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ADRENERGIC NEURONE BLOCKADE AND OTHER ACUTE EFFECTS CAUSED BY N-BENZYL-N'N''-DIMETHYLGUANIDINE* AND ITS ORTHO-CHLORO DERIVATIVE

BY

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N-Benzyl-*N'N''*-dimethylguanidine sulphate (BW 467C60) and its *ortho*-chloro derivative (BW 392C60) had adrenergic neurone blocking and sympathomimetic effects resembling those of bretylium and guanethidine in cats, dogs and monkeys, but they were more potent in blocking adrenergic mechanisms in the cat. BW 467C60 was more active than its chloro derivative. Each compound inhibited release of noradrenaline during stimulation of the splenic nerve of cats, and increased smooth muscle responses to adrenaline and noradrenaline. Pressor responses to standard doses of tyramine were also increased except when large doses of BW 467C60 or BW 392C60 were given. The adrenergic neurone block by BW 467C60 was inhibited by dopamine, cocaine and amphetamine in situations in which these amines inhibit the effects of bretylium and guanethidine. In contrast to guanethidine, BW 467C60 and BW 392C60 did not lower the pressor amine content of the iris of cats 24 hr after administration of single doses of the compounds. BW 467C60 depressed the slope of curves relating the frequency of stimuli applied to the cervical sympathetic nerves and the resulting contraction of the nictitating membrane, but the effects of the lower rates of stimulation were preferentially inhibited. Large intravenous doses of BW 467C60 and BW 392C60 blocked autonomic cholinergic mechanisms and caused neuromuscular paralysis of voluntary muscle. These effects were brief, in contrast to the adrenergic neurone blockade. Both BW 467C60 and BW 392C60 were well absorbed from the alimentary tract. In contrast to guanethidine, BW 467C60 did not cause diarrhoea in guinea-pigs.

BW 467C60, *N*-benzyl-*N'N''*-dimethylguanidine sulphate, and BW 392C60, its *ortho*-chloro derivative, are the two most active of a series of benzylguanidines found by Boura, Copp, Green, Hodson, Ruffell, Sim, Walton & Grivsky (1961a) to have powerful adrenergic neurone blocking effects. Chemically, they are related to both bretylium and guanethidine but they are more potent as blocking agents. This paper describes firstly some of the acute pharmacological actions of BW 467C60 and then summarizes analogous observations with BW 392C60.

* Approved name, bethanidine.

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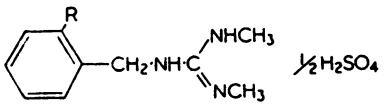
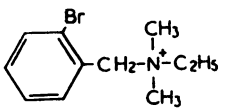
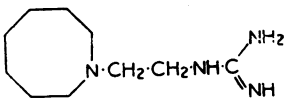


BW 467C60: R=H
BW 392C60: R=Cl



Bretylium



Guanethidine

METHODS

The methods were similar to those of Boura & Green (1959) used in the investigation of bretylium.

Experiments in vitro. The preparation of Finkleman (1930) was used to study the effects of the drugs on the depression by mesenteric nerve stimulation of the pendular movements of isolated rabbit ileum. The nerve was stimulated through platinum electrodes, with supramaximal shocks at 50 shocks/sec for 2 sec periods.

Effects on the motor responses of the isolated vas deferens of guinea-pigs and rabbits to stimulation of the hypogastric nerve were studied by the method of Huković (1961), but using stimuli at 25 instead of 80 shocks/sec to reduce fatigue of the preparation.

Isolated preparations of guinea-pig and rabbit ileum were set up in Tyrode solution containing: 8 g NaCl, 0.2 g KCl, 0.15 g $CaCl_2$, 0.05 g $NaH_2PO_4.2H_2O$, 0.2 g $MgCl_2.6H_2O$, 1 g $NaHCO_3$ and 1 g dextrose per l. For isolated rabbit uterus, the solution was the same except that it contained 0.3 g $CaCl_2$ and 0.4 g $MgCl_2.6H_2O$ per l., and no dextrose. Isolated guinea-pig uterus was suspended in a solution containing 9 g NaCl, 0.42 g KCl, 0.18 g $CaCl_2$, 0.02 g $MgCl_2.6H_2O$, 0.5 g $NaHCO_3$ and 0.5 g dextrose per l. The rat uterus was set up in the solution of Gaddum, Peart & Vogt (1949) and the ductus deferens of guinea-pigs in Krebs-Hemsleit solution (Huković, 1961). These solutions were maintained at 37° C, and except for the Krebs-Hemsleit solution, gassed with a mixture of 95% O_2 and 5% CO_2, were bubbled with pure oxygen.

Rabbit ears were perfused with Tyrode solution at 37° C, and a Thorp impulse counter measured venous outflow. Vasoconstriction was produced by injecting drugs into the lumen of the arterial cannula or by stimulating the greater auricular nerve adjacent to the medial artery.

Actions at the skeletal neuromuscular junction were tested on the rat isolated diaphragm-phrenic nerve preparation (Bülbring, 1946). Rectangular pulses of supramaximal strength and of 0.7 msec duration were applied to the nerve through fluid electrodes at the rate of 5 shocks/min.

Experiments on anaesthetized animals. Anaesthesia of cats was usually induced with ether and maintained with chloralose (about 60 mg/kg of body weight, intravenously). With dogs, pentobarbitone sodium (30 to 40 mg/kg) was injected intravenously. Urethane, 1.6 g/kg intravenously, was the anaesthetic agent used for rabbits. Blood pressure was recorded from a common carotid artery with a mercury manometer. Injections of drugs were made into a cannulated femoral vein. Contractions of the nictitating membrane were recorded by an isotonic frontal-writing lever (magnification ×15, load 7 g). Contractions of the heart were recorded with a Cushny myocardiograph attached to an isometric writing lever which closed a mercury switch, operating a Thorp impulse counter, thereby recording heart rate.

Spleen volumes were recorded by enclosing the spleen in a Perspex oncometer connected to a small float recorder. The flow of blood through the femoral vein was recorded by the method of Lindgren (1958), using a Thorp impulse counter. Changes in bronchial tone in the spinal cat were assessed by the method of Konzett & Rössler (1940).

The effect of BW 467C60 on the noradrenaline content of the venous blood from the spleen of cats during stimulation of the splenic nerve was studied by the method of Brown & Gillespie (1957). The nerve was stimulated with 10 or 25 shocks/sec for 1 min periods. Blood was collected from the splenic vein for 2 min from the start of stimulation. The pressor activity of the plasma was assayed in terms of noradrenaline on the blood pressure of the pithed rat.

The sciatic nerve-gastrocnemius muscle preparation was used with the Brown-Schuster myograph. Supramaximal rectangular pulses of 0.5 or 5 msec duration were applied to the nerve or to the muscle.

Sweat secretion of the paw of the cat was determined by the method of Pontén (1960) in which the amino acids in the sweat, absorbed on to filter paper, are stained with 1% ninhydrin.

The anaesthetized animals were always supine, except in postural hypotension experiments when they were tilted through approximately 85° head-upwards until the blood pressure fall was maximal. Spinal cats were prepared with brief ether anaesthesia.

Experiments with 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 graticule. Gastrointestinal propulsion in the rat was examined by a charcoal meal test (Green, 1959).

Local anaesthesia after intradermal injection into the shaved skin of the flank of the guinea-pig was tested by pricking the skin with a fine needle (Bülbring & Wajda, 1945).

Activity of mice was recorded with individual jiggle cages which were sensitive to limb movements. Each mouse was placed in a jiggle cage for a counting period of 10 min, starting 30 min, 2, 4 or 6 hr after various doses of the drug.

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 had been restricted to milk and water for 24 hr, and were lightly anaesthetized with ether for drug administration.

Pressor amines were extracted from the iris of the cat with 0.1 N HCl. In some experiments (Table 1, experiment 2) the iris was ground with sand in a pestle and mortar. In others (Table 1, experiment 1) a tissue homogenizer was used.

Doses of compounds are usually expressed in terms of the salts. The sulphates of BW 467C60, BW 392C60, guanethidine, atropine and amphetamine, the hydrochlorides of cocaine and tyramine, the acid tartrates of (−)-adrenaline and (−)-noradrenaline, the bromide of acetylcholine, the creatinine sulphate of serotonin, the iodide of dimethylphenylpiperazinium, and the methanesulphonate of phentolamine were used. Doses of histamine and nicotine are given as base.

RESULTS

Compound BW 467C60

Effects on the peripheral sympathetic nervous system

Rabbit ileum. The inhibition of the pendular movements of isolated rabbit ileum produced by stimulation of the visceral nerve was abolished by BW 467C60 in concentrations of 1 to 3 μg/ml. Weak responses returned about 30 min after washing. Higher concentrations of BW 467C60 (over 10 μg/ml.) were required to block the response to nerve stimulation when dopamine (10 μg/ml.) had been added to the organ bath. Cocaine (3 μg/ml.) restored the responses to nerve stimulation when they had been inhibited in the presence of 3 to 10 μg/ml. of BW 467C60.

Ductus deferens. The contraction of the guinea-pig ductus deferens produced by postganglionic nerve stimulation was reduced by 1 μg/ml. of BW 467C60 and abolished by 3 μg/ml. Since Huković (1960) has related the adrenergic neurone

block by bretylium on isolated atrial preparations to antagonism of the "nicotine-like" effect of acetylcholine that persists in the presence of atropine, we investigated the action of BW 467C60 on the responses of the ductus deferens preparation to acetylcholine. In the presence of 1 μg/ml. of atropine, BW 467C60 inhibited responses to nerve stimulation without antagonizing the effects of 500 μg/ml. of acetylcholine (Fig. 1). The responses to this high concentration of acetylcholine

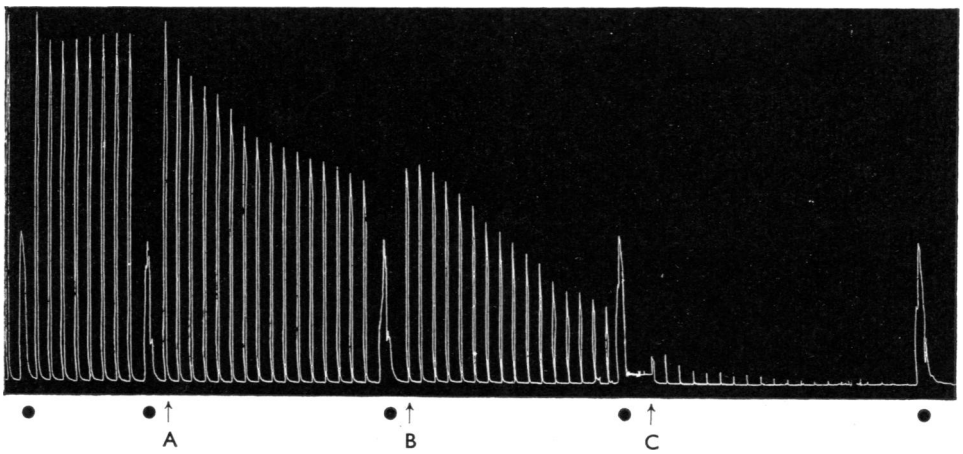


Fig. 1. Contractions of guinea-pig isolated ductus deferens preparation in the presence of atropine (1 μg/ml.). Those marked ● followed acetylcholine (500 μg/ml.), but all others were in response to supramaximal stimulation of the hypogastric nerve at 25 shocks/sec for 2 sec every min. At A, 3 μg/ml. of BW 467C60 was added to the bath, and at B, the concentration was increased to 10 μg/ml. At C the preparation was washed.

may be muscarinic, despite the presence of atropine, since they were reduced by increasing the atropine concentration. In preparations without atropine, near-maximal contractions were produced by acetylcholine (0.1 μg/ml.).

Nictitating membrane. BW 467C60 abolished the contractions of the cat nictitating membrane on stimulation of either the pre- or post-ganglionic cervical sympathetic nerves, but increased the responses to adrenaline and noradrenaline. Submaximal doses of the compound depressed the slope of the regression line relating the frequency of pre- or post-ganglionic nerve stimulation to the resulting contractions in each of three separate experiments, but the doses caused a relatively greater inhibition with low compared to high rates of stimuli (Fig. 2). Similar depressions of slope were observed with two dogs, the depression being small after 0.1 mg/kg of BW 467C60 and almost complete after 3 mg/kg.

With both dogs and cats, intravenous injections of 3 to 10 mg/kg of BW 467C60 caused well-sustained contractions of the nictitating membranes. In cats, these contractions were small and did not exceed 30% of the maximum normally obtained with nerve stimulation, but near-maximal contractions occurred with 10 mg/kg in dogs. The contractions by BW 467C60 in the cat were abolished by phentolamine (1 to 5 mg/kg).

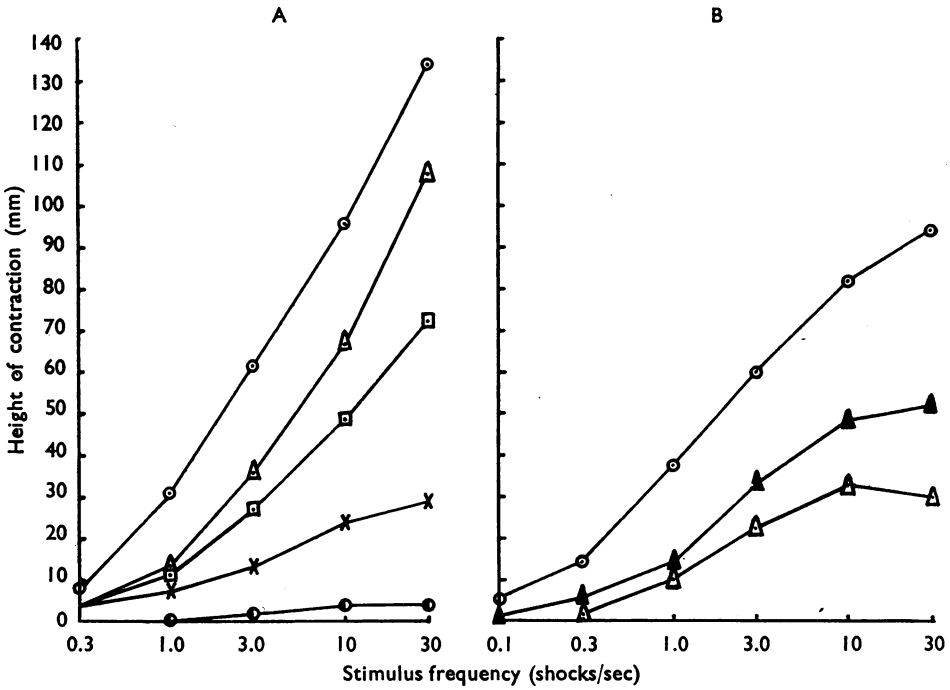


Fig. 2. Mean heights of contraction of the nictitating membranes of cats caused by stimulation of the pre- or post-ganglionic cervical sympathetic nerve at various stimulus frequencies for 1 min periods. A, effects of intravenous BW 467C60 in an acute experiment using chloralose as the anaesthetic agent and post-ganglionic stimulation: O —— O initial; Δ —— Δ after 0.1 mg/kg; □ —— □ after 0.3 mg/kg; x —— x after 1 mg/kg of BW 467C60. B, responses to pre-ganglionic stimulation 24 hr after subcutaneous injection of various doses of BW 467C60 into cats anaesthetized with pentobarbitone: O —— O controls (eleven cats); ▲ —— ▲ after 0.5 mg/kg (three cats); Δ —— Δ after 1 mg/kg of BW 467C60 (three cats). There was no response of the membranes 24 hr after 3 mg/kg of BW 467C60 (two cats).

The inhibition of the nictitating membrane responses by BW 467C60 seemed slightly less when the preganglionic nerve compared with the postganglionic nerve was stimulated in two cats.

When the drug was given subcutaneously or orally to cats, doses of 1.25 mg/kg partially, and 2.5 mg/kg fully, relaxed the nictitating membrane (Fig. 3, A). Although the action was slightly more rapid with subcutaneous than with oral dosage, the degree of relaxation was no greater. Some relaxation of the membrane persisted for 2 to 3 days. Responses of the nictitating membrane to preganglionic nerve stimulation in cats were impaired 24 hr after 1 mg/kg, and were absent after 3 mg/kg of BW 467C60. Similar relaxation was caused by rather higher doses of guanethidine (5 to 10 mg/kg subcutaneously or 10 to 20 mg/kg orally) (Fig. 3, B). Oral doses of BW 467C60 (5 mg/kg) relaxed the nictitating membrane of dogs (lower doses were not tested).

Since the adrenergic neurone block by bretylium, guanethidine and xylocholine is antagonized by (+)-amphetamine (Day, 1962), the effect of previous administration

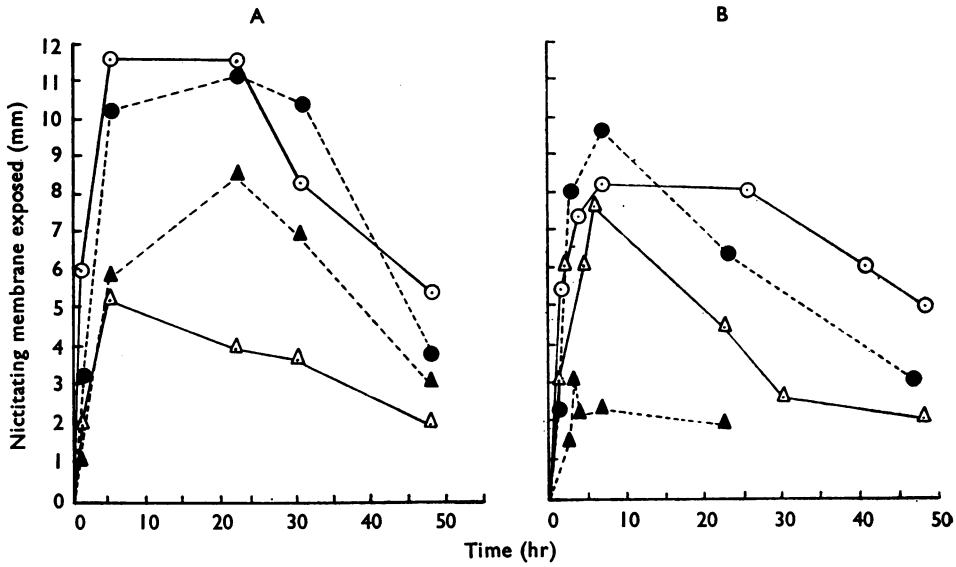


Fig. 3. Relaxation of the nictitating membrane in the unanaesthetized cat. The values are means for groups of five cats. A, Following BW 467C60: △——△ 1.25 mg/kg subcutaneously; ○——○ 2.5 mg/kg subcutaneously; ▲---▲ 1.25 mg/kg orally; ●---● 2.5 mg/kg orally. B, Following guanethidine: △——△ 5 mg/kg subcutaneously; ○——○ 10 mg/kg subcutaneously; ▲---▲ 10 mg/kg orally; ●---● 20 mg/kg orally. Values of 8 mm are maximal for some cats.

of amphetamine on block by BW 467C60 was investigated. Two anaesthetized cats showed partially contracted nictitating membranes 3 hr after receiving a total of 2.5 mg/kg of amphetamine. In each cat, 6 mg/kg of BW 467C60 then produced negligible inhibition of the membrane responses to preganglionic nerve stimulation at 1 to 10 shocks/sec and, even after a total of 12 mg/kg of BW 467C60 in one animal, a moderate response to nerve stimulation persisted. In the absence of amphetamine, 3 mg/kg of BW 467C60 abolished responses to nerve stimulation.

Cardiovascular effects

Cats. The blood pressure of supine cats anaesthetized with chloralose fell rapidly after intravenous doses of 0.3 or 1.0 mg/kg of BW 467C60, the fall with 1 mg/kg varying between 20 and 60 mm Hg and lasting for over 1 hr. In each of two cats, further doses of 1 mg/kg raised the carotid arterial blood pressure by 20 to 50 mm Hg, and caused tachycardia. The pressor effects passed off in 10 min. When the dosage of BW 467C60 was increased to 3 or 10 mg/kg the pressor responses and the tachycardia were no greater. This is in contrast with the increased responses of the nictitating membranes in cats and the increased pressor responses in dogs on elevation of dosage. Pressor responses to 1 mg/kg of BW 467C60 in the cat were abolished after giving phentolamine (1 to 5 mg/kg, intravenously).

BW 467C60 caused a rapid and persistent depression of various cardiovascular reflexes and responses to adrenergic nerve stimulation. Thus in each of two cats,

one anaesthetized with chloralose and the other with nitrous oxide, doses of 0.3 and 1 mg/kg caused marked postural hypotension. Similar doses also reduced the pressor responses to carotid occlusion, and the tachycardia normally following stimulation of the nervi accelerantes was converted to bradycardia. This bradycardia and that caused by stimulating the vagus nerve were abolished temporarily by 10 mg/kg of the drug. Vasoconstrictor responses produced in the hind limb of the cat on stimulation of the peripheral end of the cut sympathetic chain were decreased by intravenous doses of 0.1 to 0.5 mg/kg of BW 467C60. When vasoconstrictor action had been abolished by 1 mg/kg, sympathetic nerve stimulation increased the blood flow which was antagonized by 1 mg/kg of atropine sulphate. Vasoconstriction followed rapidly on injection of 1 mg/kg of BW 467C60.

Adrenaline, noradrenaline and sometimes dimethylphenylpiperazinium caused greater pressor effects after responses to stimulation of sympathetic nerves had been blocked by BW 467C60, but the pressor effect of 200 μg of tyramine was decreased by 3 to 10 mg/kg of BW 467C60 (Fig. 4).

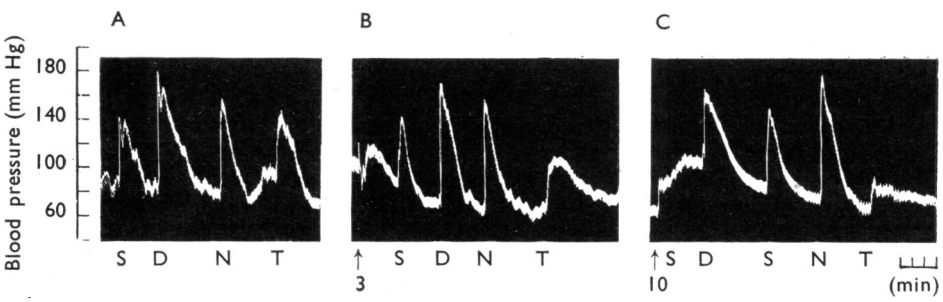


Fig. 4. Record of the blood pressure in the carotid artery of a cat anaesthetized with chloralose. At S, stimulation of the greater splanchnic nerve with supramaximal rectangular pulses at 50 shocks/sec for 20 sec; at D, 20 μg of dimethylphenylpiperazinium iodide; at N, 3 μg of noradrenaline; at T, 200 μg of tyramine hydrochloride. At "3" and "10": 3 and 10 mg/kg of BW 467C60 respectively. Between A and B 10 min, between B and C 30 min. Each drug was given intravenously. For further explanation see text.

Dogs. In three dogs anaesthetized with sodium pentobarbitone, BW 467C60 injected intravenously produced pressor effects lasting 10 to 30 min. The rise in carotid arterial blood pressure with 1 mg/kg varied between 15 and 60 mm Hg and that with 3 mg/kg between 70 and 110 mm Hg. A brief fall in blood pressure sometimes preceded the rise when doses of 3 mg/kg or more were injected. Tachycardia following BW 467C60 lasted longer than the elevation of blood pressure. Doses of 0.3 to 1.0 mg/kg caused postural hypotension and greatly reduced the pressor response to carotid occlusion (Fig. 5).

The pressor effects produced by intravenous injections of adrenaline or noradrenaline were augmented within 10 min of intravenous doses of 1, 3 and 10 mg/kg of BW 467C60. The pressor action of 5 mg of tyramine was increased by doses of 0.3 to 1 mg/kg, was reduced after 3 mg/kg and was abolished after 10 mg/kg of BW 467C60. In each of two dogs the initial pressor response to dimethylphenyl-

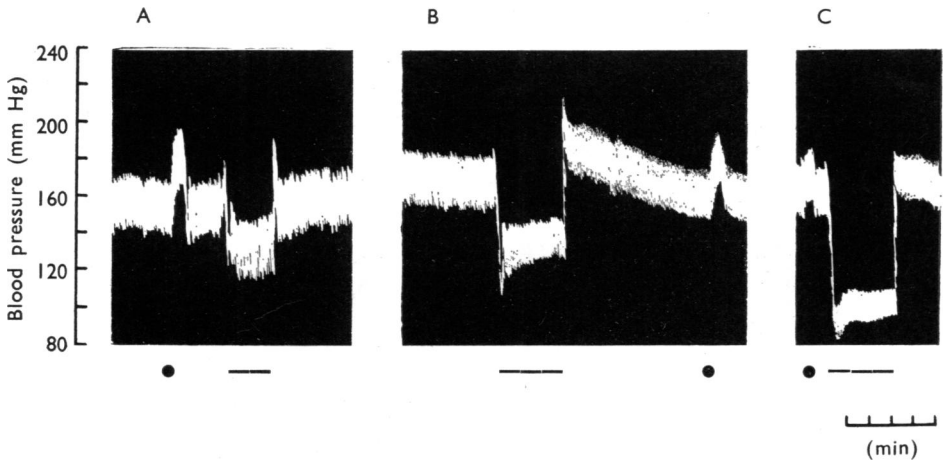


Fig. 5. Blood pressure in the left carotid artery of a dog anaesthetized with pentobarbitone sodium. The dog was supine except when tilted through 85° for the periods indicated by the lines below the records. ● =occlusion of right carotid artery for 1 min. A, before drug; B, 15 min after 1 mg/kg of BW 467C60 intravenously; C, 75 min after 3 mg/kg of BW 467C60 intravenously. The drug caused marked postural hypotension and inhibited the pressor response to carotid occlusion.

piperazinium was decreased, but the second part of the response was increased. In one dog after 4 mg/kg of BW 467C60, injection of adrenaline or noradrenaline slowed the heart rate, but after 1 mg/kg of atropine intravenously, each amine caused tachycardia.

Monkey. In a supine monkey anaesthetized with sodium pentobarbitone, intravenous doses of 1, 3 and 10 mg/kg of BW 467C60 caused brief falls of blood pressure (10 to 30 mm Hg) without change in heart rate. Pressor responses to adrenaline, noradrenaline and dimethylphenylpiperazinium increased. The pressor effect of 10 mg of tyramine was also increased by doses up to 4 mg/kg of BW 467C60 but was reduced after 10 mg/kg.

Rabbit. In rabbits anaesthetized with urethane, 1 and 3 mg/kg of BW 467C60 reduced the blood pressure by about 30 and 40 mm Hg respectively, but smaller and briefer falls of blood pressure occurred after 10 mg/kg. The pressor effects of adrenaline (10 to 20 μg), noradrenaline (30 to 60 μg), and tyramine (0.3 to 3 mg) and the depressor effect of dopamine were increased after 1 mg/kg of BW 467C60. The responses to adrenaline and noradrenaline, however, became greater when the dose of BW 467C60 was increased to 3 to 10 mg/kg, but the augmented pressor effect of 0.3 or 1 mg/kg of tyramine was diminished.

Vasoconstriction, elicited by supramaximal stimulation of the greater auricular nerve of the perfused rabbit ear, was inhibited by 30 to 100 μg of BW 467C60 injected into the arterial perfusion fluid and slight vasodilation was seen. In contrast, the vasoconstrictor effect of 1 μg of noradrenaline was increased after 100 to 300 μg of BW 467C60. Vasoconstriction, lasting from 2 to 5 min, followed the injection of more than 300 μg of BW 467C60.

The spleen and its noradrenaline output

Contractions of the spleen in response to splenic nerve stimulation were almost abolished after intravenous injection of 0.3 mg/kg of BW 467C60, the inhibitory action being greater at 30 min (Fig. 6, C) than immediately following injection (Fig. 6, B). The percentage inhibitions of the contractions were about the same at all frequencies of nerve stimulation for the one cat tested. In each of two anaesthetized

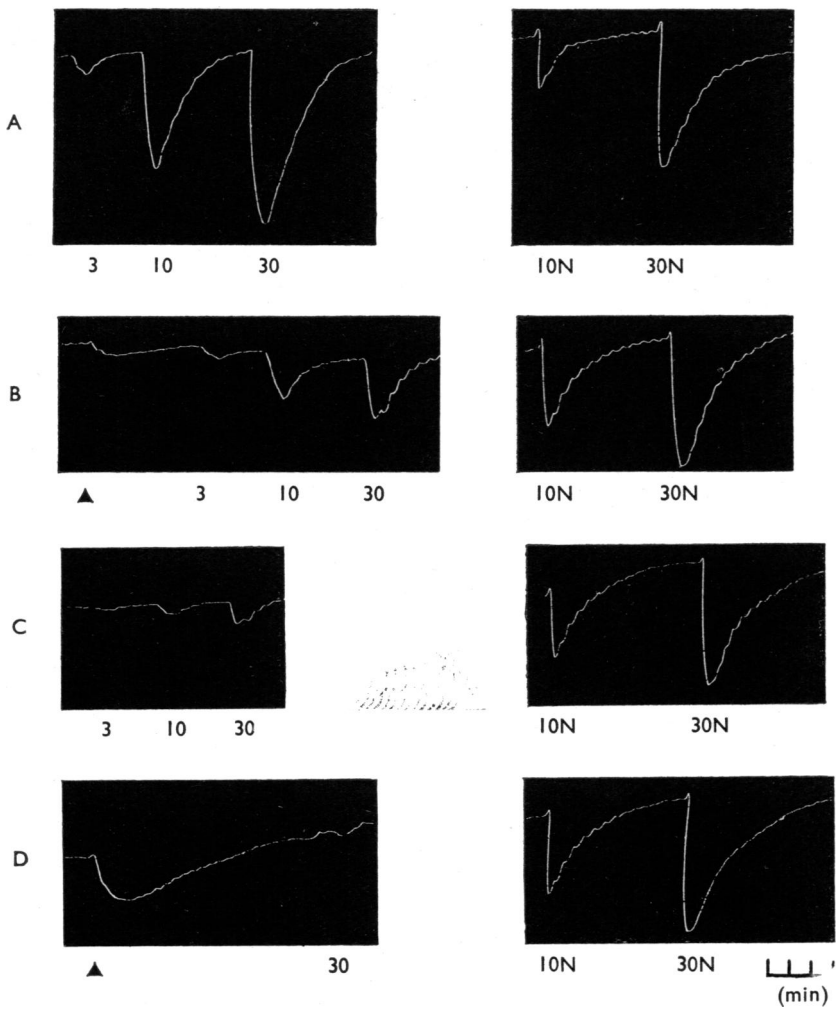


Fig. 6. Contractions of the spleen of a cat anaesthetized with chloralose. The splenic nerve was stimulated for 30 sec with rectangular supramaximal shocks at 3, 10 and 30 shocks/sec at " 3," " 10 " and " 30 " respectively. Approximately 5 min later, responses to 10 μg and 30 μg of noradrenaline intravenously were determined, at " 10N " and " 30N " respectively. A, before drug; B, early effect of 0.3 mg/kg of BW 467C60 intravenously at ▲ ; C, responses 30 min later; D, effect of 10 mg/kg of BW 467C60 at ▲. BW 467C60 caused small temporary contractions of the spleen, inhibited contractions following stimulation of the splenic nerve and increased contractions due to noradrenaline.

cats, the release of noradrenaline (assayed on rat blood pressure) into the venous effluent from the spleen, following stimulation of the splenic nerve at 25 shocks/sec for 1 min or 15 sec, was reduced from a control value between 300 and 800 pg/stimulus to 1/10 of that value 15 min after administration of 0.3 mg/kg of BW 467C60, which was approximately the amount released in the absence of stimulation.

Adrenal gland

In an adrenalectomized cat, the rapid pressor responses on stimulation of the peripheral end of the cut splanchnic nerve (10 shocks/sec for 30 sec) or after the intravenous injection of 30 μg of dimethylphenylpiperazinium iodide were abolished for over 1 hr by 0.3 to 0.5 mg/kg of BW 467C60 (Fig. 7). Although in cats with

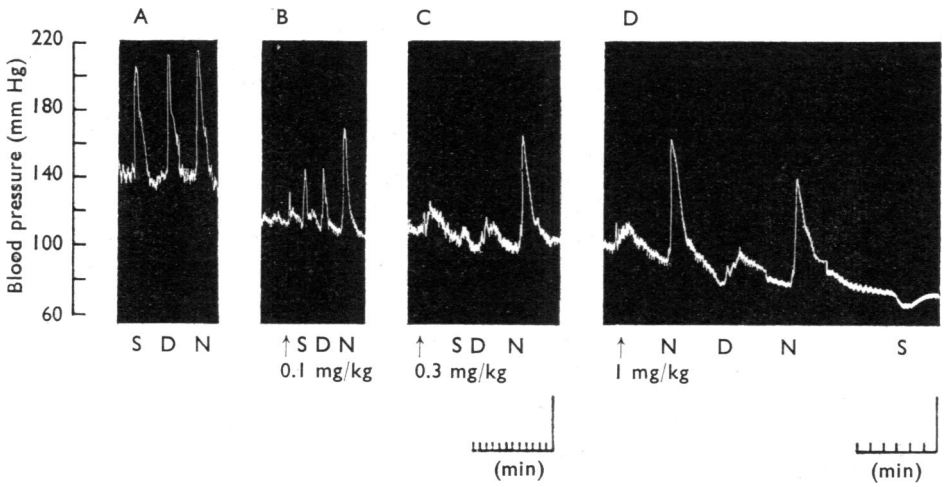


Fig. 7. Record of the blood pressure of an adrenalectomized cat anaesthetized with chloralose. The greater splanchnic nerve was stimulated with supramaximal shocks at 10 shocks/sec for 30 sec at S. Noradrenaline (2 μg) was injected intravenously at N, and dimethylphenylpiperazinium (30 μg) at D. A, control responses. B, C and D, intravenous doses of 0.1, 0.3 and 1 mg/kg of BW 467C60 respectively cause progressive diminution of the responses to splanchnic nerve stimulation and to dimethylphenylpiperazinium but not of those to noradrenaline. Between B and C, 65 min; between C and D, 12 min.

adrenals these doses did not reduce the peak heights of the pressor responses, the initial rapid component of the responses to splanchnic nerve stimulation or to injection of dimethylphenylpiperazinium were abolished, but the later pressor component remained (Fig. 4). The later response was depressed only by 3 to 30 mg/kg of BW 467C60, and then only for 11 to 15 min.

Sweat glands

Whereas piloerection from stimulation of the peripheral end of the cut sympathetic chain was abolished for at least 30 min by 0.5 mg/kg of BW 467C60, sweat secretion from the cat paw following the same stimuli was unaffected by 1 mg/kg of BW 467C60 and was reduced for 10 to 15 min by doses of 3 or 10 mg/kg.

Effects on tissue pressor amines

Guanethidine lowers the pressor amine content of the heart and spleen in rats, cats and rabbits (Sheppard & Zimmerman, 1959 ; Cass, Kuntzman & Brodie, 1960 ; Brodie & Kuntzman, 1960). Table 1 shows that the iris of cats which had received 10 mg/kg of guanethidine subcutaneously 24 hr earlier contained less pressor activity than controls. If the superior cervical ganglion on one side had been removed under short ether anaesthesia then the loss of pressor activity was about the same for the denervated and the innervated iris. In contrast, the iris of cats given 3, 10 or 30 mg/kg of BW 467C60 showed no fall of pressor amine activity 24 hr later. However, when doses of 10 mg/kg of BW 467C60 were administered daily to cats for 6 months, the pressor amine content of the hearts and spleens does fall (Boura *et al.*, 1961a).

TABLE 1

PRESSOR AMINE ACTIVITIES IN THE CAT IRIS 24 HR AFTER SUBCUTANEOUS INJECTION OF GUANETHIDINE, BW 467C60 OR BW 392C60

The pressor activities were assayed in terms of noradrenaline on rat blood pressure. In experiment 1 the superior cervical ganglion was removed on one side just before giving guanethidine except in the cat preparation marked with an asterisk. The values in experiment 2 are the means for the two irises. A modified extraction procedure was used in experiment 2 and this might account for the higher control values

	Noradrenaline equivalent (μg/g of tissue)				
	Individual cats				Mean
Experiment 1					
Controls Innervated	2·7	3·9	2·9*		3·2
Controls Denervated	3·0	5·6	—		4·3
Guanethidine (10 mg/kg) Innervated	0·7	1·4	2·1	4·1	2·1
Guanethidine (10 mg/kg) Denervated	1·5	1·5	1·1	2·7	1·7
Experiment 2					
Controls	5·4	6·2	8·9		6·8
BW 467C60 3 mg/kg	9·0	12·8			10·9
BW 467C60 10 mg/kg	3·7	5·5	6·1	7·7	5·8
BW 467C60 30 mg/kg	8·6	9·3			9·0
BW 392C60 10 mg/kg	8·4	9·6	12·6		10·2
BW 392C60 30 mg/kg	7·9	8·1			8·0

Parasympathetic nervous system

There were no effects attributable to an action on the peripheral parasympathetic nervous system except with large doses of BW 467C60. Large, near toxic, intravenous doses had a number of transitory effects resembling those produced by ganglion blocking agents. In cats which had received 15 mg/kg intravenously, the pupil dilated and its response to light was abolished for a few minutes. No mydriasis was seen after 50 mg/kg subcutaneously in cats, 50 mg/kg intraperitoneally in mice, or 100 mg/kg orally in dogs. Intravenous injection of 10 mg/kg into cats anaesthetized with chloralose reduced the bradycardia caused by stimulating the distal end of the cut vagus, but only for a few minutes.

A concentration of 300 μg/ml. of BW 467C60 decreased the sensitivity to acetylcholine of the guinea-pig isolated ileum preparation by approximately 10-fold. Concentrations of 3 to 30 μg/ml. inhibited the peristaltic reflex of the guinea-pig isolated ileum and depressed the sensitivity to acetylcholine and to nicotine (10 μg/ml.).

Alimentary tract

Gastro-intestinal propulsion, measured by the charcoal meal test of Green (1959) with fasted rats, was slower 20 to 30 min after subcutaneous injection of 10 mg/kg of BW 467C60 than in controls. The rate of propulsion 4 to 6 hr after the drug usually exceeded that in control rats. In one experiment both BW 467C60 and guanethidine were tested. Guanethidine caused a rather greater delay in gastro-intestinal propulsion than did BW 467C60, when the delay was measured soon after drug administration (Table 2). Results similar to those in Table 2 with BW 467C60 were found in two other tests.

TABLE 2

PERCENTAGE OF TOTAL LENGTHS OF SMALL INTESTINE TRAVERSED BY CHARCOAL IN 5 MIN IN FASTED RATS GIVEN BW 467C60 OR GUANETHIDINE SUBCUTANEOUSLY, 20 MIN, 2 HR OR 4 HR EARLIER

Means for groups of five rats. Only the value indicated by an asterisk was significantly different from the control ($P=0{\cdot}05$)

Interval between drug treatment and charcoal administration	BW 467C60 (10 mg/kg)	Guanethidine (10 mg/kg)	Controls
20 min	20	5*	38
2 hr	21	20	35
4 hr	49	30	31

Diarrhoea did not follow 1, 3 or 10 mg/kg of BW 467C60 administered subcutaneously to guinea-pigs. After 30 mg/kg, there was slight diarrhoea after 3 hr in 1 of 5 guinea-pigs. In contrast, 4 of 5 guinea-pigs showed diarrhoea within 2 to 3 hr of injecting 3 mg/kg of guanethidine, and with 10 mg/kg the diarrhoea was severe within 1 to 2 hr in each of 5 animals.

Neuromuscular block

Contractions of the rat isolated diaphragm in response to supramaximal stimulation of the phrenic nerve at 5 shocks/min were abolished by 2 mg/ml. of BW 467C60, but those in response to direct muscle stimulation remained. Application of a concentration of 10 mg/ml. of BW 467C60 restricted to the phrenic nerve did not modify the muscle twitch in response to indirect stimulation centrally to the site of application.

Toxicity

In cats anaesthetized with chloralose, respiratory paralysis and abolition of the response of the gastrocnemius muscle to indirect stimulation, but not to direct stimulation, occurred concomitantly after intravenous doses of 10 to 20 mg/kg of BW 467C60. Neuromuscular block seemed also to be the main toxic action in unanaesthetized cats given 10 to 15 mg/kg of BW 467C60 intravenously. These amounts caused brief respiratory arrest but were not lethal if artificial respiration was given for a few minutes. No toxic effect occurred with 50 mg/kg subcutaneously into cats, but 100 mg/kg by this route caused severe muscle weakness and incoordination. Brief respiratory failure and paralysis of the indirectly stimulated gastrocnemius muscle also occurred in an anaesthetized monkey given 10 or 20

mg/kg of BW 467C60 intravenously, but oral doses of 50 mg/kg were well tolerated in unanaesthetized monkeys. Anaesthetized dogs showed brief depression of breathing with 10 to 20 mg/kg intravenously, but no toxic effect occurred in unanaesthetized dogs given oral doses of 100 mg/kg. In albino mice, the LD50 of BW 467C60 was approximately 12 mg/kg intravenously, 150 mg/kg intraperitoneally, 260 mg/kg subcutaneously and 520 mg/kg when the drug was given by stomach tube. In chicks, 50 mg/kg intravenously caused temporary flaccid paralysis resembling that with tubocurarine (Buttle & Zaimis, 1949), while 100 mg/kg was lethal.

Sensory nerves

A 10 mg/ml. solution of BW 467C60 injected intradermally into guinea-pigs caused local anaesthesia lasting more than 5 but less than 24 hr. A 50 mg/ml. solution instilled into the conjunctival sac in rabbits caused corneal anaesthesia lasting over 2 hr.

Central nervous system

The activity of mice in jiggle cages was not significantly changed by doses of 50 mg/kg of BW 467C60 given orally ($P>0.05$), but was decreased after an intraperitoneal dose of 50 mg/kg ($P=0.03$) or an oral dose of 200 mg/kg ($P<0.01$). An oral dose of 25 mg/kg did not modify the depressant action of 2 mg/kg of reserpine given intraperitoneally 18 hr earlier ($P>0.1$). No behavioural change has been apparent in animals receiving large amounts daily: cats, 50 mg/kg subcutaneously; dogs, 100 mg/kg orally; and monkeys, 50 mg/kg orally. Prolongation of the hypnosis after 60 mg/kg of sodium pentobarbitone given intravenously to mice occurred when 12.5 mg/kg but not when 6.25 mg/kg of BW 467C60 was injected intravenously ($P<0.01$, $P=0.5$ respectively). No analgesia was caused by 50 mg/kg subcutaneously in mice.

Other effects

Concentrations of 3 μg/ml. of BW 467C60 increased the response of the rabbit isolated vas deferens preparation to adrenaline and noradrenaline (Fig. 8) and increased contractions of rabbit isolated uterus to adrenaline (10 ng/ml.), to noradrenaline (30 ng/ml.) or to acetylcholine (100 ng/ml.). A concentration of 10 μg/ml. of BW 467C60 increased the responses of the latter preparation to potassium chloride (1 mg/ml.) and to barium chloride (1 mg/ml.).

High concentrations of BW 467C60 opposed the spasmogenic action of various substances on isolated smooth muscle preparations. For instance, a concentration of 300 μg/ml. decreased the sensitivity of guinea-pig isolated ileum both to acetylcholine and to histamine by approximately 10-fold. In each instance the log dose/response curves showed a shift to the right but remained parallel to the control curve. Similarly, high concentrations of the compound (100 to 300 μg/ml.) depressed the spasmogenic effects of 5-hydroxytryptamine (0.2 μg/ml.) on rat isolated uterus and of adrenaline (0.2 μg/ml.) on rabbit isolated uterus.

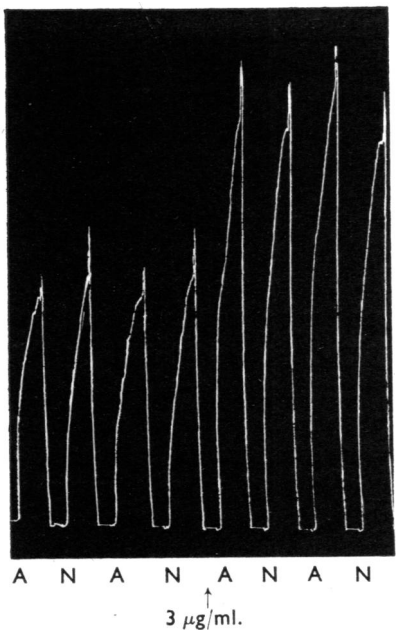


Fig. 8. Contractions of the isolated vas deferens of the rabbit caused by 5 μg/ml. of noradrenaline (at N) and by 3 μg/ml. of adrenaline (at A) were increased after adding 3 μg/ml. of BW 467C60 to the bath.

No oxytocic action with concentrations up to 300 μg/ml. of BW 467C60 was seen with the isolated uteri of the rat and guinea-pig, but increased motility occurred in 2 of 4 rabbit uteri exposed to 100 or 300 μg/ml. The vasodepressor effect of histamine in an anaesthetized dog was but little affected by 3 or 10 mg/kg of BW 467C60, but the bronchoconstrictor action of 30 μg of histamine in a spinal cat was abolished by 30 to 100 mg/kg.

Dr G. A. Stewart found that the blood sugar concentration of fasted guinea-pigs showed no change within 8 hr of administration of up to 250 mg/kg of BW 467C60 by stomach tube, and that oral doses of 500 mg/kg were toxic.

Compound BW 392C60

The effects of BW 392C60 on peripheral adrenergic nerve mechanisms were similar to those of BW 467C60. Similar concentrations antagonized the inhibitory effect of visceral nerve stimulation on the pendular movements of rabbit isolated ileum (approximately 1 μg/ml.) and the constrictor response to stimulation of the greater auricular nerve to the rabbit perfused ear (10 to 30 μg injected into the lumen of the arterial cannula). Likewise, in anaesthetized cats 0.3 to 1 mg/kg of BW 392C60 lowered the blood pressure, caused postural hypotension and inhibited (a) the vasopressor response to carotid arterial occlusion, (b) the rapid component in the vasopressor response to splanchnic nerve stimulation, (c) contractions of the nictitating membrane caused by stimulation of either the pre- or post-ganglionic cervical sympathetic nerve, and (d) piloerection caused by stimulation of the caudal end

of the cut sympathetic chain. The vasopressor response to carotid arterial occlusion was also inhibited in dogs and monkeys. Vasopressor responses and tachycardia similar to those described for BW 467C60 occurred after doses of 1 to 3 mg/kg in each of three cats and in each of two dogs. Doses of 3 mg/kg or more contracted the nictitating membrane in cats and dogs. After blockade of adrenergic nerves by 1 to 3 mg/kg of BW 392C60 in cats, dogs and monkeys, the pressor responses to adrenaline, noradrenaline, dimethylphenylpiperazinium iodide and tyramine, in doses similar to those used in testing BW 467C60, were augmented but, when the dose of BW 392C60 rose to 3 to 10 mg/kg, the responses to tyramine, but not those to the other pressor agents, decreased.

Except in high dosage BW 392C60 produced little effect on cholinergic nerve function. Sweat secretion from the paw of the cat caused by stimulation of the peripheral end of the cut sympathetic chain and the vasodepressor effect of peripheral stimulation of the vagus nerve were reduced for 10 to 15 min by 3 to 10 mg/kg of BW 392C60. The peristaltic reflex of guinea-pig isolated ileum was depressed by 10 μg/ml. and abolished by 30 μg/ml. Local anaesthesia, lasting over 3 hr, followed intradermal injection of a solution of 3 mg/ml. into guinea-pigs. Anaesthesia of the cornea lasting about 3 hr followed instillation of a solution of 50 mg/ml. into the conjunctival sac of rabbits.

The above tests were inadequate in number to distinguish between the potencies of BW 392C60 and BW 467C60, but comparison of their relaxant actions on the nictitating membranes of unanaesthetized cats showed that by the subcutaneous and oral routes the activity of BW 392C60 was about half that of BW 467C60. Also in contrast to BW 467C60, BW 392C60 depressed the slopes of curves relating stimulus frequency to the nictitating membrane contractions produced without preferential impairment of responses to low rates of stimulation (three experiments).

Intravenous doses similar to those of BW 467C60 (10 mg/kg or more) caused respiratory paralysis in mice and in anaesthetized cats and dogs, and in the cat this was accompanied by inhibition of the response of the gastrocnemius muscle to stimulation of the sciatic nerve. Subcutaneous doses of 50 mg/kg were well tolerated by cats, monkeys and mice (LD50 in mice, 150 mg/kg), no effects other than those attributable to adrenergic neurone blockade being observed. The activity counts of mice in jiggle cages were not appreciably changed by BW 392C60 except when the dose was raised to 75 to 150 mg/kg, given by stomach tube (LD50, 350 mg/kg).

DISCUSSION

These results show that the benzylguanidines BW 467C60 and BW 392C60 block smooth muscle responses to adrenergic nerve stimulation in a variety of preparations without antagonizing the effects of adrenaline or noradrenaline. Experiments using the cat spleen indicate that the block is due to inhibition of release of transmitter. The compounds also cause early but brief sympathomimetic effects. These properties of BW 467C60 are similar to those of bretylium (Boura & Green, 1959) and of guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960b ; Maxwell, Plummer, Povalski & Schneider, 1960a ; Hertting, Axelrod & Patrick, 1962). BW 467C60 is the most active of these compounds ; equivalent oral doses, required in the

cat to relax the nictitating membrane, of BW 467C60, guanethidine and bretylium are related approximately as 1 : 7 : 20.

Sympathomimetic effects of BW 467C60 and BW 392C60 include a rise of blood pressure, tachycardia, vasoconstriction in the hind limb, piloerection and contraction of the spleen and nictitating membrane. Their greater prominence is expected when sympathetic tone is low ; the pressor effects following intravenous injection were greater in dogs anaesthetized with pentobarbitone sodium than in cats with chloralose. These responses, like those due to bretylium or guanethidine, may be due to the release of sympathomimetic amines, since phentolamine abolished the pressor action and nictitating membrane contraction due to BW 467C60 in the cat. Rabbits anaesthetized with urethane showed only a depressor response to BW 467C60, as also occurs following guanethidine (Kadzielawa, 1962).

The inhibition of sympathetically mediated cardiovascular reflexes and the hypotension after BW 467C60 are probably due to adrenergic neurone blockade, since these effects occurred in cats at a time when the vasoconstrictor response in the hind limb to electrical stimulation of the peripheral end of the sympathetic chain and the tachycardia caused by stimulation of the cardioaccelerans nerve were inhibited. All the several peripheral sympathetic adrenergic nerves investigated in cats and dogs were blocked by similar doses of BW 467C60, suggesting that, as with bretylium and guanethidine, there is little specificity of action on different adrenergic mechanisms. Manifestations of adrenergic block can be masked, especially when dosage is high, by early sympathetic effects but, even when this masking is trivial (as in experiments using the spleen), the blocking effect of an intravenous dose may reach its peak only after about 30 min. This observation likewise parallels previous findings for bretylium and guanethidine. Relaxation of the nictitating membrane of unanaesthetized animals shows that the duration of adrenergic neurone blockade caused by single doses of BW 467C60 in cats was similar to that following equivalent doses of guanethidine.

Experiments using compounds that antagonize the adrenergic neurone block by BW 467C60 indicate that the drug may act in a similar way to xylocholine, bretylium and guanethidine. Thus the suppression by BW 467C60 of the inhibitory effects of visceral nerve stimulation on the pendular movements of rabbit isolated ileum was less in the presence of dopamine, as are similar actions of xylocholine (Bain, 1960), bretylium (Green, 1960b ; Day, 1962) and guanethidine (Green, 1962 ; Day, 1962). Similarly, in this preparation cocaine antagonized the blocking effect of BW 467C60 as also it antagonizes the blocking effect of the other three compounds (Nasmyth & Andrews, 1959 ; Boura & Green, 1959 ; Day, 1962). Furthermore, amphetamine antagonized the inhibitory action of intravenous BW 467C60 on responses of the nictitating membrane of cats to sympathetic nerve stimulation, as also it antagonizes the corresponding effects of xylocholine, bretylium and guanethidine (Day, 1962).

The increased sensitivity of smooth muscle in several preparations to adrenaline and noradrenaline, when sufficient BW 467C60 and BW 392C60 had been given to block adrenergic nerves, was comparable to that following bretylium (Boura & Green, 1959) and guanethidine (Maxwell *et al.*, 1960a and b). If bretylium or guanethidine are given daily to cats, the sensitivity of the nictitating membrane

becomes as great as it does after a similar period of postganglionic sympathetic nerve section (Green, 1960a ; Boura & Green, 1962), and similar changes follow daily administration of BW 467C60 (unpublished observations). The finding that pressor responses to tyramine were increased after giving sufficient BW 467C60 or BW 392C60 to impair adrenergic nerve mechanisms is attributable, at least in part, to the lowering of blood pressure. The decrease of the pressor responses after larger doses of the benzylguanidines suggests that these drugs may inhibit the releasing action of tyramine on catechol amine stores. Inhibition of pressor responses to tyramine occurs soon after administration of guanethidine to cats (Bartlet, 1962) and after bretylium to rats (Lešić & Varagić, 1961).

Though bretylium, guanethidine and the benzylguanidines have many properties in common there are some interesting differences. The slopes of the curves relating frequency of sympathetic nerve stimulation to the height of the resulting contraction of the nictitating membrane are depressed by bretylium (Boura & Green, 1959), but guanethidine (and reserpine) preferentially abolished the responses to low rates of stimulation while causing little change in the slope (Boura & Green, 1962). Whereas the effect of BW 392C60 on the frequency/response curve was similar to that of bretylium, the effect of BW 467C60 was intermediate between that of bretylium and guanethidine ; the slope was depressed but responses to low rates of stimulation were inhibited to a relatively greater extent than responses to high rates. The effects of the different compounds on tissue catechol amine contents also vary, and this action may perhaps be related to their relative effects on different rates of nerve stimulation. Thus, single doses of bretylium can increase the amount of catechol amines in the heart (Euler, 1960 ; Cass & Spriggs, 1961 ; Costa, Kuntzman, Gessa & Brodie, 1962) and similarly, but with lower doses, BW 392C60 increases the noradrenaline content of the heart of the rat (Costa *et al.*, 1962 ; Kuntzman, Costa, Gessa & Brodie, 1962) and of the cat iris in the experiments reported here. In contrast, the adrenergic neurone blockade caused by guanethidine and manifested as a parallel shift of the nerve stimulus frequency/response curve is followed by a fall of the noradrenaline content of the heart in the rat (Cass & Spriggs, 1961) and of the cat iris, as found by us. The small depletion of noradrenaline in rats given BW 467C60 (Costa *et al.*, 1962) accords with the intermediate effect of this drug on stimulus frequency/response curves, but no depletion of the iris of the cat was found.

Bretylium and guanethidine (and reserpine) reduce the uptake of isotopic noradrenaline by slices of cat spleen (Dengler, Spiegel & Titus, 1961) and by rat heart *in vivo* (Hertting *et al.*, 1962). If there is a continuous leakage of catechol amines from storage sites the reduction in uptake would tend to lower the catechol amine stores. Such an explanation has been suggested for the loss of catechol amines caused by reserpine (Euler & Lishajko, 1961). That catechol amine stores are not reduced, or at least not so rapidly, by single doses of bretylium adequate to block adrenergic neurones may be related to the finding that this compound also inhibits spontaneous release of noradrenaline from rat heart (Hertting *et al.*, 1962), since this latter effect may be expected to oppose changes resulting from inhibition of catechol amine uptake. Such a stabilizing action may also account for the ability of bretylium to reduce the depletion of noradrenaline caused by reserpine (Hertting *et al.*, 1962).

and for guanethidine causing less depletion in animals given bretylium, BW 392C60 or BW 467C60 (Kuntzman *et al.*, 1962 ; Costa *et al.*, 1962). BW 392C60 is effective in lower doses than bretylium, both in blocking adrenergic nerves and in preventing the depleting action of guanethidine, and this suggests that its activity in stabilizing stores of catechol amines is correspondingly greater. The stabilizing action, possibly on membranes enveloping catechol amine granules, may be analogous to the effect exerted by local anaesthetic agents on the axonal membrane. The benzylguanidines, like bretylium, exert a long-lasting local anaesthetic action. An analogy between the effects of bretylium in preventing release of catechol amines from adrenergic nerve terminals and in impairing conduction in adrenergic nerve trunks has been drawn (Boura & Green, 1959 ; Boura, Copp, Duncombe, Green & McCoubrey, 1960).

The benzylguanidines are highly specific adrenergic neurone blocking agents. Cholinergic mechanisms are impaired only by large doses in the intact animal, and then briefly. The specificity of action of bretylium, or of *N*-(2-4′-benzoyl-2′:6′-dimethylphenoxyethyl)-*N*: *N*: *N*-trimethylammonium methosulphate (BW 172C58), on adrenergic mechanisms has been related to selective accumulation of these compounds by adrenergic nerves following systemic administration (Boura *et al.*, 1960 ; Boura, Duncombe & McCoubrey, 1961b). A similar preferential uptake of BW 467C60 has been found (Boura, Duncombe, McCoubrey & Robson, 1962). That this may be the main cause of the specificity of BW 467C60 is suggested by the brief cholinergic blocking effects which follow intravenous injection when the concentration of the drug in blood is likely to be high, and by the relative slowness of onset of full adrenergic neurone blockade and its persistence in cats. Also supporting this conclusion is the finding, using isolated preparations, that the concentration of BW 467C60 that blocked the adrenergic innervation of the rabbit ileum or guinea-pig vas deferens was similar to that which blocked the cholinergic innervation of guinea-pig ileum. Effects produced by high concentrations *in vitro* are less dependent upon the presence of cumulative mechanisms. It is also pertinent that in isolated organs, adrenergic neurone blocking effects, in contrast to the cholinergic blockade, persist for a long time after washing the preparations.

Both BW 467C60 and BW 392C60 would seem to be potentially useful hypotensive agents. BW 467C60 has the greater activity in cats, and some advantages over other adrenergic neurone blocking agents might be expected. That the absorption of orally administered BW 467C60 is considerably greater than that of guanethidine or bretylium is indicated by the ratios of the subcutaneous to oral doses in cats and may allow greater uniformity of effect. Guanethidine sometimes causes diarrhoea, and animal experiments show this is less likely to occur with BW 467C60. Boura & Green (1962) have suggested that, because of contrasting effects on responses to different rates of sympathetic nerve stimulation, bretylium might be more liable than guanethidine to cause exertional hypotension, whereas guanethidine would be expected to cause a relatively greater fall of blood pressure in supine subjects and be more likely to cause bradycardia. The nictitating membrane experiments suggest that the corresponding effects of BW 467C60 may be intermediate between those of bretylium and guanethidine.

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