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THE EFFECTS OF SOME CENTRALLY-ACTING DRUGS ON GANGLIONIC TRANSMISSION IN THE CAT

BY

D. A. BROWN AND J. P. QUILLIAM

From the Department of Pharmacology, Medical College of St. Bartholomew's Hospital, London, E.C.1

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Transmission through the cat superior cervical ganglion was studied by recording the response of the nictitating membrane to both pre- and postganglionic cervical sympathetic nerve stimulation. The intra-arterial injection of central depressant drugs to the ganglion through the lingual artery depressed transmission through the ganglion. The central depressant drugs tested were (in decreasing order of activity): amylobarbitone, pentobarbitone, carbromal, benactyzine, mephobarbitone, hydroxyzine, phenobarbitone, azacyclonol, methylpentynol carbamate, paraldehyde, phenytoin, mephenesin, chlorbutol, troxidone, methylpentynol and barbitone. All were weaker ganglion-blocking agents than tetraethylammonium. The intra-arterial injection of the central stimulant drugs leptazol, bemegride, amiphenazole and 5-(1,3-dimethylbut-2-enyl)-5-ethylbarbituric acid (McN 481) also depressed ganglionic transmission. Leptazol or bemegride did not antagonize the ganglion-blocking action of amylobarbitone or troxidone. The intra-arterial injection of pecazine and perphenazine, and the intravenous injection of barbitone, benactyzine, azacyclonol, hydroxyzine, mephenesin, methylpentynol and paraldehyde impaired the response of the nictitating membrane to both post- and preganglionic stimulation. The implications of these observations are discussed.

The action of central depressant drugs on transmission through sympathetic ganglia is of interest both as an index of their possible effects on central synapses and as a means of obtaining further information regarding the nature of synaptic transmission in general.

This paper reports the effects of a number of centrally-acting drugs on transmission through the cat superior cervical ganglion *in vivo*, using the conventional nictitating membrane preparation. To reduce side-effects due to the systemic accumulation of these drugs, which are weak ganglion-blocking agents, the effects of the drugs were localized to the ganglion by injecting them intra-arterially through the lingual artery, according to the method of Morrison & Paton (1953). Some effects of intra-arterially injected methylpentynol and paraldehyde have been reported previously (Quilliam, 1957, 1959).

METHODS

Cats were anaesthetized with chloralose (60 mg/kg) administered intravenously after induction with ethyl chloride and ether, or with an intraperitoneal injection of sodium pentobarbitone (35 to 45 mg/kg). The pre- and postganglionic cervical sympathetic nerve trunks

were isolated and prepared for stimulation through bipolar platinum electrodes. The nerves were stimulated repetitively at either 10 or 50 shocks/sec. Stimulation was either continuous (at 10 shocks/sec only) or intermittent, with bursts lasting 15 or 30 sec and delivered once each minute. A relay device controlled two electronic stimulators and delivered the trains of stimuli alternately to pre- and postganglionic trunks. The electronic equipment was developed from that designed by Bell (1957). Rectangular pulses of 0.2 msec duration at a voltage more than sufficient to evoke maximal contracture of the nictitating membrane at the frequency chosen (" supramaximal stimuli ") were used. The contractures of the nictitating membrane were recorded on smoked paper with a frontal-writing lever giving an eight-times magnification.

The mean arterial blood pressure was recorded from a femoral artery or from the contralateral carotid artery using a mercury manometer. Heparin (Pularin, 100 U/kg) was used as an anticoagulant. Respiratory movements were recorded by attaching a thread from the abdominal wall at the level of the diaphragm to a lever writing on the smoked paper so that inspiration produced a downward movement on the trace.

For intra-arterial injection a blunt-tipped needle cannula was inserted retrogradely into the lingual artery. Other branches of the carotid artery in the vicinity of the superior cervical ganglion, except for those carrying the blood supply to the ganglion, were divided between ligatures. During intra-arterial injection, the external carotid artery distal to the lingual artery was occluded with a clip. Drugs were dissolved in 0.9% (w/v) saline. The injection volume was 0.5 ml. Intravenous injections were made through a cannula in a femoral vein.

The ganglion, cervical sympathetic nerves and adjacent blood vessels were submerged in a pool of medicinal liquid paraffin B.P. maintained at 37° C.

The following drugs were administered as the salts: acetylcholine chloride, physostigmine salicylate, atropine sulphate, hyoscine hydrobromide, tetraethylammonium bromide, sodium amylobarbitone, sodium pentobarbitone, sodium mephobarbitone, sodium phenobarbitone, sodium barbitone, benactyzine hydrochloride, azacyclonal hydrochloride and hydroxyzine hydrochloride. Doses of these compounds refer to the weights of salts. The following pure compounds were also used: methylpentynol, methylpentynol carbamate, paraldehyde, chloral hydrate, methyprylone, ethinamate, carbromal, 5-(1,3-dimethylbut-2-enyl)-5-ethylbarbituric acid (McN 481), perphenazine, pecazine, meprobamate, mephenesin, troxidone, phenytoin, bemegride, leptazol and amiphenazole.

RESULTS

The intra-arterial injection of methylpentynol carbamate to the cat superior cervical ganglion during continuous supramaximal stimulation of the preganglionic cervical sympathetic nerve produced a sharp fall-off in the response of the nictitating membrane, lasting several minutes (Fig. 1,*a*). This effect was due to an action of the drug on transmission through the ganglion because no effect was observed when the drug was injected during stimulation of the postganglionic cervical sympathetic trunk (Fig. 1,*b*). The intra-arterial injection of methylpentynol or of paraldehyde also produced a transient block of ganglionic transmssion, as previously reported (Quilliam, 1959).

Ganglion-block could be more conveniently studied by delivering short bursts (15 or 30 sec) of stimuli alternately to pre- and postganglionic nerve trunks. Fig. 2 shows how the intra-arterial injection of ethinamate or of methyprylone to the superior cervical ganglion selectively blocked the response of the nictitating membrane to preganglionic stimulation without affecting the response to stimuli applied postganglionically.

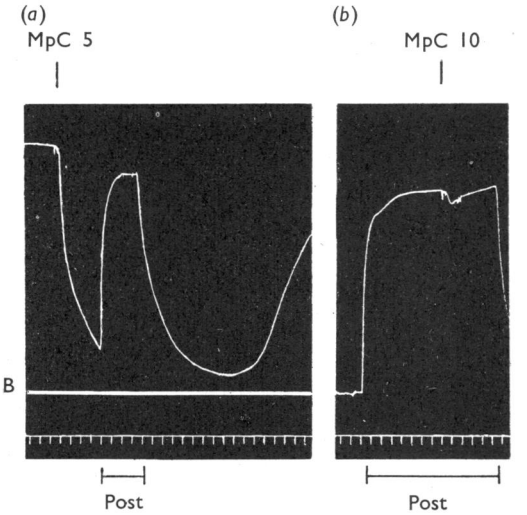

Fig. 1. The blocking action of methylpentynol carbamate on ganglionic transmission in a cat (3·1 kg., chloralose anaesthesia). Records of the contracture of the cat nictitating membrane produced by continuous supramaximal stimulation at 10 shocks/sec. of (*a*) the preganglionic and (*b*) the postganglionic cervical sympathetic nerve trunks. The postganglionic nerve was also stimulated briefly in (*a*) for the duration of the line marked Post. The fully relaxed state of the nictitating membrane in record (*a*) is indicated by the line B. At MpC 5 and MpC 10, doses of 5 and 10 mg respectively of methylpentynol carbamate were injected intra-arterially to the superior cervical ganglion through the lingual artery while the external carotid artery was occluded. Time marks, 30 sec.

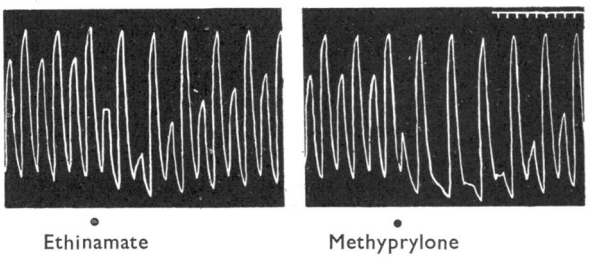

Fig. 2. The blocking actions of ethinamate and methyprylone on ganglionic transmission in a cat (2·5 kg, chloralose anaesthesia). Records of the contractures of the cat nictitating membrane elicited by supramaximal stimuli delivered at 10 shocks/sec alternately to the pre- and postganglionic cervical sympathetic nerves for 30 sec each, with 30 sec rest periods in between. The responses to preganglionic stimulation are the smaller. 0·5 ml of a saturated aqueous solution of ethinamate, containing approximately 0·5 mg, and 30 min later, 250 μg of methyprylone were injected intra-arterially to the superior cervical ganglion. Time marks, 30 sec.

Selective block of the response of the nictitating membrane to preganglionic stimulation was also observed following the intra-arterial injection of the following drugs: tetraethylammonium, hexamethonium, atropine, benactyzine, azacyclonol, mephenesin, chlorbutol, carbromal, amylobarbitone, pentobarbitone, mephobarbitone, phenobarbitone, barbitone, barbituric acid, troxidone, phenytoin and hydantoin.

The intra-arterial injection of chloral hydrate did not block, but potentiated, the response of the nictitating membrane (Brown, 1962).

The phenothiazine compounds, pecazine and perphenazine, reduced the response of the nictitating membrane to both pre- and postganglionic cervical sympathetic stimulation. Fig. 3 contrasts the typical selective ganglion-blocking action of tetraethylammonium with the nonselective action of intra-arterially injected perphenazine on the alternately stimulated preparation. The action of perphenazine was also notable for its prolonged duration, so that in Fig. 3 the effects of the first injection of perphenazine had not completely worn off by the time the second injection was made 45 min later.

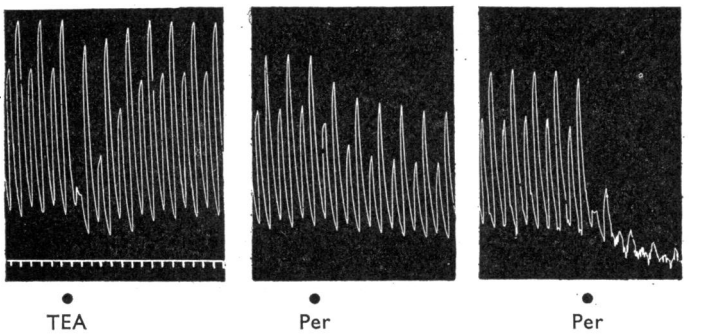

Fig. 3. Comparison of the action of tetraethylammonium and perphenazine on the nictitating membrane preparation of a cat. (3·9 kg, chloralose anaesthesia). Records of the contractures of the cat nictitating membrane elicited by supramaximal stimuli at 10 shock/sec delivered alternately to pre-and post-ganglionic cervical sympathetic nerve trunks for 15 sec. each, with rest periods of 15 sec between bursts of stimuli. The responses to preganglionic stimulation are the smaller. At TEA, 50 μg of tetraethylammonium, and, 2 hr later, at Per 50 μg of perphene azine were injected intra-arterially to the superior cervical ganglion. An interval of 45 min elapsed between each injection of perphenazine. Note that perphenazine depressed the respons- of the membrane to both pre- and postganglionic stimulation, whereas tetraethylammonium affected only that to preganglionic stimulation. Time marks, 30 sec.

The intra-arterial injection of hydroxyzine also frequently depressed the response of the nictitating membrane to postganglionic stimulation, but this postganglionic action did not account entirely for its effect on the response to preganglionic stimulation.

The technique of intra-arterial injection requires the drug tested to have a high aqueous solubility. Saturated solutions of glutethimide, thalidomide, captodiamine and meprobamate, which are poorly soluble in water, failed to produce any material block of ganglionic transmission when injected by this route.

Intravenous injections

The intravenous injection of methylpentynol or of its carbamate ester decreased the response of the nictitating membrane to both pre- and postganglionic stimulation. These compounds also produced a fall of blood pressure and a profound depression

of breathing necessitating artificial ventilation, effects which have been noted by Marley (1959). The intravenous injection of some other drugs whose action after intra-arterial injection was restricted to the superior cervical ganglion also produced a nonselective depression of the responses to both pre- and postganglionic stimulation —notably, paraldehyde, mephenesin, barbitone, azacyclonol, benactyzine and hydroxyzine. However, even by the intravenous route of injection, tetraethylammonium, hexamethonium, atropine, amylobarbitone, pentobarbitone, mephobarbitone, phenobarbitone and troxidone preferentially blocked the effects of preganglionic stimulation.

Experiments using tetraethylammonium showed that the intravenous dose required to block ganglionic transmission was about fifty-times higher than the intra-arterial dose (mean, 48-times; range, 22 to 120; n=14).

Quantitative estimation of ganglion-blocking activity

The extent to which ganglionic transmission was impaired by the intra-arterial injection of central depressant drugs depended closely upon the dose of drug injected (Fig. 4). This allowed fairly accurate estimations of relative activities. Since very

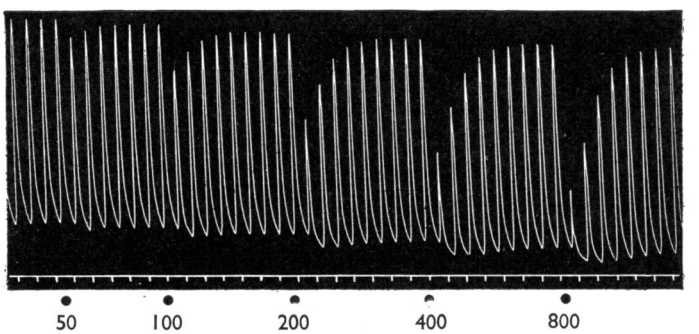

Fig. 4. The effect of successively doubling the dose of pentobarbitone injected intra-arterially to the superior cervical ganglion on the response of the cat nictitating membrane to preganglionic cervical sympathetic stimulation. (Cat, 4·0 kg, chloralose anaesthesia.) The preganglionic nerve trunk was stimulated supramaximally at 50 shocks/sec for 15 sec during each minute. The numerals below the record indicate the doses of pentobarbitone injected (μg). Injections were made 15 sec before the onset of a burst of stimulation. Time marks, 1 min.

high intra-arterial doses of some of the weaker agents were necessary to block transmission (see Fig. 1), preparations highly sensitive to block were used to reduce the systemic accumulation of drug. The sensitivity to block can be enhanced by stimulating the preganglionic nerve at the high frequency of 50 shocks/sec (Morrison & Paton, 1953). In our experiments, raising the frequency from 10 to 50 shocks/sec had the effect of approximately halving the dose of methylpentynol or tetraethylammonium required to block transmission. To obtain a steady response at this frequency, short bursts of stimuli separated by periods of rest were necessary; a burst of stimuli applied for 15 sec each minute to the preganglionic trunk proved satisfactory (Fig. 4). Intermittent stimulation had the added advantage of ensuring a smooth and rapid recovery from block.

The activity of central depressant drugs on transmission was estimated by comparison with tetraethylammonium. In each cat, dose/response relations were established for two or three central depressant drugs in the manner illustrated in Fig. 4. A dose/response relation for tetraethylammonium was obtained before and after the dose/response relation for each of the central depressant agents. The reduction of the membrane contracture with each dose was expressed as a percentage of the initial contracture height and plotted graphically against the dose. Fig. 5

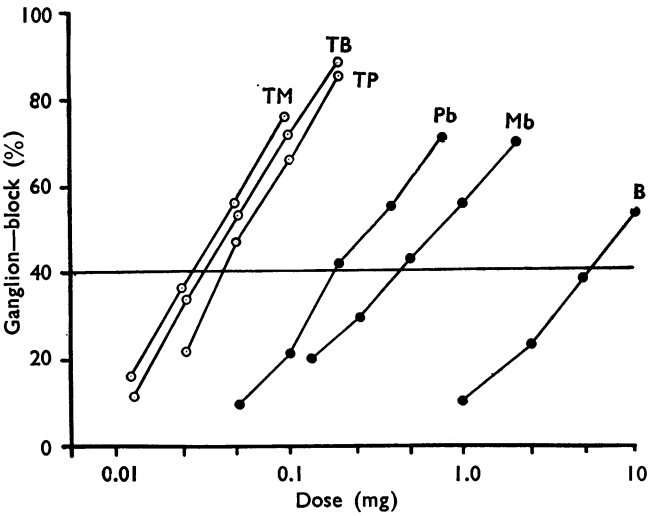

Fig. 5. A set of dose response curves obtained from one preparation in the course of an assay of the ganglion-blocking activity of three barbiturates using the technique illustrated in Fig. 4. Ordinate : % reduction of the response of the cat nictitating membrane to preganglionic cervical sympathetic stimulation. Parameters of stimulation as in Fig. 4. Abscissa : dose of drug (mg) injected intra-arterially in 0·5 ml. of fluid. Pb = pentobarbitone ; Mb = Mephobarbitone; B = barbitone. TP, TM and TB are three dose/response curves for tetraethylammonium. Each point is the mean of values estimated before and after establishing the dose/response relation for each barbiturate.

shows dose/response curves for the ganglion-blocking action of three different barbiturates, together with the corresponding dose/response curves for tetraethylammonium, obtained from a single experiment. Each point on the dose/response curves for tetraethylammonium is the mean of two estimates, one made before and one after the corresponding series of barbiturate injections.

The dose of drugs reducing ganglionic transmission by 40% (the ED40 value) was determined from the dose/response curves and converted to molar units, and the activity of the central depressant drug expressed as a percentage of the activity of tetraethylammonium. This is termed throughout the text " relative molar activity." The relative molar activity of pentobarbitone in Fig. 5 was 27.8% of that of tetraethylammonium, that of mephobarbitone 8.24%, and that of barbitone 0.66%. Thus, all three barbiturate compounds were less active than tetraethylammonium. The mean relative molar activities of fifteen different central depressant drugs are listed in Table 1.

TABLE 1

GANGLION-BLOCKING ACTIVITY OF SOME CENTRAL DEPRESSANT DRUGS

The activity of the drugs in depressing transmission through the cat superior cervical ganglion is expressed as a percentage of the activity of tetraethylammonium, estimated from the relative molar concentrations of drug and tetraethylammonium required to reduce transmission by 40%. Each drug was compared directly with tetraethylammonium in the same cat. *The mean intra-arterial ED40 of tetraethylammonium was 33 ± 1 μg (0·16 m.mole).

Drug	Activity % Mean	Standard error	No. of determinations
Tetraethylammonium*	100		
Amylobarbitone	74·1	1·2	3
Pentobarbitone	33·9	4·5	3
Mephobarbitone	9·56	1·03	3
Phenobarbitone	6·74	0·82	3
Barbitone	0·78	0·05	3
Barbituric acid	0·45	0·09	3
Carbromal	6·97	0·52	4
Methylpentynol carbamate	1·20	0·17	4
Paraldehyde	0·71	0·07	3
Methylpentynol	0·24	0·05	3
Chlorbutol	<1·0		2
Benactyzine	17·5	3·7	3
Hydroxyzine	10·1		2
Azacyclonol	6·63		1
Mephenesin	1·12		1
Troxidone	0·38		1

TABLE 2

RELATION BETWEEN ANAESTHETIC ACTIVITY AND GANGLION-BLOCKING ACTIVITY

The anaesthetic blood level in column 3 was obtained from the sources indicated below. For mephenesin, benactyzine, hydrozyxine and azacyclonol, which do not produce true anaesthesia, a half-lethal dose was used. The value for the ganglion-blocking concentration is the mean experimentally observed ED40. References : [1] Tatum, Nelson & Kozelka, 1941 (rabbit) ; [2]Goldbaum, 1948 (rabbit); [3]Mark, Burns, Brand, Campomanes, Trousof, Papper & Brodie, 1958 (dog) ; [4]Butler 1952 (dog) ; [5]Dille, Linegar & Koppanyi, 1935 (dog) ; [6]Sollman, 1957 (man) ; [7]Levine, Gilbert & Bodansky, 1939 (dog) ; [8]Maynert & Klingman, 1960 (dog) ; [9]Wyngaarden, Woods & Seevers, 1947 (dog) ; [10]Marley, 1959 (cat) ; [11]Hutcheon *et al.*, 1956 (dog) ; [12]Lynes & Berger, 1957 (cat) ; [13]Jacobsen & Skaarup, 1955 (cat) ; and [14]Brown, Braun & Feldman, 1956 (dog).

Drug	ED40 on ganglion (mM)	Anaesthetic blood concentration (mM)	Concentration ratio : Anaesthesia / Ganglion ED40
Amylobarbitone	0·25	0·25[1,2]	1·00
Pentobarbitone	0·49	0·2[2,3]	0·42
Phenobarbitone	3·0	0·6[4]	0·20
Mephobarbitone	1·6	0·25[2,3,4]	0·13
Barbitone	18·7	0·8[3,5]	0·04
Carbromal	2·5	1·8[6]	0·70
Paraldehyde	7·1	3·0[7,8]	0·43
Mephenesin	9·8	3·5[9]	0·36
Methylpentynol carbamate	4·2	1·4[10]	0·30
Methylpentynol	24	2·1[10]	0·09
Hydroxyzine	1·7	0·1[11,12]	0·06
Benactyzine	0·95	0·03[13]	0·03
Azacyclonol	3·4	0·1[14]	0·03

It is of interest to see how far the activity of these drugs on ganglionic transmission compares with their activity as central depressant agents. In Table 2 are listed the mean molar concentrations of drug found to depress ganglionic transmission by 40% (column 2) and the molar blood concentrations obtaining during surgical anaesthesia induced by these agents (column 3). These values are expressed as a ratio in column 4. The anaesthetic blood concentration was chosen because, with most of these agents, anaesthesia provides a standard measure of central depression for which blood concentrations are available. However, mephenesin, benactyzine, hydroxyine and azacyclonol do not produce true anaesthesia, so half the lethal blood concentration is given for these drugs. The anaesthetic or lethal blood concentrations refer to direct measurements in animals, or were derived from the therapeutic doses in man.

Whereas with most agents the concentration affecting ganglionic transmission clearly exceeds the estimated anaesthetic blood concentration, in the case of amylobarbitone the two values are similar. Exley (1954) noted the pronounced ganglion-blocking activity of amylobarbitone and suggested that this might account for its hypotensive action. We have observed that the intravenous injection of doses of

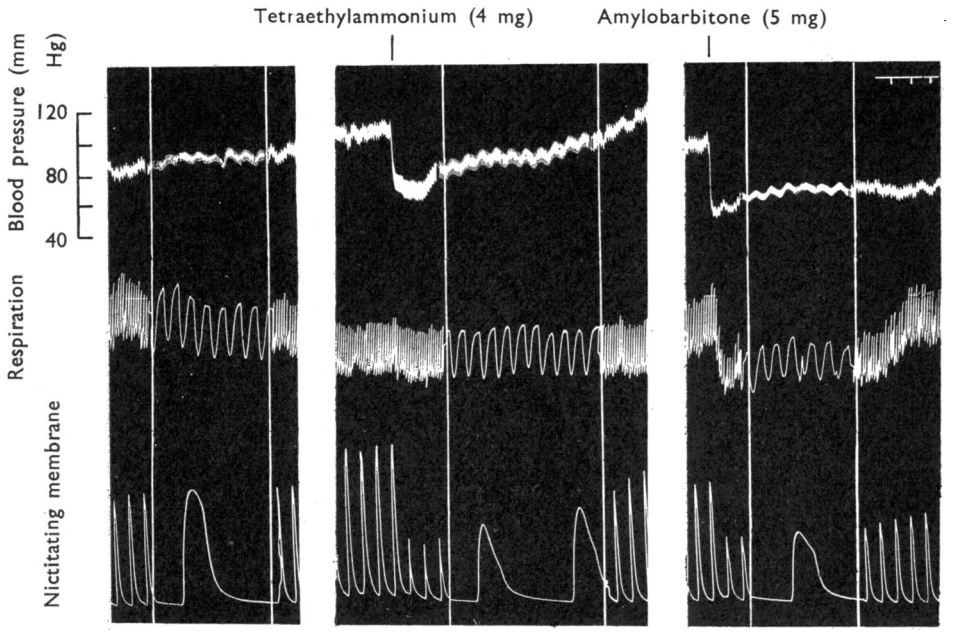

Fig. 6. A comparison of the effects of tetraethylammonium, and amylobarbitone on blood pressure respiration, and ganglionic transmission in an anaesthetized cat (2·5 kg, chloralose anaesthesia) Records, from above down, are : mean femoral arterial blood pressure ; abdominal respiratory movements, inspiration being denoted by a downward movement of the lever ; and contractures of the nictitating membrane elicited by supramaximal stimulation (50 shocks/sec) of the preganglionic cervical sympathetic nerve for 15 sec in each minute. The drugs were injected intravenously in the total doses indicated. Two minutes after injection, the drum speed was increased for short periods (between the vertical white lines) to facilitate observation of respiration. Time marks (referring to the slower drum speed only), 1 min.

amylobarbitone considerably below the anaesthetic dose of 50 mg/kg suggested by Garry (1930) produced a clear depression of ganglionic transmission, accompanied by a sharp fall of blood pressure. Fig. 6 shows a comparison of the effects of amylobarbitone and tetraethylammonium. An injection of 5 mg of amylobarbitone depressed the blood pressure by 45 mm Hg and reduced ganglionic transmission by 50%, whereas 4 mg of tetraethylammonium depressed the blood pressure by 40 mm Hg, but produced a greater decrease of ganglionic transmission (63%). The injection of amylobarbitone, but not that of tetraethylammonium, also depressed breathing.

The graphs in Fig. 7 relate the fall of blood pressure to the reduction of ganglionic transmission following the intravenous injection of amylobarbitone and tetraethylammonium into the same cat. The dose of amylobarbitone was progressively

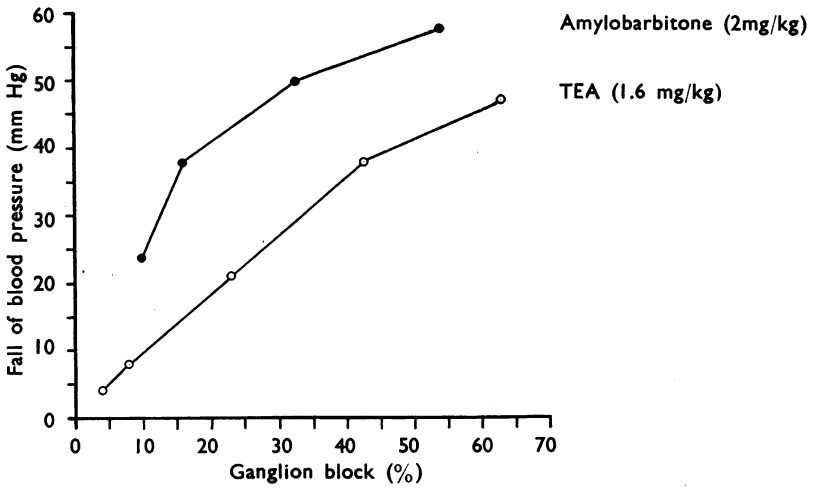

Fig. 7. The relation between the fall of blood pressure and the block of ganglionic transmission produced by intravenous injections of amylobarbitone and tetraethylammonium (TEA), determined by the technique illustrated in Fig. 6. Ordinate : blood pressure fall (mm Hg). Abscissa : % reduction of the response of the nictitating membrane to preganglionic cervical sympathetic nerve stimulation. Each point represents the effect of one dose of amylobarbitone (○) or of tetraethylammonium (●). The doses of amylobarbitone and of tetraethylammonium were progressively doubled to maxima of 2·0 and 1·6 mg/kg respectively.

increased by a factor of two to a maximum intravenous dose of 2 mg/kg, and the dosage of tetraethylammonium was likewise doubled to a maximum of 1.6 mg/kg. With both amylobarbitone and tetraethylammonium the magnitude of both fall of blood pressure and ganglion-block increased in proportion with the increase of dose, but amylobarbitone produced a greater fall of blood pressure for a given degree of ganglion-block than did tetraethylammonium. Whereas the slopes of the two curves are similar in the upper dose range, the slope of the curve for amylobarbitone is greater than that for tetraethylammonium in the lower dose range.

Similar curves were constructed to compare the effects of pentobarbitone, phenobarbitone and barbitone with those of tetraethylammonium. The curve with each

barbiturate compound was steeper than that with tetraethylammonium, and in all instances the difference exceeded that observed between amylobarbitone and tetraethylammonium. The barbiturate compounds may be arranged in the order in which the fall of blood pressure becomes progressively greater with a given percentage ganglionic block, as follows: amylobarbitone $\gg$ phenobarbitone $>$ pentobarbitone $\gg$ barbitone.

The action of central stimulant drugs on ganglionic transmission

Camp (1928) concluded that the sympathetic discharge produced by the central stimulant drug leptazol was central in origin, since the pupillary dilation produced by leptazol in rabbits was abolished by section of the cervical sympathetic trunk. However, Eckenhoff (1949) has claimed that leptazol can facilitate transmission through sympathetic ganglia and can oppose the ganglion-depressant action of barbiturate drugs.

We have investigated the effects of four central stimulant drugs on transmission through the cat superior cervical ganglion; leptazol, bemegride, amiphenazole and McN 481, the last being a barbiturate drug with convulsant actions in mammals (Cain & Kleis, 1959). To detect any facilitation of transmission, the ganglion was stimulated submaximally by partially transecting the preganglionic cervical

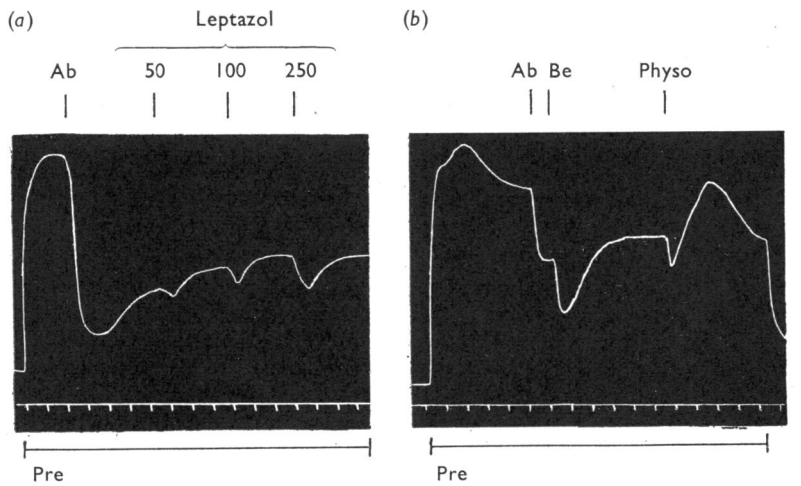

Fig. 8. The effect of analeptic drugs on the ganglion-blocking action of amylobarbitone. Records of the contracture of the cat nictitating membrane elicited by continuous supramaximal stimulation of the preganglionic cervical sympathetic nerve at 10 shocks/sec for the duration indicated by the horizontal line (denoted Pre) below each trace. Each record is from a different experiment. Time marks, 1 min.

(*a*) At Ab, 6 mg/kg of amylobarbitone were injected intravenously into a cat (4·9 kg, chloralose anaesthesia), causing a profound block of transmission. The subsequent injection of 50, 100, and 250 μg of leptazol intra-arterially to the superior cervical ganglion only increased the block.

(*b*) At Ab, 2 mg/kg of amylobarbitone were injected intravenously into a cat (1·8 kg, chloralose anaesthesia). The intra-arterial injection 1 min later of 200 μg of bemegride (Be) to the superior cervical ganglion increased the block of transmission. The subsequent intra-arterial injection of 50 μg of physostigmine (Physo) initially depressed, then transiently improved transmission.

sympathetic trunk between the point of pre-ganglionic stimulation and the ganglion (Kamijo & Koelle, 1952).

None of the four central stimulant drugs, in intra-arterial doses from 1 μg to 1 mg, augmented the response of the nictitating membrane to submaximal preganglionic cervical sympathetic stimulation. Instead, such effects as they had were to depress transmission, even though the largest dose of each evoked symptoms of central nervous stimulation such as bilateral twitches of the facial muscles, vibrissae and pinnae, or occasionally tremors or general convulsions. In all these experiments, control intra-arterial injections of 1 to 5 μg of nicotine always facilitated transmission.

Antagonism to the action of central depressant drugs on ganglionic transmission

The action of hexamethonium on the cat superior cervical ganglion can be reversed by the subsequent injection of physostigmine, neostigmine or nicotine (Mason, 1962 ; Brown, 1962). The intra-arterial injection of nicotine or of physostigmine (Fig. 8,*b*) also reversed the ganglion-blocking action of amylobarbitone. However, the intra-

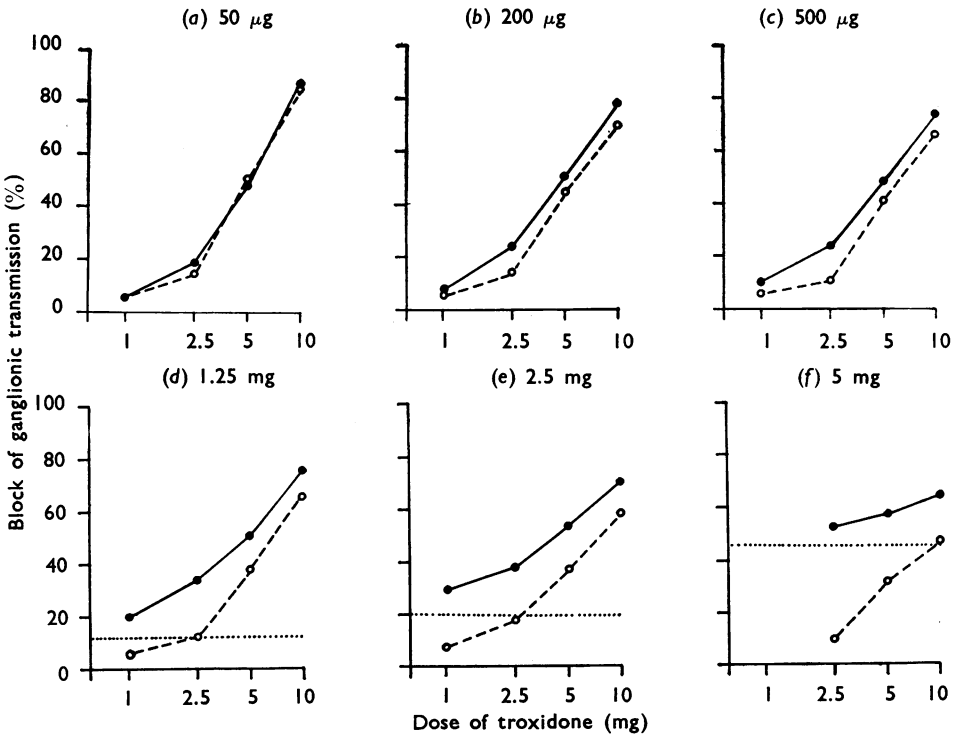

Fig. 9. Dose/effect curves for the ganglion-blocking action of troxidone in the absence and in the presence of leptazol. Ordinates : % depression of the response of the nictitating membrane to stimulation of the preganglionic cervical sympathetic nerve. Parameters of stimulation as in Fig. 4. Abscissae : dose of troxidone (mg) injected intra-arterially to the superior cervical ganglion in 0·5 ml. of fluid. Control curves (○ - - - ○) show the effect of troxidone alone, others (●——●) the effects of troxidone mixed with the amount of leptazol indicated above each graph. The effect of leptazol itself is indicated by the horizontal dotted lines in the lower three graphs.

arterial injection of bemegride or leptazol (Fig. 8) did not reverse, but tended to intensify, the action of amylobarbitone on the superior cervical ganglion. Likewise, leptazol did not modify the ganglion-blocking action of troxidone.

The interaction of troxidone and leptazol on ganglionic transmission was further studied by injecting troxidone mixed with various concentrations of leptazol intra-arterially to the ganglion (Fig. 9). The addition of 50 μg of leptazol to troxidone (graph *a*, full line) did not modify the action of troxidone (graph *a*, broken line). Addition of larger quantities of leptazol intensified the blocking action of troxidone alone (graphs *b* to *f*). This can be accounted for by the fact that large doses of leptazol could depress transmission in the absence of troxidone (graphs *d* to *f*, dotted lines); the blocking effect of smaller doses of leptazol, though insufficient to produce overt depression, was presumably manifest in the augmented depressant effect of the troxidone-leptazol mixture (graphs *a* to *c*).

It was also found that the admixture of various amounts of leptazol to amylobarbitone did not reduce, but only increased, the ganglion-blocking action of amylobarbitone.

DISCUSSION

The intra-arterial injection of many central depressant drugs to the cat superior cervical ganglion reversibly blocked the response of the nictitating membrane to preganglionic cervical sympathetic nerve stimulation without affecting the response of the membrane to postganglionic stimulation. This effect can be ascribed to a depression of transmission through the ganglion. On the other hand, the intra-arterial injection of pecazine and perphenazine, and occasionally of hydroxyzine, produced a prolonged reduction of the response of the nictitating membrane to postganglionic stimulation. This postganglionic action may have been due to the pronounced antiadrenaline properties of these compounds (Nieschulz, Popendiker & Sack, 1954; Hutcheon, Scriabine & Morris, 1956; Roth, Irwin, Eckhardt, Tabachnik & Govier, 1959).

The response of the membrane to postganglionic stimulation was also depressed when some of the less active agents, such as methylpentynol or barbitone, were injected intravenously, though not when they were administered intra-arterially. Since tests with tetraethylammonium indicated a fiftyfold difference between the intra-arterial and intravenous doses required to block ganglionic transmission, the intra-arterial dose of 10 to 20 mg of methylpentynol found necessary in our experiments to block ganglionic transmission would correspond to an intravenous dose of 0.5 to 1 g. It was hardly surprising that attempts to inject such large doses of methylpentynol intravenously resulted in nonspecific effects. This serves to show the advantage of using the method of intra-arterial injection to restrict the effects of drugs to the superior cervical ganglion.

There have been several previous reports that some anaesthetic or hypnotic drugs can depress ganglionic transmission (Elio, Jalon & Obrador, 1949; Larrabee & Posternak, 1952; Exley, 1954; Quilliam, 1957, 1959; Marley, 1959). The study of barbiturate drugs by Exley (1954) is of particular interest in that the method of measuring effects on transmission was identical to that which we have used, but

the route of injection was intravenous instead of intra-arterial. In Table 3 our own findings with regard to the activity of barbiturate drugs are compared with some of those of Exley (1954). It would seem that the method of intravenous

TABLE 3

COMPARISON OF THE RELATIVE ACTIVITIES OF SOME BARBITURATE DRUGS ON THE RESPONSE OF THE CAT NICTITATING MEMBRANE TO PREGANGLIONIC CERVICAL SYMPATHETIC NERVE STIMULATION WHEN MEASURED USING INTRA-ARTERIAL AND INTRAVENOUS INJECTIONS

The values for mean intra-arterial activity are taken from Table 1 but expressed as percentages of the activity of amylobarbitone (w/w). This allows direct comparison with the values for intravenous activity, which are taken from Exley (1954). Values are means with standard errors.

	Activity	
Drug	Intra-arterial	Intravenous
Amylobarbitone	100	100
Pentobarbitone	43·2 ± 4·8	42·5 ± 1·5
Mephobarbitone	13·0 ± 1·4	28·0 ± 3·6
Phenobarbitone	8·9 ± 1·1	14·2 ± 0·6
Barbitone	1·28 ± 0·1	17·1 ± 1·3

injection leads to higher estimates for the ganglion-blocking activity of the weaker barbiturates. The difference between the two sets of determinations is particularly marked with barbitone. Since barbitone was one of the drugs found by us to impair the response of the nictitating membrane to postganglionic stimulation when injected intravenously, an additional effect of barbitone on the membrane may in part account for the higher value for its activity on this preparation estimated by intravenous injection.

An analogous investigation into the action of several hypnotic drugs on neuromuscular transmission, using the frog iliofibularis muscle, has been undertaken by Quilliam (1955a). Comparison with the present study shows that chlorbutol, carbromal, methylpentynol and paraldehyde depressed both neuromuscular and ganglionic transmission. Chloral hydrate potentiated ganglionic transmission, probably because of an anticholinesterase action (Brown, 1962), but depressed the response of the frog iliofibularis muscle to motor nerve stimulation. Since the response of the muscle to direct stimulation was also reduced, depression of muscle fibres may have masked potentiation of nerve-muscle transmission. In contrast, barbiturates enhanced the response of the iliofibularis muscle to both direct and indirect stimulation. However, this was a mechanical result of the slowed muscle action potential (Quilliam, 1955b), so that the action of barbiturates on both nerve and muscle appears to be basically depressant.

Actions of central depressant drugs on ganglionic transmission may be regarded as possible side-effects to their therapeutic actions. According to the estimates in Table 2, only with amylobarbitone, pentobarbitone, paraldehyde, carbromal, mephenesin and methylpentynol carbamate do the anaesthetic blood concentrations approach within 30% or more of the concentration found to depress transmission through the cat superior cervical ganglion by 40%. Of these drugs, the last four are usually administered in doses so far below the anaesthetic dose that it is highly

unlikely that they would depress ganglionic transmission in man. On the other hand, pentobarbitone is regularly employed as an anaesthetic agent for laboratory animals. Since we have observed the intravenous injection of as little as 5 mg/kg of pentobarbitone to impair seriously ganglionic transmission, some depression of transmission might be expected in animals anaesthetized with 35 to 45 mg/kg of pentobarbitone. Support for this view is afforded by the observations of Covian & Funes (1955) that pentobarbitone anaesthesia elevated the threshold voltage of preganglionic cervical sympathetic stimulation required to elicit contractures of the cat nictitating membrane, and radically altered the relationship between stimulation threshold and stimulation frequency.

Although amylobarbitone is now rarely used as an anaesthetic in the laboratory, the ganglion-blocking activity of this drug is sufficiently great to suggest that some impairment of transmission might accompany the use of amylobarbitone as a sedative or hypnotic in man. Exley (1954) drew attention to the striking hypotensive action of amylobarbitone, and suggested that this might be due to ganglion-block. This suggestion is supported to some extent by the close temporal relation between the fall of blood pressure and block of ganglionic transmission produced by intravenous amylobarbitone (Fig. 6), and by the parallel increase of both effects as the dose of amylobarbitone is increased (Fig. 7). However, the hypotensive action of amylobarbitone is somewhat greater than that of tetraethylammonium for a similar degree of ganglion-block. This might be held to imply some hypotensive action of amylobarbitone additional to ganglion-block, perhaps central or perhaps directly on the blood vessels (Sollmann, 1957 ; Price, 1960). In this connexion, the failure of bemegride or leptazol to antagonize the ganglion-blocking action of amylobarbitone is interesting, for it suggests that the ability of these drugs to ameliorate the circulatory depression produced by barbiturates arises entirely from a central stimulant action.

The observations reported in this paper suggest that the reactions of ganglionic neurones to centrally-acting drugs differ considerably from those of central neurones. For instance, leptazol has been reported to antagonize the depressant action of troxidone on monosynaptic transmission in the spinal cord (Esplin & Curto, 1957), but no such antagonism was detected at the superior cervical ganglion. Neither were the central stimulant actions of lepazol, bemegride, amiphenazole or McN 481 reflected in a stimulant action on ganglion cells. Even the blocking action of central depressant drugs on ganglionic transmission showed no clear relationship to their anaesthetic potency. Further analysis of the mode of action of some of these drugs on ganglionic transmission may help to show the cause of these differences.

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