbit uterus caused by stimulation of the hypogastric nerve. The vertical lines indicate supramaximal stimulation (50 pulses/sec. for 1 min.). From **B10**, the nerve bath contained bretylium at 10 μg./ml. and from **B50** it contained 50 μg./ml. Time, 1 min.

3 μg./ml. to the bath fluid (Fig. 1*a*). With these concentrations the effect took about 20 min. to develop fully, but higher concentrations acted more rapidly. The effect persisted after washing the tissue several times. The inhibitory effect of bretylium was reduced by adding cocaine (100 μg./ml.). The response to nerve stimulation could also be abolished by immersing the nerve in bretylium (10 to 100 μg./ml.) between the point of stimulation and the intestine (Fig. 1*b*). It was important to choose a preparation without much fat around the nerve.

The inhibitory effects of adrenaline and noradrenaline (10 to 100 ng./ml.) on the pendular movements of isolated rabbit ileum were materially unaltered by previous treatment with bretylium (30 μg./ml.).

Rabbit Uterus.—Stimulation of the hypogastric nerve supplying an isolated horn of the rabbit uterus caused contractions. This response to nerve stimulation was abolished by concentrations of 3 to 10 μg./ml. of bretylium in the bath fluid or by adding bretylium (10 to 50 μg./ml.) to the Ringer solution in the nerve bath situated between the point of stimulation and the muscle (Fig. 2). The sensitivity of the uterus to 20 ng./ml. of adrenaline or noradrenaline was not reduced unless large concentrations of bretylium (100 μg./ml.) were added to the bath.

Vasodilatation in the Rabbit.—The subcutaneous injection of 10 mg./kg. of bretylium was followed by vasodilatation, which was seen most readily in the ears. The vasoconstriction in perfused rabbit ear, caused by stimulation of the greater auricular nerve, was blocked by the injection of 30 to 100 μg. of bretylium into the perfusion fluid (Fig. 3*a*) or by local application to the nerve of a concentration of 100 μg./ml. between the stimulating electrodes and the ear. In contrast, the vasoconstriction following the injection of 0.1 to 1.0 μg. of noradrenaline into the perfusion fluid

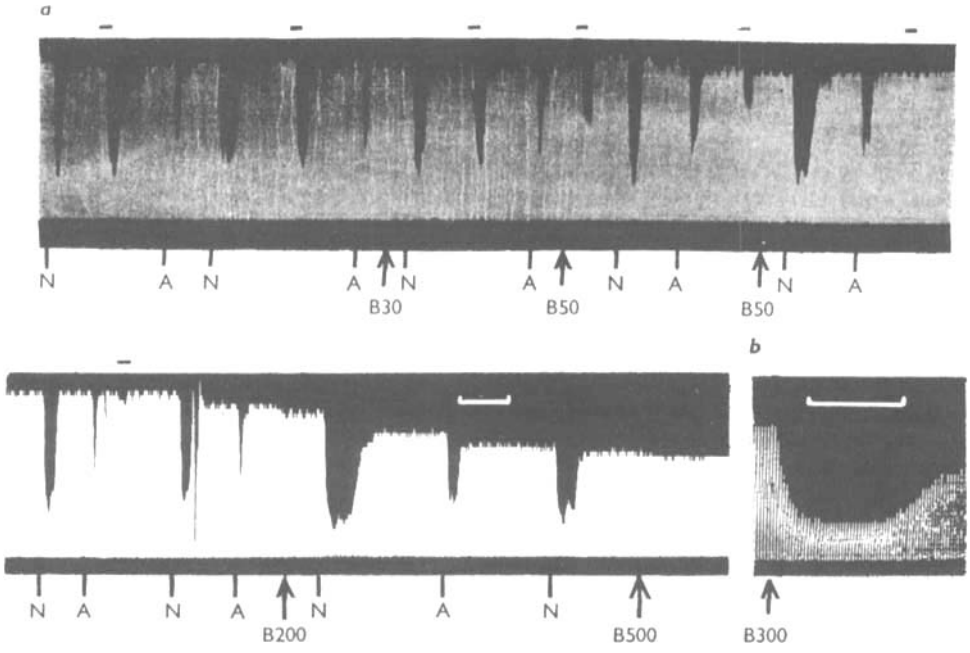


FIG. 3.—Venous outflow from a perfused rabbit ear, recorded with a Thorp impulse counter. The horizontal bars indicate supramaximal stimulation of medial nerve (50 pulses/sec. for 1 min.). At **N**, 0.2 μg. of noradrenaline, and at **A**, 0.1 μg. of adrenaline were injected into the arterial cannula. At **B30**, **B50**, **B200**, **B500**, and **B300**, 30, 50, 200, 500, and 300 μg. of bretylium respectively were injected into the arterial cannula. Records *a* and *b* were made from different ears. Time, 5 min.

was increased after the injection of 50 to 300 μg. of bretylium (Fig. 3*a*). The response to adrenaline (0.1 to 1.0 μg.) was increased to a lesser extent and, in some experiments, was unchanged. Vasoconstriction occurred after injecting large amounts of bretylium (100 to 300 μg.) into the perfusion fluid. In most experiments the response was similar to that in Fig. 3*b*.

Nictitating Membrane. — The subcutaneous injection of 5 to 10 mg./kg. bretylium into cats caused relaxation of the nictitating membrane and narrowing of the palpebral fissure. The pupils became smaller but dilated in dim light. The relaxation of the nictitating membrane was the most characteristic effect. Usually it did not appear until 3 hr. had elapsed and was greatest between 12 and 24 hr. Normal tone of the membrane did not usually return for 36 to 48 hr. When the drug was administered by stomach tube, larger amounts of bretylium were needed to produce these effects. The onset of action tended to be slower and the duration longer with oral than with subcutaneous injections (Fig. 4). In cats in which the membranes had been relaxed by administration of bretylium, the injection of adrenaline, noradrenaline or a further amount of bretylium caused transient contractions of the membranes.

The nictitating membranes of dogs were also relaxed by injection of bretylium, in amounts similar to those which were effective in cats. Again the effect was slow in onset and persisted at least 24 hr.

In cats anaesthetized with chloralose, the nictitating membrane retracted after the intravenous injection of large amounts of bretylium. The effect was small or absent after 5 mg./kg. or less, but 10 to 20 mg./kg. often caused retractions ranging between 20 and 80% of those due to maximal stimulation of the cervical sympathetic nerve. The action persisted after cutting the pre- or post-ganglionic nerves, and removal of the adrenals had little effect. Retraction of the membrane was often maintained for over 1 hr. A second injection of drug given when the membrane had relaxed caused a temporary contraction. These findings accord with those observed in unanaesthetized animals.

After administration of bretylium the nictitating membrane failed to maintain the tonic retraction

in response to electrical stimulation of the cervical sympathetic nerve, irrespective of whether the stimulation was applied proximally or distally to the superior cervical ganglion. High rates of stimulation were affected to a relatively greater extent than were low rates of stimulation, as is seen in Fig. 5. The responses of the membranes on each side were recorded during continuous stimulation of both the postganglionic nerves at each of five frequencies five times in random order. Bretylium (2 to 6 mg./kg.) reduced the regression of response on the log. of the frequency of stimulation and, after a total dose of 10 mg./kg. of bretylium, the sensitivity to all rates of stimulation was practically abolished. Such membrane tone seen in Fig. 5 after 10 mg./kg. was largely accountable to the effect of bretylium itself. After bretylium had depressed the response of the nictitating membrane to nerve stimulation, resting the preparation was followed by a temporary increase of the response. The intravenous injection of adrenaline, noradrenaline or dimethylphenylpiperazinium in the non-adrenalectomized cat continued to cause contractions of the membrane after administration of bretylium in amounts sufficient to abolish its response to nerve stimulation.

The intravenous injection of 1 mg./kg. of cocaine reduced the effect of intravenous bretylium on the membrane response to nerve stimulation (Fig. 5). In other experiments when 1 mg./kg. of cocaine reduced the depressant effect of 5 mg./kg. of bretylium on the response to nerve stimulation, the response to injected adrenaline was increased to a similar extent (Fig. 6). Increasing the amount of cocaine administered caused no further increase in either response whereas, at this stage, a further dose of 5 mg./kg. of bretylium depressed the response to nerve stimulation but not that to adrenaline.

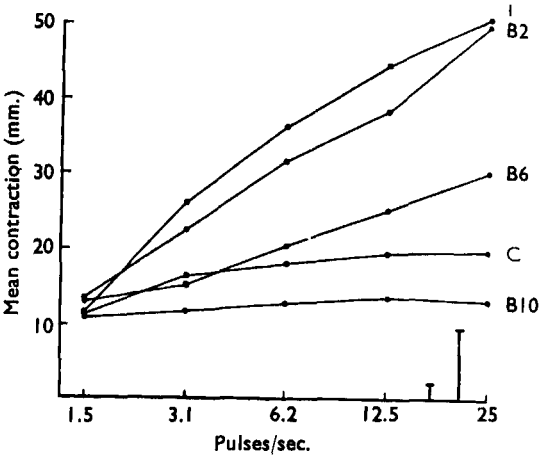


FIG. 5.—Contractions of the nictitating membranes of a cat under chloralose anaesthesia during stimulation of the postganglionic cervical sympathetic nerves at various frequencies by supra-maximal rectangular pulses. Each point represents the mean of five responses on each of the two membranes. I, Initial; B2, after 2 mg./kg. of bretylium intravenously; B6, after a total of 6 mg./kg.; B10, after a total of 10 mg./kg.; C, after 1 mg./kg. of cocaine in addition. The vertical lines represent the heights of the sustained contractions of the membranes caused by 6 and 10 mg./kg. of bretylium respectively in the absence of nerve stimulation.

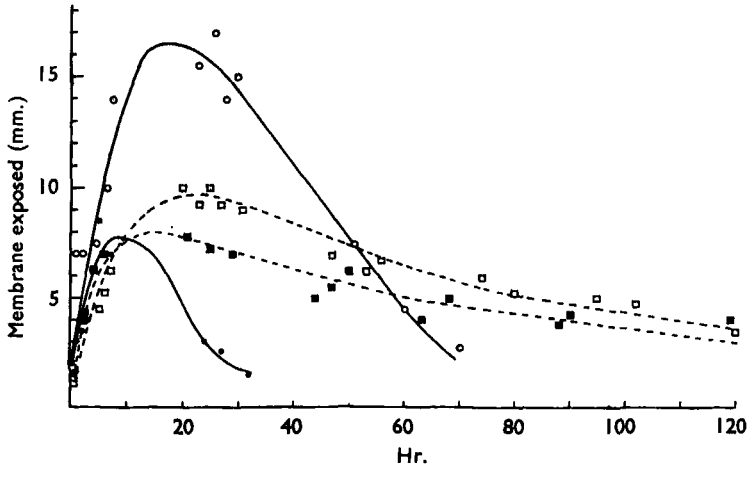


FIG. 4.—Relaxation of the nictitating membrane after bretylium in the unanaesthetized cat. Each point is the mean of observations on four animals. The higher oral and subcutaneous doses were maximal in effect for the method of administration. O——O, 10 mg./kg. subcutaneously. ●——●, 2.5 mg./kg. subcutaneously. □ - - - - □, 50.0 mg./kg. orally. ■ - - - - ■, 12.5 mg./kg. orally.

The injection of 1 mg. of bretylium into the carotid artery of an adrenalectomized cat (3.8 kg. body weight) caused a small retraction of the nictitating membrane (10% of maximal) lasting about 3 min., and 15 min. later it had reduced the response to stimulating the postganglionic nerve by about 50%. When a further 2 mg. was given the membrane retracted to about 20% of maximum and regained its normal tone after about 15 min. This quantity reduced the response to nerve stimulation by 95%. A further 3 mg. produced a similar degree of membrane

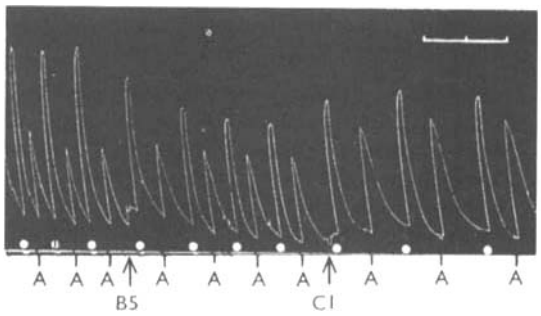


FIG. 6.—Contractions of the nictitating membrane of a cat anaesthetized with chloralose. Supramaximal rectangular pulse stimuli 25 sec. were applied to the postganglionic nerve for 30 sec. at the white dots. **A**, 50 μg. of adrenaline intravenously; **B5**, 5 mg./kg. of bretylium intravenously; **C1**, 1 mg./kg. cocaine intravenously. Time, 10 min.

retraction lasting 25 min. and eliminated the response to nerve stimulation.

In the perfused superior cervical ganglion preparation in the cat, injection of 100 μg. of bretylium caused a small temporary retraction of the nictitating membrane followed by a temporary impairment of the effects of preganglionic stimulation. The addition of bretylium to the perfusion fluid depressed the response to preganglionic nerve stimulation in the manner characteristic of ganglionic blocking agents (Fig. 7). Return to a perfusion fluid containing no bretylium led to a full restoration of the membrane retractions.

In an adrenalectomized cat which had received atropine (2 mg./kg.), intravenous injection of 5 or 10 mg. of acetylcholine caused retractions of the acutely denervated nictitating membranes which, though diminished, continued after 9 mg./kg. of bretylium but ceased after a further 3 mg./kg. of atropine.

Cardiovascular Effects.—The blood pressure of supine cats under chloralose anaesthesia usually increased temporarily after the intravenous injection of 3 to 10 mg./kg. of bretylium and then, after a period of 2 to 10 min., gradually fell. The rise in pressure varied considerably in different cats, but seldom exceeded 30 mm. Hg after 3 mg./kg. of bretylium or 60 mm. Hg after 10 mg./kg. It was accompanied by tachycardia. The delayed lowering of blood pressure was so gradual that in experiments in the anaesthetized animal we could not always be sure that the effect was distinct from a possible deterioration of the preparation. However, in most experiments the pressure had fallen 30 to 60 mm. Hg below the pre-injection level within 30 to 60 min. after injection of 5 to 10 mg./kg. A return to pre-injection levels was never attained in any experiment, but some recovery was observed within 6 hr. on two occasions. The pressor effect of bretylium was greatest in animals with a low blood pressure, as in the spinal preparation. It also persisted after ganglionic blockade or adrenalectomy.

Postural hypotension was produced by bretylium in doses below those which materially changed the supine pressure. When supine cats were tilted towards the vertical position, the mean carotid blood pressure usually fell about 20 mm. Hg. After 1 to 3 mg./kg. of bretylium, the postural fall was as much as 50 to 100 mm. Hg (Fig. 8). Cardio-acceleration caused by stimulation of the inferior cardiac nerve was

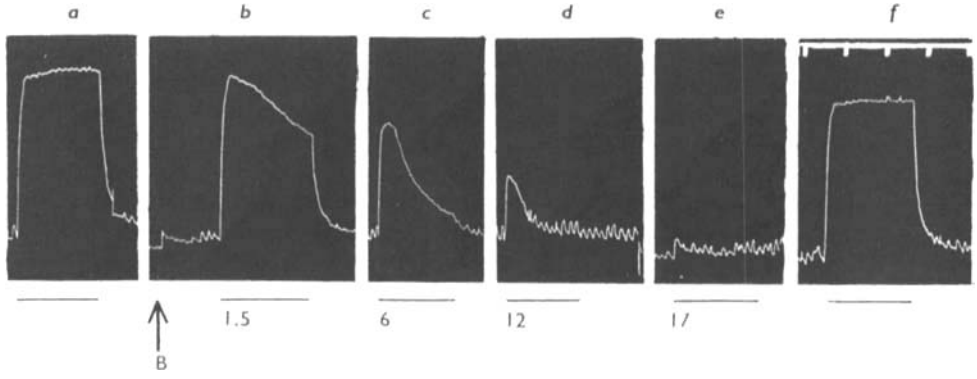


FIG. 7.—The effect of bretylium added to the fluid perfusing the isolated superior cervical ganglion of the cat. Record of the nictitating membrane responses caused by supramaximal stimulation of the preganglionic cervical sympathetic nerve (10 pulses/sec.). The presence of continuous stimulation is indicated by the horizontal lines. *a*, Contraction before the drug. *b* to *e*, contractions during the perfusion with Tyrode solution containing 100 μg./ml. of bretylium started at B; the numerals give the time in minutes from changing to this solution. *f*, Contraction 8 min. after returning to normal Tyrode solution. Time, 1 min.

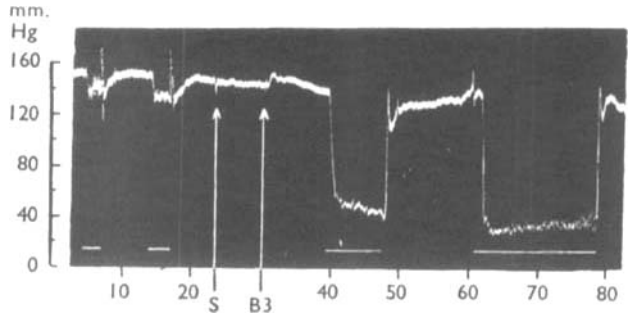


FIG. 8.—Postural hypotension after bretylium in a cat anaesthetized with chloralose. The cat was supine except when tilted through 75°, for the periods indicated by the horizontal white lines. Saline was injected intravenously at S and 3 mg./kg. of bretylium at B3. The numerals 10 to 80 indicate the time in min.

gradually abolished after the intravenous injection of 1 to 3 mg./kg. of bretylium (Fig. 9). When bretylium had blocked the cardiac acceleration in such experiments, stimulation of the nerve caused some slowing of the heart which continued after increasing the bretylium dose to 10 mg./kg. but was abolished by 1 mg./kg. of atropine.

Bretylium also reduced the reflex hypertension caused by stimulating the central end of the cut vagus nerve and the hypertension caused by carotid occlusion in the cat, the minimal effective intravenous dose being about 3 mg./kg. Further, the pressor effect of intravenous acetylcholine (5 mg.) in an atropinized adrenalectomized cat was abolished by 3 mg./kg. bretylium. On the other hand, the pressor action of intravenous noradrenaline, and to a lesser extent that of adrenaline, increased after giving sufficient bretylium to block adrenergic nerve transmission, and adrenaline continued to cause tachycardia (Fig. 9).

In a dog, 3 mg./kg. of bretylium intravenously caused hypertension lasting about 20 min. The pressor response to carotid occlusion was abolished but that to noradrenaline was increased.

The Spleen and its Noradrenaline Output.—Contraction of the spleen caused by bursts of stimuli at 25/sec. applied to the splenic nerve ceased after injection of 5 to 10 mg./kg. of bretylium. This was accompanied by a reduction in the noradrenaline content in the venous blood from the spleen. Fig. 10 shows the amount of noradrenaline released by each stimulus to the nerve, in each of two experiments.

Sweat Glands.—Bretylium in intravenous doses of up to 10 mg./kg. did not prevent sweating of the paws of anaesthetized cats following stimulation of the peripheral sympathetic chain.

Adrenal Gland.—No change in the functioning of the adrenal medulla was observed after the administration of bretylium. In cats, splanchnic nerve stimulation continued to exert a pressor effect even after 10 mg./kg. of the drug (Fig. 11). In none of three cats was there a rapid component in the hypertensive response attributable with certainty to contraction of the splanchnic vessels, but in the one cat where the record indicated a likelihood of its presence, the rapid component disappeared after giving bretylium.

Intravenous doses of 20 to 50 μg. of dimethylphenylpiperazinium cause a liberation of catechol amines from the adrenal medulla in the cat, and the hypertension so produced was enhanced by bretylium (Fig. 11), like that of injected adrenaline or noradrenaline. After adrenalectomy larger doses of dimethylphenylpiperazinium (100 μg.) are needed to cause

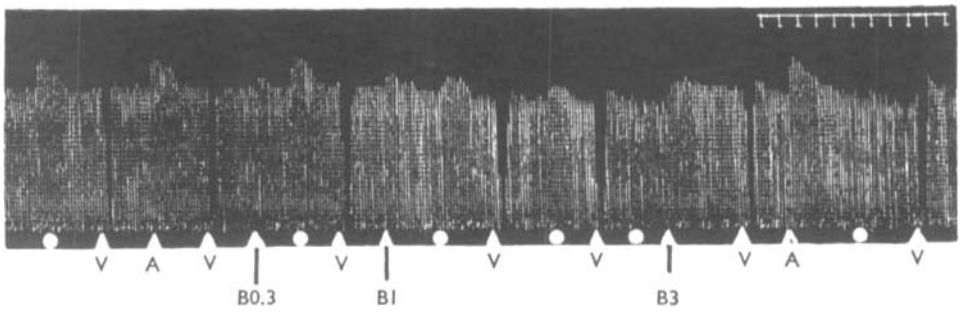


FIG. 9.—The heart rate of a cat anaesthetized with chloralose recorded with a Thorp impulse counter. Bretylium (0.3, 1.0, and 3 mg./kg.) was injected intravenously at B0.3, B1, and B3 respectively. It abolished the cardioacceleration caused by supramaximal stimulation of the inferior cardiac nerve for 30 sec. with 20 pulses/sec. (at white dots), but not the responses to vagal stimulation for 20 sec. at 20/sec. (at V). The response to 5 μg. of adrenaline intravenously (at A) was unchanged. Time, 1 min.

hypertension, and this effect (presumably due to stimulation of the sympathetic ganglia) was antagonized by 3 mg./kg. of bretylium (Fig. 12),

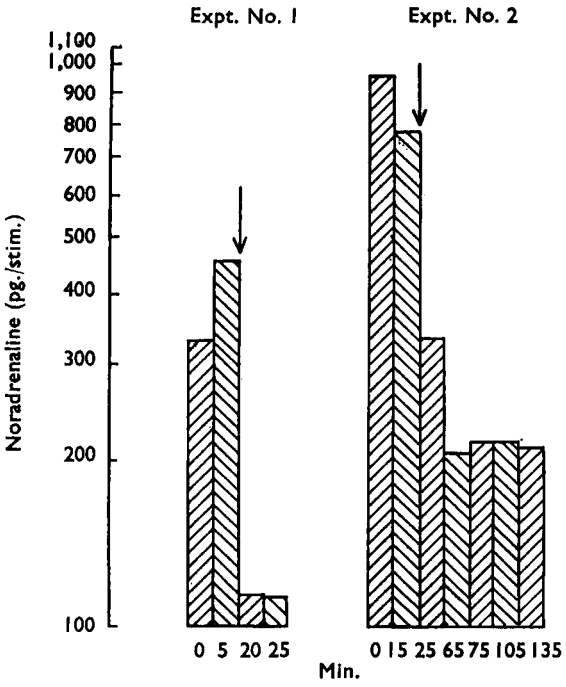


FIG. 10.—The effect of bretylium on the noradrenaline content of the venous blood from the spleen during stimulation of the splenic nerve. Stimuli were applied to the nerve at the rate of 25/sec. for 10 sec. in Expt. No. 1 and for 60 sec. in Expt. No. 2. The venous blood was collected for 30 sec. from the beginning of stimulation in Expt. No. 1, and for 2 min. in Expt. No. 2. The output of noradrenaline, assayed in the pithed rat, is expressed in pg./stimulus. The arrows indicate the intravenous injection of 10 mg./kg. of bretylium in Expt. No. 1 and 5 mg./kg. in Expt. No. 2.

like that of acetylcholine in the atropinized adrenalectomized cat.

Actions Associated with the Parasympathetic Nervous System

No effects attributable to an action on the peripheral parasympathetic nervous system have been observed, except with high concentrations of bretylium. Large, near toxic, intravenous doses caused a number of transitory effects resembling those produced by ganglionic blocking agents. In cats, 15 mg./kg. intravenously dilated the pupil and abolished its response to light for a few minutes. The nictitating membrane temporarily relaxed, but this was distinct from the later, slowly developing, persistent effect on the membrane, which we associate with a depression of the adrenergic nerve function. No mydriasis was seen after amounts of up to 30 mg./kg. subcutaneously in cats. In mice given 12.5 mg./kg. intravenously, the pupils measured under a powerful light source rapidly dilated to about half the diameters found after fully effective doses of ganglionic blocking agents, but returned to the control size in 5 to 10 min. Mydriasis was not seen after 50 mg./kg. intraperitoneally in mice, nor after 15 mg./kg. intramuscularly in dogs. Similarly, after the intravenous injection of 10 mg./kg. in the cat anaesthetized with chloralose, the bradycardia caused by stimulating the distal end of the cut vagus was reduced, but only for a few minutes. The depressor effect of intravenous acetylcholine was not appreciably changed.

Bretylium had no effect on the tone of the isolated guinea-pig or rabbit ileum except in very high concentrations. In a comparative test, 200 μg./ml. caused a spasm of guinea-pig ileum equivalent to those caused by 40 ng./ml. of

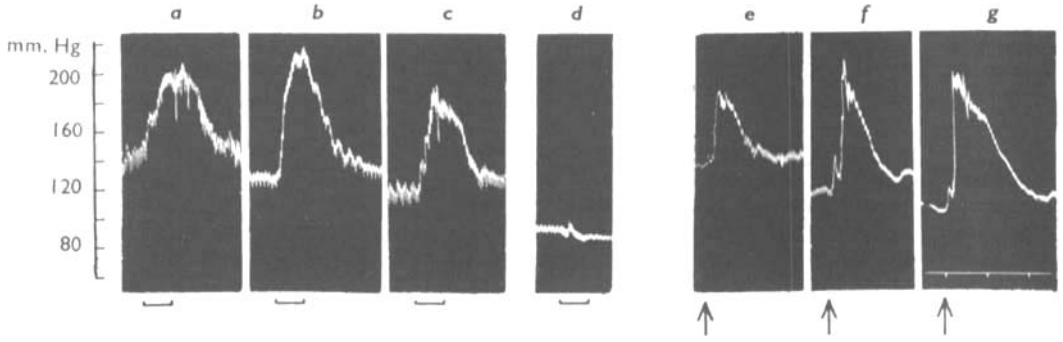


FIG. 11.—Records of the blood pressure of cats anaesthetized with chloralose. The first group shows the pressor responses to supramaximal stimulation of the splanchnic nerve at 15/sec. for 40 sec. (horizontal bars). *a*, Before the drug; *b* and *c*, 50 min. after 3 and 10 mg./kg. of bretylium intravenously respectively; *d*, 8 min. after 1 mg./kg. pentacynium intravenously. The second group shows the pressor responses to 20 μg. dimethylphenylpiperazinium intravenously in another cat (at arrows). *e*, Before the drug; *f*, 35 min. after 3 mg./kg.; and *g*, 45 min. after 10 mg./kg. of bretylium. Time, 1 min.

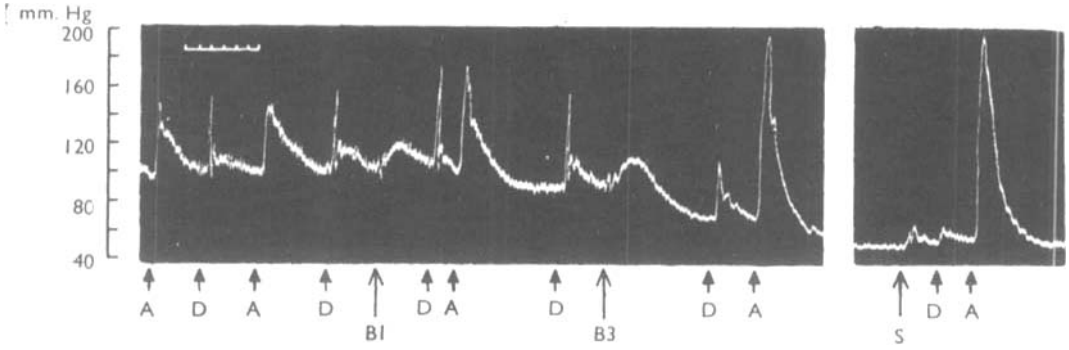


FIG. 12.—Record of the blood pressure of an adrenalectomized cat anaesthetized with chloralose. Adrenaline (10 μg.) was injected intravenously at A and 100 μg. of dimethylphenylpiperazinium at D. Bretylium was injected intravenously, 1 mg./kg. at B1 and 3 mg./kg. at B3. S represents an injection of saline. Time, 1 min.

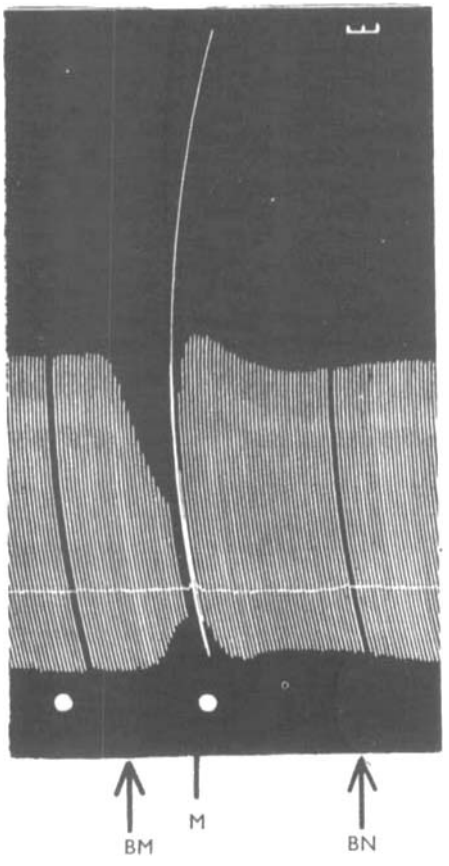


FIG. 13.—Record of twitch responses of an isolated rat diaphragm. Stimuli (5/min.) were applied to the phrenic nerve except at M which is a response to direct muscle stimulation. Bretylium was added to the organ bath to give 400 μg./ml. at BM and to the nerve only to give 10 mg./ml. at BN. The bath fluid was changed at white dots. Time, 1 min.

acetylcholine and 50 μg./ml. of choline 2,6-xylyl ether bromide. This action was inhibited by atropine (0.1 μg./ml.) and slightly reduced by hexamethonium (0.5 mg./ml.). A concentration of 100 μg./ml. did not reduce the sensitivity of the gut to acetylcholine (50 ng./ml.) or to 5-hydroxytryptamine (50 ng./ml.).

Neuromuscular Toxicity

Contractions of the isolated rat diaphragm in response to stimulation of the phrenic nerve were abolished by 400 μg./ml. of bretylium in the organ bath, whereas the sensitivity to direct muscle stimulation remained unchanged (Fig. 13). It was not antagonized by neostigmine. Topical application of 10 mg./ml. of bretylium to the phrenic nerve trunk did not depress conduction. Therefore the block seemed to be at or near to the neuromuscular junction.

In cats anaesthetized with chloralose, respiratory paralysis and inhibition of the response of the gastrocnemius muscle to indirect stimulation, but not to direct stimulation, occurred concomitantly after intravenous doses of 15 to 30 mg./kg. of bretylium. Thus, neuromuscular block seemed to be the main toxic effect of the drug. Subcutaneous doses of 100 mg./kg. caused muscular weakness in unanaesthetized cats but 50 mg./kg. was well tolerated. In monkeys, ptosis and a diminution in muscular effort often occurred after 50 mg./kg. In albino mice the LD50 of bretylium was approximately 16 mg./kg. intravenously, 68 mg./kg. subcutaneously and 270 mg./kg. when the drug was given by stomach tube. In two chicks 25 mg./kg. of bretylium intravenously caused a flaccid paralysis resembling that with curare. A dose of 37.5 mg./kg. intravenously was toxic in two other chicks.

Sensory Nerves

Local anaesthesia followed the intracutaneous injection of bretylium in concentrations of 5 mg./ml. or more in guinea-pigs. The effect was slow in onset but very prolonged even compared with that caused by cinchocaine (Fig. 14). Injection of a 30 mg./ml. solution left the site of injection not fully sensitive to stimulation after 48 hr. Similar observations were made using concentrations of 2.5 mg./ml. in man, a small area at the injection site remaining insensitive for over 24 hr.

Central Nervous System

No important behavioural change was observed in cats injected with amounts sufficient to relax the nictitating membrane, though lethargy was sometimes apparent after 10 to 30 mg./kg. of bretylium subcutaneously. No change in the activity counts of mice was detected at 15 to 25 min. after 50 mg./kg. of bretylium intraperitoneally nor at 2 hr. after 2.5 to 40 mg./kg. of bretylium tosylate intraperitoneally. However, at 4 and 6 hr. after 10 or 20 mg./kg. of bretylium tosylate, the mean activity counts were only about half those of the control mice and these differences were highly significant. The reduction in activity with 40 mg./kg. tended to be less than with 10 or 20 mg./kg. The duration of hypnosis following intravenous pentobarbitone sodium (60 mg./kg.) was slightly prolonged by the intravenous injection of 12.5 mg./kg. of bretylium at the same time, but not by 6.25 mg./kg. These effects could be due to peripheral changes.

In rats, no analgesic action was detected up to 6 hr. after 50 mg./kg. bretylium subcutaneously.

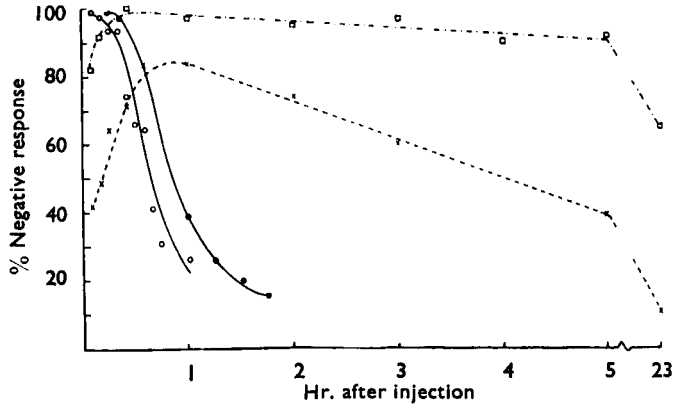


FIG. 14.—Local anaesthesia after the intradermal injection of bretylium and cinchocaine hydrochloride in guinea-pigs. Ordinate: the % of negative responses to six pricks on four wheals in each of three guinea-pigs (total of 72 responses). × and □, Bretylium 10 and 30 mg./ml. respectively; O and ●, Cinchocaine hydrochloride 0.5 and 1.5 mg./ml. respectively.

The intracisternal injection of 0.5 mg. in 0.05 ml. of saline caused severe tremors for about 1 hr. The pupil diameters were unchanged.

Other Effects

Alimentary Tract.—No salivation or gastro-intestinal disturbance was apparent in cats. Salivation occurred after 10 to 30 mg./kg. of bretylium intravenously in the dog, and faecal evacuation after 30 to 100 mg./kg. subcutaneously in the rabbit and guinea-pig. Whether these effects should be attributed to parasympathomimetic action requires further investigation. The salivation in the dog could be due to the early sympathomimetic effect of the drug, and the faecal evacuation in the other two species could be consequent to a diminution in sympathetic tone. The proportion of the small intestine traversed by a charcoal meal administered to fasted rats was not materially changed by the previous administration of bretylium. In an experiment with groups of six rats the mean percentage of the small intestine traversed in 10 min. was 34 in controls given saline. In the groups given 10 and 40 mg./kg. of bretylium 1 hr. before the charcoal the values were 34 and 32% respectively. When 10 and 40 mg./kg. were given 6 hr. before the meal the values were 45 and 41%. The differences are not statistically significant, though bretylium might have accelerated gastro-intestinal propulsion. The peristaltic reflex elicited by distending the isolated guinea-pig ileum was abolished by 30 μg./ml. bretylium.

Isolated Heart Preparations.—In each of three Langendorff preparations of the rabbit heart, the injection of 0.05 to 0.5 mg. of bretylium into the arterial cannula caused small transient reductions in the coronary flow and the strength of the ventricular contractions. No change in heart rate was detected. Strips of guinea-pig ventricles, driven at the rate of two contractions/min. by direct muscle stimulation (Stewart, 1958), showed a small positive inotropic response with bretylium (10 to 20 μg./ml.).

Uterus.—No change in the tone of isolated guinea-pig or rabbit uterus was observed until the concentration of bretylium was increased to between 100 and 300 μg./ml., at which a small contraction occurred. Rat uterus showed no response to this concentration, but its sensitivity to

5-hydroxytryptamine (0.1 μg./ml.) was impaired.

Histamine.—No antihistamine effect on the blood pressure was detected in cats after the injection of 10 mg./kg. of bretylium intravenously, but concentrations between 1 and 10 μg./ml. reduced the submaximal contractions of the isolated guinea-pig ileum preparation stimulated by histamine.

Bretylium Tosylate

The tosylate (*p*-toluenesulphonate) is preferred to the bromide for clinical use and was chosen because it was the least hygroscopic of the many alternative salts examined. The anion did not appear to influence the pharmacological action of the bretylium cation. The toxicity of bretylium tosylate was equivalent on a molar basis to that of the bromide in comparative tests. The LD50 of the bretylium tosylate in mice was approximately 20 mg./kg. by intravenous injection, 72 mg./kg. subcutaneously and 400 mg./kg. orally. The tosylate anion was not expected to contribute to the toxicity of the bretylium salt, since in mice the intravenous LD50 of sodium paratoluene sulphonate is 1,700 mg./kg. Comparison of bretylium tosylate and bretylium bromide in cats showed that they had equivalent effects on blood pressure and on the nictitating membrane and that their toxicities were equivalent. Amounts of up to 400 mg./kg. of bretylium tosylate were tolerated when given by stomach tube to rats, as was 200 mg./kg. by the same route in the monkey.

Discussion

Bretylium specifically depressed the peripheral sympathetic nervous system. Since the responses to stimulation of several different postganglionic sympathetic nerves were depressed as readily as responses to preganglionic stimulation, the depressant action is peripheral to the ganglia. While bretylium depressed the end-organ responses to stimulation of all the postganglionic adrenergic nerves of the sympathetic nervous system investigated, it did not depress the function of the postganglionic cholinergic nerves of this system. Thus the production of sweat from the paw of the cat during stimulation of its postganglionic nerve supply continued after administration of the drug. Further, after bretylium had been given in sufficient amounts to prevent the tachycardia caused by stimulation of the cardioaccelerans nerve, stimulation of the latter caused a bradycardia which was resistant to further doses of bretylium but abolished by atropine. The parasympathetic nervous system was unaffected by amounts of bretylium sufficient to depress the sympathetic, but large intravenous doses caused signs of a block in synaptic transmission in parasympathetic and sympathetic ganglia. These effects were brief and occurred only with doses which impaired neuromuscular transmission. No antimuscarine action was detected.

The block of responses mediated by adrenergic nerves was not due to an antagonism of adrenaline or noradrenaline. The effects of these compounds were enhanced after giving bretylium, as they are after sympathectomy. Experiments on the spleen have shown that its adrenergic nerve failed to release noradrenaline after bretylium. This failure might be attributable to an impairment of transmission along the neurone since bretylium could block adrenergic nerve trunks when applied topically. As mentioned in preliminary reports (Boura *et al.*, 1959a and b), bretylium administered systemically does accumulate selectively in adrenergic nerves in sufficient concentrations to impair conduction (Boura, Copp, Duncombe, Green and McCoubrey, unpublished observations). That the finer terminal nerve endings may be more sensitive or accessible than the nerve trunks to the drug is suggested by experiments on the isolated rabbit intestine and uterus and on the perfused rabbit ear. Higher concentrations were needed to cause a local block of nerve trunks than were needed to depress the end-organ response when the bretylium was applied to the whole preparation and consequently gained access to the nerve endings. The cell body of the adrenergic neurone might be expected to be sensitive to bretylium, though experiments in which the drug was given by close arterial injection or into the fluid perfusing the isolated ganglion do not differentiate between effects at ganglionic synapses and effects on adrenergic neurones.

Bretylium also depressed transmission in other nerves when applied locally. Local anaesthesia of long duration was produced by the intracutaneous injection of the drug in guinea-pig and man, though the concentrations needed were higher than those which depressed adrenergic nerves. This correlates with the observation that many local anaesthetics depress conduction in adrenergic nerves (such as vasoconstrictor fibres) in lower concentrations than are needed to depress sensory conduction (Gaddum, 1953). The phrenic nerve of the rat was insensitive to the local application of high concentrations of bretylium, perhaps because the myelin sheath prevents the penetration of the bretylium cation.

The pressor response to stimulating the adrenal medulla, either by splanchnic nerve stimulation or by the intravenous injection of dimethylphenylpiperazinium, was increased rather than impaired by bretylium and this is a further contrast between bretylium and ganglionic blocking agents. The increased response to stimulation of the medulla may be accounted for by an enhancement of the effect of the released adrenaline and noradrenaline. The contrast between the effect of the drug on the adrenal medulla and the effect on adrenergic neurones is in keeping with the contention that the drug affects nerve conduction and not the reactivity of chromaffin tissue in general. In this connexion, it is of interest that, while bretylium did not inhibit the action of dimethylphenylpiperazininum on the adrenal medulla, it antagonized the pressor effects of larger doses of this drug in the adrenalectomized cat and likewise the pressor effect of acetylcholine in the atropinized adrenalectomized cat.

Many of the effects of bretylium on sympathetic function resemble those of choline 2,6-xylyl ether bromide (Hey and Willey, 1954; Exley, 1957; Edge, Mason, and Wyllie, 1957) but, in contrast, bretylium even in near toxic concentrations did not cause parasympathomimetic effects *in vivo*. However, high concentrations of bretylium (200 μg./ml.) might be regarded as muscarine-like in action, for they cause a spasm in isolated strips of guinea-pig ileum which was affected but little by hexamethonium but was antagonized by atropine. This action is less than that of choline 2,6-xylyl ether bromide.

An inhibition of conduction in adrenergic neurones was thought by Hey and Willey (1954) to explain the depressant effects of choline 2,6-xylyl ether bromide on the peripheral sympathetic system. They showed that this drug had a powerful long-lasting local anaesthetic effect, and that injected procaine would inhibit the effect of stimulating the postganglionic cervical sympathetic nerves. However, the apparent failure of the drug to impair action potentials on adrenergic nerves (Exley, 1957), the reductions in the catechol amine content of the rat adrenal medulla found after its administration (Coupland and Exley, 1957; Coupland, 1958) and a reduction in the synthesis of noradrenaline by phaeochromocytoma tissue (Bain and Fielden, 1957) led to the suggestion that choline 2,6-xylyl ether bromide acted by inhibiting the synthesis of noradrenaline in adrenergic nerves. Bretylium does not seem to act specifically in this way (Boura, Green, and McCoubrey, unpublished observations). Its mechanism of action seems to be similar to that first postulated for choline 2,6-xylyl ether bromide by Hey and Willey (1954).

The reduction caused by bretylium in the regression of the effect on the nictitating membrane of the frequency of stimulation applied to its adrenergic nerve supply (Fig. 5) may be of considerable significance. At the low rates of stimulation a small increase in the stimulation rate compensated for the administration of a threshold dose of bretylium (2 to 6 mg./kg.); but at the higher rates a large increase, often beyond normal physiological limits, was required. This situation may allow functional specificity of action on different adrenergic nerves, the impairment being greatest in those subjected to the greatest traffic of impulses. It could be a factor in postural hypotension, the frequency of sympathetic impulses being higher when the animal is tilted towards an erect position. It suggests that supine hypertensive patients show a greater fall in the blood pressure after bretylium than do the normotensive individuals (Boura *et al.*, 1959b) because their sympathetic tone is greater. Similar arguments have been used in relation to the action of the ganglionic blocking agents since, under similar circumstances, they block more readily when the frequency of stimulation is high (Paton, 1951; Green, 1956), but, however, the analogy between the two drugs ends here. Ganglionic blocking agents antagonize acetylcholine competitively at the ganglionic synapses and have a greater effect on high rates of stimulation applied continuously because the acetylcholine released at the ganglion falls during the period of stimulation. Because of this the nictitating membrane of a cat which has received a ganglionic blocking agent may show a characteristic spike contraction during a period of continuous tetanic stimulation of the preganglionic nerve. After bretylium has been given to cats in which the membrane is stimulated through its postganglionic nerve, such spike contractions do not occur. The membrane contraction may be greatly reduced but is fairly well sustained during continuous stimulation.

Cocaine antagonized the effect of bretylium, blocking adrenergic nerves to the isolated rabbit gut and the nictitating membrane of the cat, in doses which increased the sensitivity to adrenaline, noradrenaline, and nerve stimulation. Nasmyth and Andrews (1959) have shown that cocaine antagonizes the adrenergic nerve block caused by choline 2,6-xylyl ether bromide.

Sympathomimetic effects were seen after the administration of large doses of bretylium. They followed rapidly after intravenous injection into

the cat, were brief on the blood pressure and heart rate, but last longer on the nictitating membrane. They occurred in adrenalectomized animals and those with spinal transection. The effect on the nictitating membrane persisted after cutting the postganglionic nerve and could be produced by intra-arterial injection either into the region of the ganglion or into the internal carotid artery. Vasoconstriction occurred after large amounts were injected into the perfused rabbit ear. It is therefore concluded that the stimulatory actions, like the inhibitory effects of bretylium on the sympathetic nervous system, may be due to an action of the drug on the adrenergic neurones.

High concentrations of bretylium caused a curare-like paralysis, and this was the main toxic effect of the drug. In the chick, bretylium caused flaccid paralysis, similar to that which Buttle and Zaimis (1949) found distinguished the action of curare from that of depolarizing drugs. On the other hand, the neuromuscular paralysis produced in the rat diaphragm preparation, in contrast to that caused by curare, was not antagonized by neostigmine.

When bretylium was given orally, the adrenergic neurone blocking action in the cat and the toxic effects in the cat, mouse, and rat occurred only after three to five times the doses which produced these effects by the subcutaneous route. This indicates that only about a third of the dose may be absorbed, a not uncommon proportion with a quaternary ammonium salt. This does not, however, introduce the complication encountered in the clinical use of ganglionic blocking agents. In contrast to them, bretylium lacked the parasympathetic blocking effect of delaying intestinal transport which may result in the accumulation of unabsorbed drug in the intestine and consequent paralytic ileus. No untoward disturbance of gastrointestinal function was found after administration of bretylium in man (Boura *et al.*, 1959b).

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