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THE PHARMACOLOGY OF NEW ADRENERGIC NEURONE BLOCKING AGENTS, *N*-*p*-CYCLOHEXYLBENZYLTROPINIUM DERIVATIVES

BY

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The three *N*-*p*-cyclohexylbenzyltropinium derivatives studied gave rise to lasting hypotension after transient stimulation and block of sympathetic ganglia in anaesthetized and conscious animals. The drugs inhibited the contractions of the nictitating membrane and spleen evoked by pre- or postganglionic nerve stimulation. In adrenalectomized animals the pressor effect of tetramethylammonium, the hypertensive response to carotid arterial occlusion and the effect of tyramine on the nictitating membrane were also reduced. The drugs have slight or no parasympathomimetic, parasympatholytic or curare-like side-effects. The bretylium-like action of the compounds is discussed.

In recent years new compounds have been added to the group of drugs active on the autonomic nervous system. Substances have been described which block adrenergic neuronal transmission without inhibiting the action of adrenaline or noradrenaline on the effector organ. The first of these compounds was xylocholine (Hey & Willey, 1954), followed by bretylium (Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim, 1959; Boura & Green, 1959) and guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960). The last two drugs have a long duration of action and no parasympatholytic side-effects, which make them useful in the treatment of human hypertension.

We have shown that the potent ganglionic stimulant drug, N-361 (*N*-*p*-phenylbenzyl-3α-benzoyltropinium bromide), described by Gyermek & Nádor (1955), caused a prolonged hypotension after a preliminary hypertension, due to a diminution in the activity of sympathetic postganglionic neurones. We attempted to alter the structure of the molecule so that the marked ganglionic stimulant action would be diminished or abolished without reducing the neuronal blocking action. It seemed that the partial hydrogenation of the aromatic system of the quaternerizing group of N-361 would be the method of choice; in other words the quaternerizing aralkyl group would become *p*-cyclohexylbenzyl.

There were approximately twenty-five compounds among nearly fifty of similar chemical structure (Nádor, György & Dóda, 1963) that had a lasting hypotensive effect. The pharmacology of three of these compounds, the ones most favourable with regard to potency and toxicity, has been studied in most detail.

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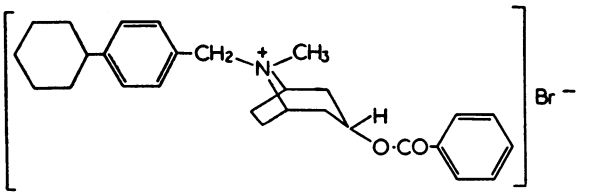
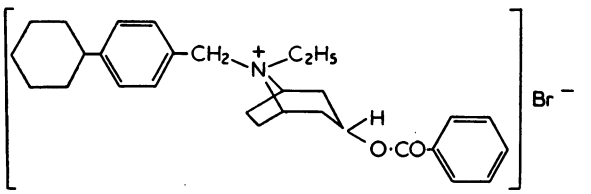
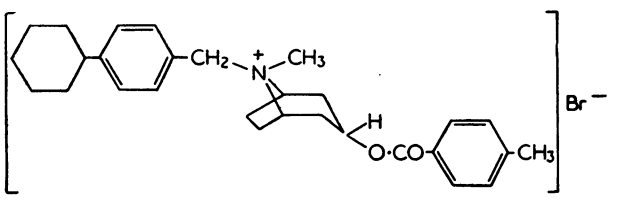


N-718. 3α-O-benzoyl-*N*-*p*-cyclohexylbenzyltropinium bromide



N-830. 3α-O-benzoyl-*N*-*p*-cyclohexylbenzyl-*N*-ethylnortropinium bromide



N-856. 3α-O-*p*-toluyl-*N*-*p*-cyclohexylbenzyltropinium bromide

METHODS

Cats anaesthetized with chloralose (50 mg/kg) and urethane (400 mg/kg), or with chloralose alone (70 mg/kg), or hexobarbitone (40 to 50 mg/kg) alone, as well as conscious cats (light ether anaesthesia followed by local anaesthesia with lignocaine) were used. Blood pressure was measured by means of a mercury manometer or a Statham P 23 Db pressure transducer, coupled to a Hellige electromanometer (type TFV 3).

The hypotensive effect was greatest in animals with a high blood pressure. Because of this the dose of drug required to reduce the blood pressure to 70 mm Hg was used as the criterion of assessment. The hypotensive response to the compounds developed rapidly and was maximal within 5 min of intravenous injection. After a few initial experiments we gave doses of each compound which would cause the blood pressure to fall to or below 70 mm Hg. The so-called ED-70 mm Hg value was read from the dose/response curve plotted. Since the effect was prolonged, doses administered within 15 min were added and a cumulative dose/response curve was plotted.

The compounds to be tested were dissolved in 0.9% saline and injected intravenously, intraperitoneally, subcutaneously or into the stomach or duodenum through appropriately inserted tubes, in the form of a 2% gum acacia suspension.

Close-arterial injections were made through a cannula in the lingual artery. In some experiments the superior cervical ganglion was painted with lignocaine solution to rule out ganglionic effects.

Respiration was recorded from a tracheal cannula connected to a Marey tambour.

Contractions of the nictitating membrane were recorded by attaching it to an isotonic lever (magnification ×12).

Contractions of the tibialis anterior muscle of the cat were recorded with two Marey tambours connected by a tube; contractions of the muscle were elicited by stimulation of the sciatic nerve (0.1 to 0.6 V, 0.1 msec pulse-duration and 0.5 shocks/sec).

The carotid sinus reflex was assessed by the blood pressure response to occlusion and release of the common carotid arteries, the blood pressure being measured from the femoral artery.

Contractions of the spleen were studied by the method of Ahlquist (1953) and György & Dóda (1960). The splenic nerve, together with the splenic artery, was placed on the electrode and the contractions of the spleen were elicited by stimulating the splenic nerve (5 to 20 V, 0.1 to 0.2 msec pulse-duration, 20 to 40 shocks/sec for periods of 10 to 30 sec). The cervical sympathetic nerve was stimulated through electrodes placed on the pre- or postganglionic trunk (2 to 5 V, 0.1 msec pulse-duration, 50 shocks/sec for 0.4 sec in every 0.5 min). The above two stimulations were carried out with submaximal voltages. Action potentials of the superior cervical ganglion were led through a single electrode, into a resistance-capacity amplifier coupled to an oscilloscope. In these experiments the preganglionic trunk was stimulated with supramaximal rectangular pulses of 1 msec duration and at 2 to 8 shocks/sec.

The cervical vagus nerve on the right side was stimulated for 1 sec in every 0.5 or 1 min with the same parameters which were used for stimulating the cervical sympathetic trunk, but with 4 to 6 V intensity. Heart rate was recorded in the following way: a polyethylene tube was inserted into the left iliac artery through the femoral artery, and the pulsations of blood pressure were recorded from a Statham pressure transducer and Hellige electromanometer by a Piesker pulse-frequency integrator (type 301/60). The heart rate was registered by a Hellige six-channel recorder (Multiscriptor, type 9400/6).

In some experiments the adrenal glands were ligated on both sides through a mid-line abdominal incision.

Parasympatholytic effects on the cat's blood pressure were studied by a method described by György, Dóda & Nádor (1961).

The blood pressure of rats (anaesthetized with 800 mg/kg of urethane or lightly with ether) was measured with a Hellige electromanometer, via a hypodermic needle tied into a carotid artery.

Changes in pupil diameter were measured by means of a stereoscopic microscope (magnification $\times 16$), after administering compounds subcutaneously, intraperitoneally or intravenously.

Dogs were anaesthetized with morphine (10 mg/kg) and chloralose (60 mg/kg); the carotid arterial blood pressure was recorded by a mercury manometer and registered on a kymograph.

Cat and rat isolated guts and the cat spleen were suspended in Krebs phosphate-nutrient fluid at 37° C, and were used for examining how the compounds tested antagonized acetylcholine and adrenaline.

The toxicity of the compounds was studied in albino mice and in rats, after intravenous, subcutaneous, intraperitoneal and oral administration. The results were evaluated as described by Litchfield & Wilcoxon (1949). The compounds were administered as a 1 to 2 mg/ml. solution, or as a suspension in 5% gum acacia solution.

RESULTS

Effects on blood pressure

Anaesthetized cats. In these preparations 0.5 to 3 mg/kg of N-718 or N-830 increased blood pressure for 0.5 to 1 min (Figs. 1, 2 and 3); for N-718 the increase was about 80 mm Hg for a 1 mg dose, and for N-830 it was 25 mm Hg. N-856 increased blood pressure for 15 to 30 sec and only in doses of 2 mg/kg or higher. This pressor response could be elicited repeatedly when N-718 was administered at intervals of 20 min or more; it diminished or ceased if the interval between the doses was as short as 4 or 5 min. Immediately after the pressor effect a strong and long depressor one developed: 1 or 2 mg/kg of any of the compounds produced a hypotension lasting 1 to 2 hr or longer. Table 1 shows the mean values for the

TABLE 1

BLOOD PRESSURE OF THE ANAESTHETIZED CAT BEFORE AND AFTER ADMINISTRATION OF TROPEINE DERIVATIVES

Blood pressures are means ± standard errors. For chemical formulae see text. Injections were intravenous

Compound	Doses (mg/kg)	No. of cats	Blood pressure	
			Before injection (mm Hg)	After injection (mm Hg)
N-718	0·5	3	131±46	115±15
	1·0	17	167±34	111±27
	1·5	5	151±17	82± 4
	2·0	20	145±41	75±27
	3·0	4	108±17	46±28
N-830	1·0	9	146±33	101± 8·5
	2·0	18	142±29	58±21
	3·0	12	155±19	48±14
N-856	1·0	7	139±15	100±19
	1·5	8	143±22	78±19
	2·0	26	133±18	69±15
	3·0	9	135±17	56± 7·6

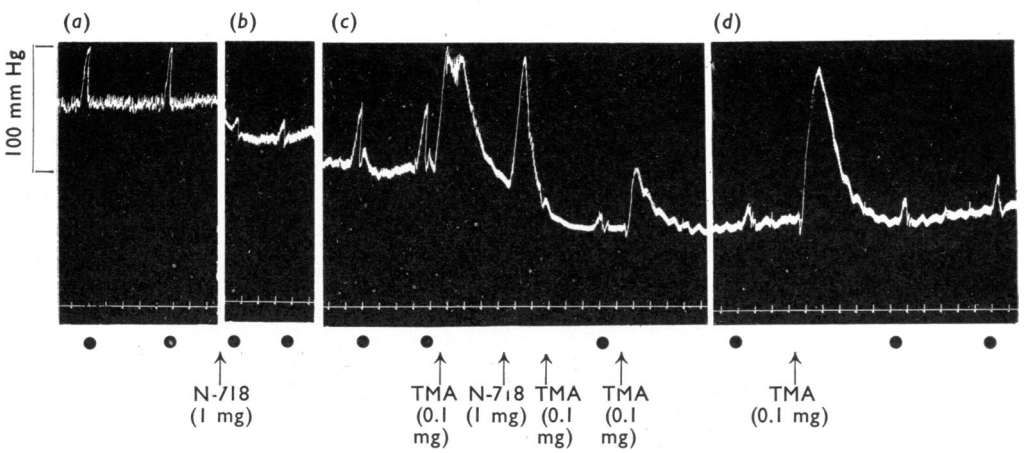


Fig. 1. Blood pressure responses of a cat (3 kg) anaesthetized with chloralose. Time (min). Dots indicate bilateral carotid arterial occlusion for 30 sec ; (*a*) after 3 to 11 min ; (*b*) 45 to 50 min ; (*c*) 91 to 113 min ; (*d*) 118 to 135 min. 100 μg of tetramethylammonium was given intravenously at TMA. Between (*a*) and (*b*) 1 mg/kg of N-718 was given intravenously. Doses are expressed per kg body weight.

depressor responses to different doses of the compounds tested. The ED-70 mm Hg values obtained from the dose/response curves are: N-718 2.1 mg/kg, N-830 1.65 mg/kg and N-856 1.95 mg/kg.

Infusion of the compounds into the femoral vein at a rate of 40 to 50 μg/kg/min began to decrease blood pressure after the administration of total doses of about 1 to 1.5 mg/kg, and the ED-70 mm Hg values thus obtained were about 30 to 60% higher than those given above. Intraperitoneal administration caused a strong and lasting depression of blood pressure (down to 70 or 80 mm Hg) with doses of 2 to 6 mg/kg. The effective subcutaneous doses were 4 to 10 mg/kg for compounds N-718 and N-830, and 8 to 20 mg/kg for compound N-856. With intragastric or

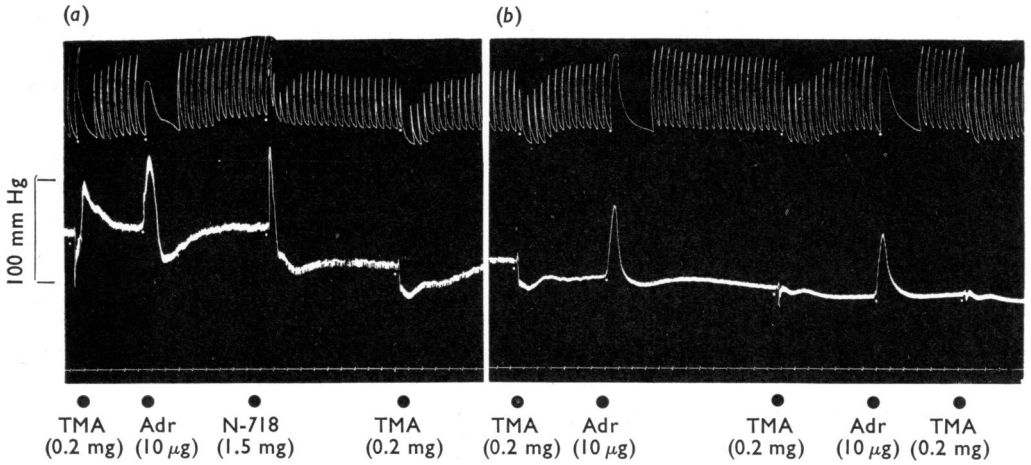


Fig. 2. Cat (3·5 kg), anaesthetized with chloralose and urethane and adrenalectomized. Contractions of the nictitating membrane (above) to submaximal stimulation of the cervical preganglionic sympathetic nerve for 0.4 sec twice per min, blood pressure (below), and time (in min). There was an interval of 6 min between (*a*) and (*b*). Doses of drugs are expressed per kg body weight, and were given intravenously. Adr=adrenaline ; TMA=tetramethylammonium.

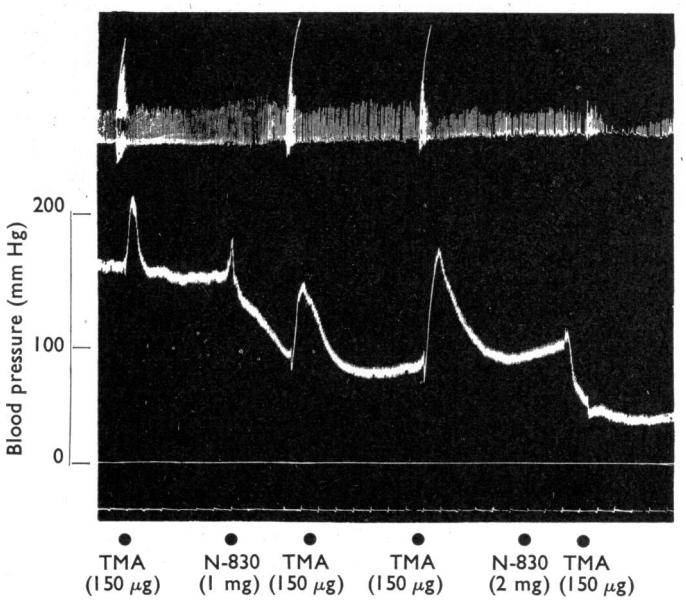


Fig. 3. Cat (2·5 kg) anaesthetized with chloralose and urethane. Records of respiration, blood pressure and time (in min). Doses of drugs are expressed per kg body weight, and were given intravenously. TMA=tetramethylammonium.

intraduodenal administration, 20 to 25 mg/kg were required of any compound to elicit a lasting depressor response (Fig. 4). No pressor effect has been noted with the last four routes of administration.

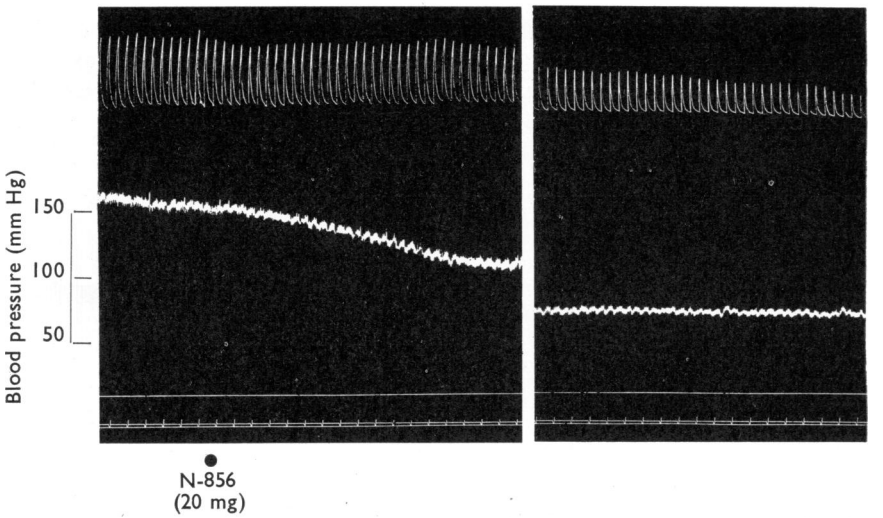


Fig. 4. Cat (1·6 kg) anaesthetized with chloralose and urethane. Records of contractions of the nictitating membrane in response to stimulation of the cervical sympathetic nerve for 0·4 sec twice per min, blood pressure and time (in min). 33 min between records. The dose of N-856 is expressed per kg body weight, and was given by duodenal instillation.

Unanaesthetized cats. In these preparations the effect was the same as, although weaker than, in the cats anaesthetized with chloralose and urethane. The intravenous ED-70 mm Hg values were about 50% higher than those in the anaesthetized animals.

Dogs. In this species N-718 had a much more marked pressor effect than in the cat. The 1 mg/kg dose increased blood pressure to 180 to 200 mm Hg, and this effect lasted a few minutes. The blood pressure then fell to 30 to 50 mm Hg. The pressor responses to N-830 and N-856 were weaker, but the effect was still more marked than in the cat. Of the last two compounds doses of 3 to 6 mg/kg were required to elicit a strong hypotensive response lasting several hours.

Rat. In this species the pressor effect of N-718 was small: N-718 and N-856 (4 to 8 mg/kg) and N-830 (2 to 3 mg/kg) produced lasting hypotension (Fig. 5).

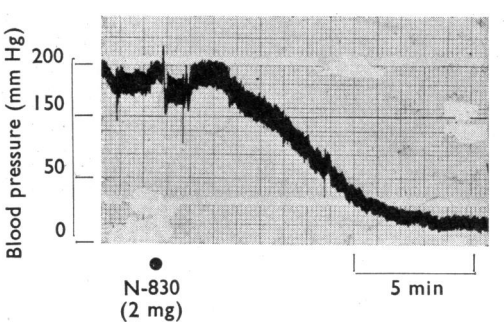


Fig. 5. Rat (220 g) anaesthetized with urethane. Record of blood pressure. The dose of N-830 is expressed per kg body weight, and was given intravenously.

Occlusion of the common carotid arteries. This test caused a pressor response which was diminished by 1 mg/kg of the drugs intravenously, and was strongly or completely blocked by 2 mg/kg. This inhibition was prolonged, though it was shorter in duration than the depressor response (Figs. 1 and 6).

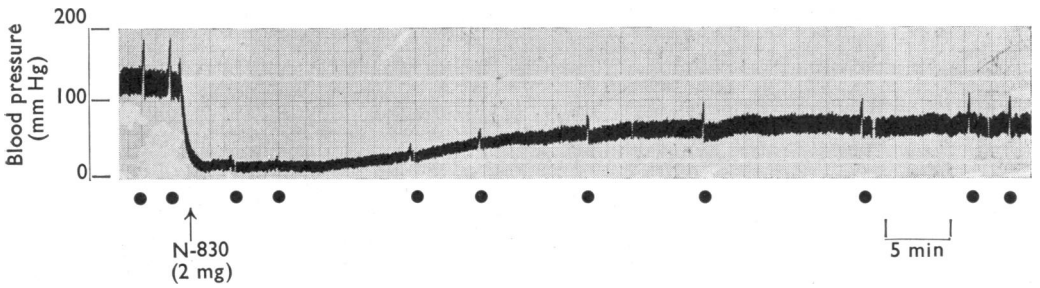


Fig. 6. Cat (2·7 kg) anaesthetized with chloralose. Record of blood pressure. Dots indicate bilateral carotid arterial occlusion for 30 sec. The dose of N-830 is expressed per kg body weight, and was given intravenously.

Effects on the nictitating membrane

Simultaneously with the pressor response, N-718 caused transient contractions of the nictitating membrane, which were completely inhibited by 1 mg/kg of tetraethylammonium bromide. N-830 and N-856 did not contract the nictitating membrane.

Each of the three compounds inhibited the contraction of the nictitating membrane elicited by stimulation of the preganglionic cervical sympathetic nerve. Immediately after the administration of 1 to 2 mg/kg the contractions diminished, but increased again after a few minutes. The contractions usually did not return to the control level, but a lasting inhibition of 20 to 80% was observed. In other experiments the effect was not biphasic; the administration of the compound was followed immediately by the development of lasting inhibition (Figs. 2, 7 and 8).

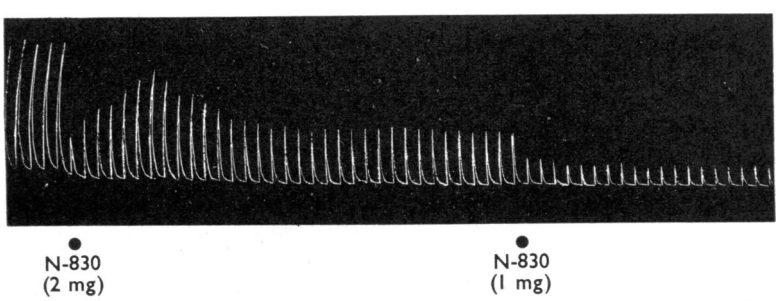


Fig. 7. Cat (2·8 kg) anaesthetized with chloralose and urethane. Contractions of the nictitating membrane in response to submaximal stimulation of the preganglionic cervical sympathetic nerve for 0·4 sec twice per min. Doses of N-830 are expressed per kg body weight.

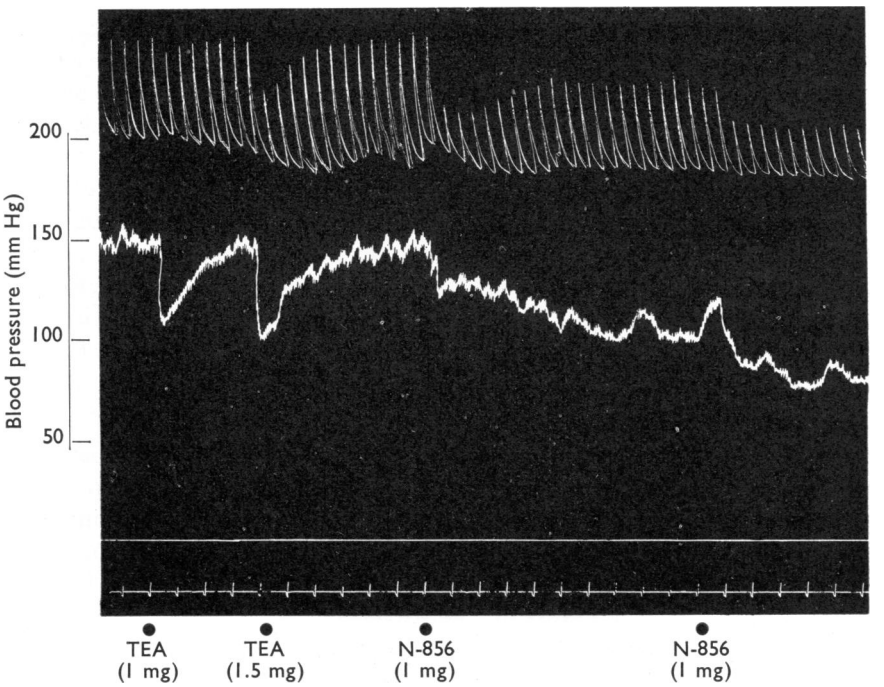


Fig. 8. Cat (3·2 kg) anaesthetized with chloralose and urethane. Records of contractions of the nictitating membrane in response to submaximal stimulation of the preganglionic cervical sympathetic nerve for 0·4 sec twice per min, blood pressure and time (in min). Doses of drugs are expressed per kg body weight. TEA=tetraethylammonium.

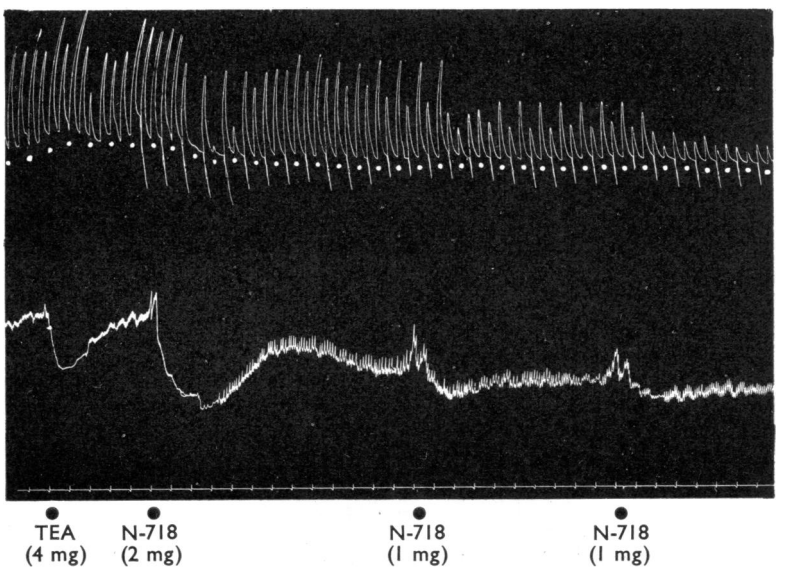


Fig. 9. Cat (3·5 kg) anaesthetized with chloralose and urethane. Records of contractions of the nictitating membrane in response to alternate stimulation of the pre- (at white dots) and postganglionic cervical sympathetic nerves for 0·4 sec twice per min, blood pressure and time (in min). Doses of drugs are expressed per kg body weight. TEA=tetraethylammonium.

The compounds also inhibited the responses to stimulation of the postganglionic cervical sympathetic nerve. Fig. 9 shows results obtained in such an experiment, in which the cervical sympathetic nerve was stimulated alternately pre- and postganglionically; 4 mg/kg of tetraethylammonium inhibited the effect only of preganglionic stimulation. Following the intravenous administration of 2 mg/kg of N-718 the response to preganglionic stimulation was totally abolished for a short while, but subsequently the contractions increased rapidly in size. The same dose diminished the contractions in response to postganglionic stimulation only slightly, but in an increasing measure. Further doses of N-718 had a similar effect, and ultimately the responses to both the pre- and postganglionic stimulations were strongly diminished. The other compounds gave a similar response. From the above facts we conclude that the compounds possess transient ganglionic stimulant and ganglionic blocking actions, as well as a third effect peripheral to the ganglion.

Acetylcholine (20 to 40 μg), injected into the lingual artery after treatment of the superior cervical ganglion with lignocaine, contracted the nictitating membrane. This effect was strongly or completely inhibited by the intravenous administration of 2 mg/kg of N-718 (Fig. 10), was not influenced by hexamethonium, but was blocked by hyoscine or by 1 to 2 mg/kg intravenously of phentolamine.

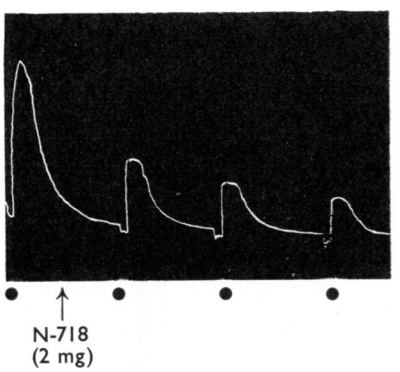


Fig. 10. Cat (3·8 kg) anaesthetized with chloralose and urethane. Contractions of the nictitating membrane after 5 mg/kg of hexamethonium (stimulation of the preganglionic cervical sympathetic nerve was ineffective). At the dots injections of 40 μg of acetylcholine were made into the lingual artery. The dose of N-718 is expressed per kg body weight.

Effects on action potentials in the superior cervical ganglion

After the injection of 50 to 100 μg/kg of N-718 into the lingual artery the following changes were observed. In some experiments the synaptic potential increased for a few minutes followed by depression of the spike potentials S_1 and S_2 (S_1 is the spike potential of the nerve cells in the ganglion, S_2 is due to activity in the postganglionic fibres running through the ganglion). The synaptic potential (S_y) showed either no depression or a small and brief depression. These effects disappeared in 5 to 30 min, and could be evoked again by repeated injections (Fig. 11). Likewise, 1 to 2 mg/kg intravenously of N-718 caused only a temporary depression of the potentials.

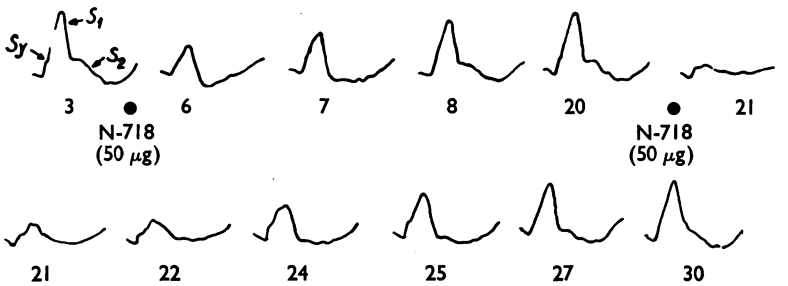


Fig. 11. Cat (2·5 kg) anaesthetized with chloralose and urethane. Action potentials of the post-ganglionic cervical sympathetic nerve before and after injection of N-718, expressed per kg body weight, into the lingual artery. Numbers give times after the beginning of the experiment.

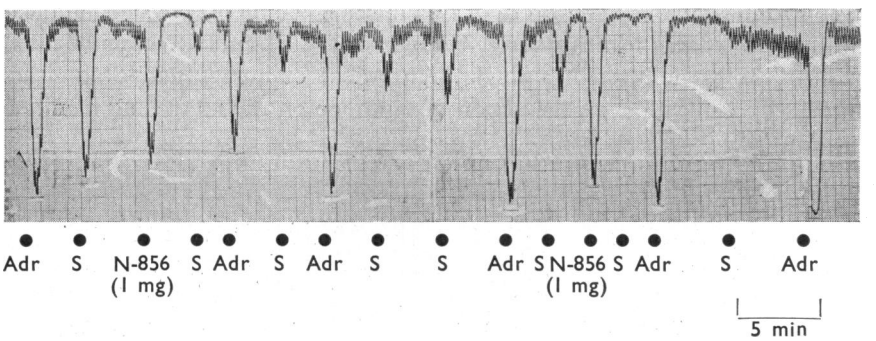


Fig. 12. Cat (1·7 kg) anaesthetized with chloralose and urethane. Records of contractions of the spleen. S, stimulation of the splenic nerve ; Adr, injection of 1 μg/kg of adrenaline intravenously. Doses of N-856 are expressed per kg body weight.

Effects on the spleen

The compounds caused brief contractions of the spleen and each inhibited the contractions elicited by splenic nerve stimulation (Fig. 12).

Effects on the action of tetramethylammonium bromide

Each of the three compounds lessened (in doses of 1 to 1.5 mg/kg) and completely inhibited (in higher doses) the pressor response to 100 to 150 μg/kg of tetramethylammonium administered intravenously. This inhibition was temporary and the pressor effect of tetramethylammonium returned fully within 5 to 20 min. The compounds lowered blood pressure and inhibited the carotid sinus reflex for a much longer time than the duration of the inhibition of the pressure effect induced by tetramethylammonium (Fig. 1). After ligation of the adrenal glands much higher doses (200 μg/kg) of tetramethylammonium were required to evoke a significant pressor response; under such conditions each compound inhibited this pressor effect for periods of 1 to 1.5 hr. The inhibition was greater on the blood pressure than on the nictitating membrane (Fig. 2).

Effects on the action of adrenaline

The three compounds either enhanced or did not influence the actions of adrenaline on the blood pressure and spleen. N-718 and N-830 did not inhibit the effect of adrenaline on the nictitating membrane but N-856 had a weak action. The adrenolytic effects of these compounds do not account for their effects (Figs. 2, 12 and 13).

The contractions of the cat isolated spleen induced by adrenaline (2 to 4×10^{-7}) were inhibited by N-718 and N-856 at concentrations of 10^{-6} to 10^{-7}, while for N-830 10^{-5} was required for this inhibition. These effects were 100- to 1,000-times less potent than that of ergotoxine, could be washed out readily and were reversible.

Effects on the action of tyramine

N-718 and N-830 did not inhibit the pressor response to the intravenous administration of 0.2 to 1 mg/kg of tyramine, while a slight inhibition followed N-856. Each compound inhibited the effect of tyramine on the nictitating membrane. For N-856 this action was less evaluable, because of the adrenolytic component of the effect. As shown in Fig. 13, a single higher dose of noradrenaline abolished the inhibitory effect of N-830 on the action of tyramine on the nictitating membrane.

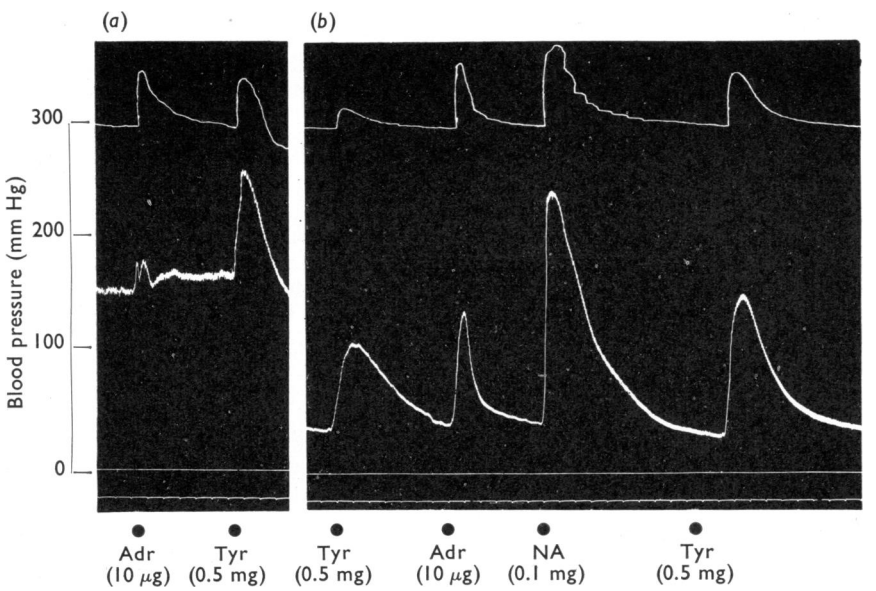


Fig. 13. Cat (3·8 kg) anaesthetized with chloralose and urethane. Records of contractions of the nictitating membrane, blood pressure and time (in min). Adr, adrenaline; NA, noradrenaline; Tyr, tyramine. Doses are expressed per kg body weight. 4 min after (*a*) N-830 (4 mg/kg) was given intravenously. 20 min between (*a*) and (*b*).

Effects on parasympathetic ganglia

The compounds did not increase pulse rate, except that with N-718 a transient increase sometimes occurred. Following the administration of the compounds, cats gradually developed a bradycardia which ran parallel with the depressor

response; the pulse rate decreased from 180 to 190 down to 130 to 140 beats/min. The bradycardia elicited by stimulation of the cervical vagus nerves was not influenced by low doses of the compounds (Fig. 14), but it was inhibited for 5 to 20 min by doses of 1.5 to 2 mg/kg.

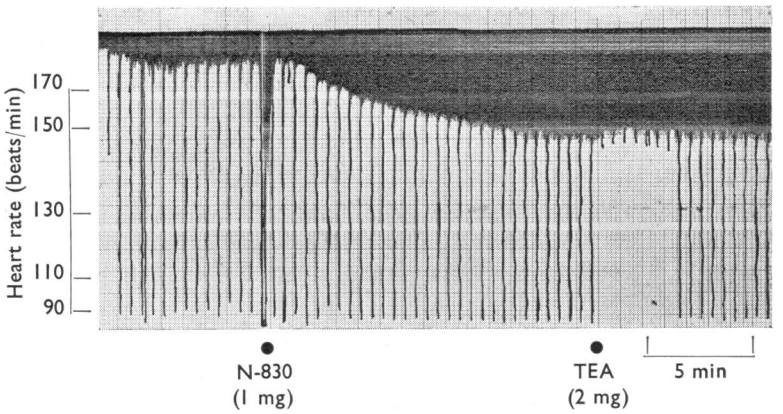


Fig. 14. Cat (2·7 kg) anaesthetized with chloralose and urethane. Record of pulse frequency (beats/min). The right vagus was stimulated for 1 sec twice per min. The effect of the stimulation (decrease of the heart rate to 90 to 100 beats/min) was blocked by tetraethylammonium (TEA) but not by N-830. Doses of drugs are expressed per kg body weight.

In the rat no mydriasis was evoked by subtoxic subcutaneous or intraperitoneal doses; this effect developed only after intravenous injection. 5 mg/kg intravenously of N-718 dilated the pupil by 300%; N-830, in intravenous doses of 5, 8 and 12 mg/kg, caused 190, 220 and 290% mydriasis; and 8 and 12 mg/kg intravenously of N-856 increased the diameter of the pupil by 235 and 417%, respectively. The duration of the mydriatic action was 3 to 4 (maximum 5) min with each compound.

Atropine-like actions

Intravenous doses of 0.5 to 1 mg/kg of the compounds had no influence on the depressor action of 0.001 to 0.1 μg/kg of acetylcholine in the cat.

At a concentration of 10^{-6}, N-718 inhibited the contractions of the rat isolated intestine induced by acetylcholine; the other two compounds had a similar effect at concentrations of 10^{-5} or higher. These effects were 1/1,000th and 1/10,000th of that of atropine, respectively. In the rabbit intestine a concentration of 3 to 7×10^{-6} of N-718 so strongly diminished the pendulum movements that adrenergic effects could not be studied.

Effects on striated muscle activity

In the vasodepressor-doses employed the compounds had no curare-like activity. Doses of 3 to 5 mg/kg did not influence the contractions of the tibialis anterior muscle induced by stimulation of the sciatic nerve, but in some experiments a slow diminution of the contractions, not antagonized by neostigmine, was observed.

Effects on respiration

When administered intravenously, N-718 increased respiratory rate for a few minutes concurrently with the increase in blood pressure and the contraction of the nictitating membrane; this effect was inhibited by 1 to 2 mg/kg of tetraethylammonium administered intravenously. Compounds N-830 and N-856 had no such effect. The increased respiration caused by 150 μg/kg intravenously of tetramethylammonium was temporarily inhibited by the compounds (Fig. 3).

Toxicity

In cats anaesthetized with chloralose and urethane artificial ventilation had to be started following the intravenous administration of 2.5 to 3 mg/kg of N-718. For N-830 and N-856 this was necessary only when the doses were as high as 5 to 7 mg/kg. In cats anaesthetized with hexobarbitone or conscious (under local anaesthesia) and in anaesthetized dogs and rats, doses higher than 10 mg/kg made it necessary to employ artificial ventilation. Intravenous doses of 5 to 6 mg/kg of N-718 were well tolerated by rabbits after a period of muscle weakness, but 10 mg/kg was lethal. In the unanaesthetized cat, 30 to 40 mg/kg of N-718 injected intraperitoneally in 2 or 3 doses within 24 hr caused muscular weakness, relaxation of the nictitating membrane and, in some instances, an increase of respiratory rate. No sign of a central nervous effect was noted either in these species or in rodents.

According to cardiometric, cardioplethysmographic and electrocardiographic studies the compounds had no direct effect on the heart; in acute cases the animals died of paralysis of the respiratory muscles or of collapse. With the intraperitoneal,

TABLE 2

ACUTE TOXICITY OF TROPEINE DERIVATIVES IN MICE AND RATS

I.v. = intravenous ; i.p. = intraperitoneal ; s.c. = subcutaneous ; * approximate

		Mice		Rats	
Route	Time (hr)	LD50 (mg/kg)	95% confidence limits	LD50 (mg/kg)	95% confidence limits
(*A*) *N*-718					
I.v.	24	21·6	19·0–24·5	22·7	19·4–26·6
I.p.	24	34·7	28·2–42·6	37·4	30·4–46·0
	48	33·0	28·2–38·6	27·0	18·1–40·5
S.c.	24	50·8	44·9–57·5		
	48	47·0	41·2–53·6		
Oral	24	1200*			
	48	1000*			
(*B*) *N*-830					
I.v.	24	23·0	19·4–27·4		
	48	21·5	18·5–25·0		
S.c.	24	45·0	39·4–51·3		
	48	33·0	28·6–37·9		
Oral	24=48	800	710–920		
(*C*) *N*-856					
I.v.	24	24·7	22·4–27·0		
	48	23·0	21·2–24·9		
S.c.	24	60*			
	48	51·9	46·7–57·5		
Oral	24	1140	926–1400		
	48	990	811–1208		

subcutaneous and oral routes of administration, collapse, muscle weakness and probably the marked decrease of body temperature were the most important factors in toxicity. The LD50 values obtained in rodents are shown in Table 2.

DISCUSSION

The compounds studied have very complex autonomic nervous effects. N-718, intravenously in cats, causes first a rise of blood pressure of short duration; this effect is different from that seen following administration of bretylium or guanethidine, which elevate blood pressure for as long as 2 to 20 and 30 to 120 min respectively, while the pressor response to our tropinium derivatives is usually over in 1 min. The pressor effect of bretylium is due to an action on adrenergic neurones (Boura & Green, 1959) whereas guanethidine mobilizes catechol amines and its pressor effect cannot be blocked by ganglionic blocking agents (Maxwell *et al.*, 1960b). The pressor effect of N-718 is ascribed to a stimulation of ganglia, which is indicated by the facts that tachyphylaxis develops rapidly to the pressor effects of the doses given intravenously in succession, and that the resulting elevations of blood pressure, contractions of the nictitating membrane and increases in respiration are inhibited by tetraethylammonium. The cause of the very transient mydriasis in the rat after intravenous administration may be partly excitation of ganglia or mobilization of adrenaline, though ganglionic-block may also play a role. The pressor effect in the cat and the mydriatic effect in the rat have in common their short duration and their occurrence exclusively following intravenous administration.

As mentioned in the introduction, the compounds studied here were synthesized in an attempt to diminish the pressor effect of N-361 (Gyermek & Nádor, 1955), which possesses also a strong hypotensive activity. This attempt was to a great extent successful for N-718, and almost so for N-856.

After the stimulation of ganglia is over, a secondary ganglionic-blocking effect develops in the cat. The contractions of the nictitating membrane in response to preganglionic sympathetic stimulation, the action potentials of the superior cervical ganglion and the pressor and respiratory effects of tetramethylammonium diminish, while a transient paralysis of vagus nerves and sympathetic ganglia develops; these effects partly account for the mydriasis. This ganglion-block disappears so quickly that it cannot be responsible in any way for the lasting depressor effect of the compounds.

The intravenous administration of 1 to 2 mg/kg of the drugs is followed by hypotension lasting several hours. At the same time the contractions of the nictitating membrane elicited by pre- or postganglionic stimulation, the splenic response to the stimulation of the splenic nerve, the carotid sinus reflex and, in the adrenalectomized animal, the pressor effect of tetramethylammonium decrease. Meanwhile, the effects of adrenaline on blood pressure, spleen and nictitating membrane remain unchanged or enhanced.

It seemed possible that our compounds acted in the way suggested by Hey & Willey (1954) and Boura & Green (1959); that is by accumulation in nerve fibres and thereby block of conduction. Studies of the action potentials of the superior

cervical ganglion have shown that N-718 did not block the development of the synaptic potential (the essential part of the process of transmission) but it inhibited the summation of the local potentials which had developed under the presynaptic endings and the initiation of the spike potential mechanism. Thus N-718 is active on the postsynaptic elements, in the first place, but these effects are much shorter in duration than are the depressor and the other effects mentioned above; thus, the effects of N-718 cannot be explained by a "local anaesthetic" action. N-718 is therefore not different in this respect from xylocholine (Exley, 1957) or from guanethidine (Maxwell *et al.*, 1960b), which cause no lasting depression of action potentials. More recently this behaviour has been shown also for bretylium (Exley, 1960), though it is absolutely certain that this drug acts on postganglionic sympathetic fibres, but at much higher concentrations than when applied to the area of the end-plate (Boura & Green, 1959). According to Boyd, Chang & Rand (1961) the effect of bretylium is substantially different from that of procaine.

The principal effect of guanethidine is a reserpine-like, catechol amine depleting activity (Sheppard & Zimmerman, 1959; Cass, Kuntzman & Brodie, 1960; Cass & Spriggs, 1961; Krayer, Alper & Paasonen, 1962; Kroneberg, Schümann & Eckardt, 1962; Sanan & Vogt, 1962; Pfeifer, Vizi & Sâtory, 1962). This behaviour is responsible for the fact that guanethidine inhibits the actions, pressor and other, of tyramine, amphetamine and other indirectly acting phenylalkylamine derivatives (Maxwell, Plummer, Povalski & Schneider, 1960a; Kroneberg *et al.*, 1962; Bartlet, 1962), although this has been questioned (Vernikos-Danellis & Zaimis, 1960; Zaimis, 1960).

This antagonism of tyramine resembles the action of reserpine. Bretylium does not deplete catechol amines from its stores and it is questionable whether it antagonizes tyramine. According to Burn & Rand (1960) it does not inhibit the actions of tyramine, while Lešić & Varagić (1961) claim that there is an antagonism between the two compounds. Our compounds inhibit exclusively the action of tyramine on the nictitating membrane, but do not influence or enhance its action on blood pressure. At present, it is not reasonable to draw far-reaching conclusions from the experiments with tyramine, because the effect of tyramine itself is not yet fully clarified and it is not certain whether tyramine releases noradrenaline from the same sources as does nervous stimulation (Schmitt & Schmitt, 1961), although it has been proved that the compounds depleting catechol amines are also antagonists of tyramine. Our compounds presumably cause no depletion of noradrenaline; at least this is not a primary part of their effect.

The depletion of catechol amines brought about by guanethidine develops slowly and the blood pressure also decreases slowly. At the same time, guanethidine inhibits very rapidly the contractions of the nictitating membrane in response to stimulation of the cervical sympathetic nerve. It has therefore been suggested that, besides its reserpine-like action, guanethidine has another effect (Burn, 1961), like that of bretylium. Burn & Rand (1960) and Burn (1961) have indicated that acetylcholine can be liberated in the sympathetic postganglionic fibre and this liberation would release noradrenaline from its stores. According to Burn (1961) this new, fourth action of acetylcholine is antagonized specifically by bretylium. It is possible that N-718 and our other compounds have a site of action similar to that

of bretylium. In support of this view is the fact that N-718 inhibits the contractions of the nictitating membrane in response to injection of acetylcholine into the carotid artery, although this problem requires further investigation because this effect of acetylcholine is not fully clarified. According to Trendelenburg & Weiner (1962) this action of acetylcholine is little diminished by phenoxybenzamine, while according to our investigations it is strongly inhibited by phentolamine and therefore the adrenergic component cannot be ruled out.

These experiments make it clear that our compounds inhibit the actions of post-ganglionic sympathetic stimulation on the nictitating membrane and on the spleen and do not inhibit the actions of adrenaline on these organs, that they have weak anti-tyramine activity and that probably neither catechol amine depletion nor a "local anaesthetic" activity plays any significant role in their effect. Our compounds appear to act in the same way as bretylium.

Our compounds have the advantageous property of lowering blood pressure immediately after a very short pressor effect. As has been shown by infusion experiments, the depressor effect develops even when the blood concentration is relatively low. This also explains why large doses of the compounds introduced into the stomach or duodenum can exert a therapeutic effect although, being quaternary compounds, they are not readily absorbed following oral administration.

Our compounds have no parasympathomimetic, parasympatholytic, ganglionic-blocking or curare-like side-effects, or else the duration of these is negligibly short as compared with that of the main effect.

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