Br. J. Pharmac. (1974), 52, 51-59

THE EFFECTS OF ANTICHOLINESTERASES ON SYNAPTIC TRANSMISSION THROUGH NICOTINIC AND MUSCARINIC RECEPTORS IN RAT SYMPATHETIC GANGLIA *in vivo*

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1 Stimulation of the entire spinal sympathetic outflow at supramaximal voltage and 25-100 Hz, in the chlorisondamine-treated, pithed, adrenalectomized rat produced a delayed pressor response (late pressor response; LPR).

2 The LPR was abolished by phenoxybenzamine, bretylium or a small dose of atropine (25-50 μg/kg), suggesting the involvement of ganglionic muscarinic receptors.

3 In the presence of atropine at a dose level (15 μg/kg) which did not influence the LPR, the anticholinesterases physostigmine, neostigmine and Ro 02-0683 but not BW 284C51 markedly enhanced and prolonged the LPR, whereas all of them reduced the pressor responses to AHR-602.

4 After blockade of the ganglionic muscarinic receptors with a large dose of atropine (250 μg/kg) the four anticholinesterases did not influence responses to DMPP or noradrenaline and only slightly enhanced responses to preganglionic nerve stimulation at 6 Hz in the absence of chlorisondamine.

5 It is concluded that inhibition of butyrylcholinesterase accounts for the enhancement and prolongation of the LPR by anticholinesterases.

Introduction

Repetitive preganglionic nerve stimulation of sympathetic ganglia blocked by curare or nicotine (Eccles & Libet, 1961; Dunant & Dolivo, 1964) leads to the appearance of a triphasic ganglion potential. The initial N wave (the summated synaptic potential) is followed by a hyperpolarization (P wave or s.i.p.s.p.) (Eccles & Libet, 1961; Koketsu & Nishi, 1967) which in turn is followed some 200-500 ms later by a small, slowly arising but prolonged depolarization (the LN wave or s.e.p.s.p.). Both the s.i.p.s.p. and the s.e.p.s.p. are abolished by atropine at a dose level 1/100-1/1000th of that depressing the N wave (Eccles & Libet, 1961).

The s.i.p.s.p. may lead to reduced cellular sensitivity following preganglionic nerve stimulation (Koketsu & Nishi, 1968) whereas the s.e.p.s.p. can give rise to postganglionic nerve discharge (Nishi & Koketsu, 1966; Haefely, 1970). The latter responses may be enhanced following anticholinesterase administration (Gillis, Flacke, Garfield & Alper, 1968).

In the present experiments, blood pressure and heart rate increases, sensitive to small doses of atropine (25-50 μg/kg), were observed following a period of high frequency stimulation of the spinal sympathetic outflow in the 'ganglion blocked', pithed, adrenalectomized rat. In addition, the effects of anticholinesterases on these and other forms of ganglionic stimulation were examined, in order to try to establish the role of the sympathetic muscarinic ganglionic receptors in the process of ganglionic transmission.

Methods

C.S.E. rats (240-275 g) were used throughout the study. In experiments involving anticholinesterases, only male rats were used. Two to three hours after bilateral adrenalectomy, and treatment with corticosterone (5 mg/kg, i.m.) (Drew & Leach, 1971) the rats were pithed under halothane/ether anaesthesia, by means of a 16 gauge stainless steel wire, coated with Shellac varnish. A cleared area of the pithing rod was used to stimulate the entire spinal sympathetic pre-

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ganglionic nerve outflow (Gillespie & Muir, 1967). The stimulation parameters were 3-100 Hz; 20 V or 50 V; 0.03 ms pulse duration.

Pithed rats were artificially respired with a Palmer miniature ideal pump which delivered 1 ml/100 g of room air at a rate of 50 strokes/minute.

Blood pressure was recorded from the left common carotid artery with a Bell & Howell pressure transducer (type 4-326-L212). The arterial pulse was used to trigger an integrator in circuit with a Devices rate meter, thus providing an instantaneous heart rate recording.

Before experimentation tubocurarine (1 mg/kg i.v.) was administered to reduce skeletal muscle movement during the periods of stimulation. Heparin (2000 units/kg i.v.) was injected to prevent blood clotting.

Drugs, dissolved in 0.9% w/v NaCl solution (saline), were injected into the femoral vein via a polythene cannula. Anticholinesterases, however, were injected i.a. via a polythene cannula (pp 50) pushed down to the aortic arch through the right common carotid artery. This technique enabled small volumes ($\geq$50 μl) of concentrated solutions of the anticholinesterases to be injected directly into the arterial blood supplying the sympathetic ganglia.

Diastolic rather than systolic blood pressure changes were used for assessing cardiovascular responses, thus eliminating any influences of changes in cardiac output or contractile force.

Closely reproducible responses to electrical stimulation of the spinal sympathetic outflow were obtained if the stimulation periods were separated by intervals of 5 minutes. Injections of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), N-benzyl-3-pyrrolidylacetate methobromide (AHR-602) and noradrenaline were separated by intervals of 7.5 minutes.

In those experiments in which anticholinesterases were injected, the first test response to either electrical stimulation or drug administration was obtained 60 s after anticholinesterase injection. Hence test responses to sympathetic outflow stimulation were obtained 1, 6, 11, 16, 21 and 26 min after injection, whereas responses to DMPP, AHR-602 and noradrenaline were obtained 1, 8.5 and 16 min after anticholinesterase injection.

During the course of those experiments in which atropine (10-15 μg/kg) was administered to reveal the potentiation of the delayed pressor response (late pressor response; LPR) by anticholinesterases, the atropine was injected 12-13 min after the anticholinesterase; thus potentiation of the LPR was measured at 16, 21 and 26 min after anticholinesterase administration.

Statistical analysis

The significance of changes produced in the response to the various forms of ganglionic stimulation following anticholinesterase administration was assessed by the paired t test. Values of P of >0.05 were not considered significant.

Drugs used

N-benzyl-3-pyrrolidylacetate methobromide (AHR-602, A.H. Robins), atropine sulphate (Northern Pharmaceuticals), 1 : 5-bis(4-allyldimethylammonium phenyl)pentan-3-one diiodide (BW 284C51, Burroughs Wellcome), chlorisondamine hydrochloride (Ciba), corticosterone (Organon and Sigma), 1,1 dimethyl-4-phenylpiperazinium iodide (DMPP, R.N. Emanuel), physostigmine sulphate (B.D.H.), halothane (Fluothane, I.C.I.), mepyramine maleate (May & Baker Ltd.), neostigmine methylsulphate (Roche), noradrenaline acid tartrate (Hoechst), phenoxybenzamine (Smith, Kline & French), (2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide (Ro 02-0683, Roche), tubocurarine hydrochloride (Tubarine, Burroughs Wellcome).

Doses quoted in the text refer to the salts of the above compounds, with the exception of noradrenaline which is given as the base. Noradrenaline was stored as a 1 mg base/ml stock solution in 0.01M HCl and diluted as required.

Results

Entire spinal sympathetic outflow stimulation

It has previously been shown that sympathetic outflow stimulation at 3-12 Hz for 15 s at submaximal voltage (20 V) produces frequency related increases in blood pressure, sometimes accompanied by an increase in heart rate (Drew & Leach, 1971). In the present experiments it was found that atropine (10 μg-1 mg/kg) did not alter the magnitudes of these pressor responses, but that responses were abolished by bretylium (5-10 mg/kg) or phenoxybenzamine (2-5 mg/kg).

Chlorisondamine (0.5 mg/kg i.v.) completely abolished the pressor responses observed during sympathetic outflow stimulation at 3-12 Hz for 15 s at both submaximal (20 V) and supramaximal (50 V) stimulation voltages. Increasing the frequency of stimulation to 25, 50 or 100 Hz, at 20 V or 50 V, while reducing the period of stimulation to 5 s, produced a complex change in blood pressure, consisting of:

(1) a small (5-10 mmHg) initial increase in blood pressure which occurred *during* the period of stimulation.

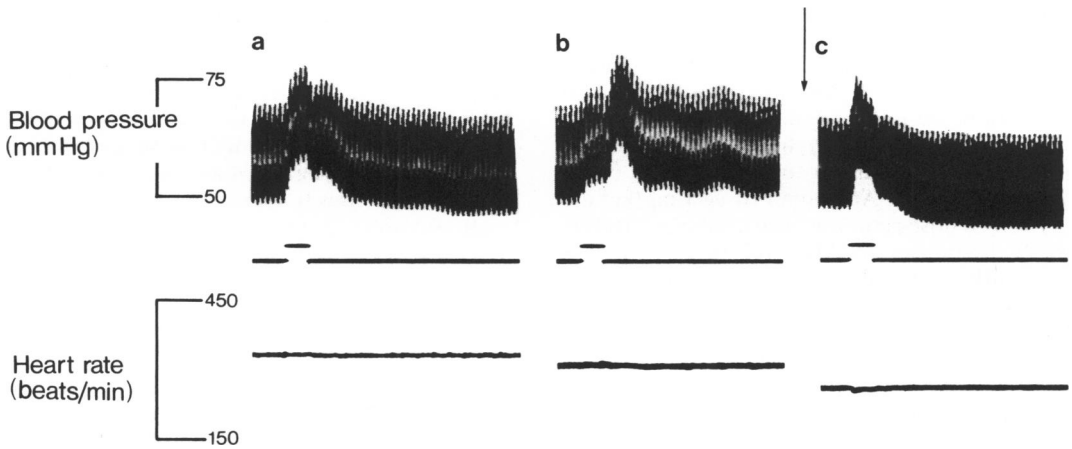

Fig. 1 Blood pressure (mmHg) and heart rate (beats/min) responses to stimulation of the entire spinal sympathetic outflow of the pithed adrenalectomized rat, for 5 s at 50 V and 50 Hz in the presence of chlorisondamine (0.5 mg/kg). Time marker bar = 5 s during stimulation period. (a) First stimulation: note the small rise in pressure produced during the period of stimulation which is followed by a delayed response which arises only after cessation of stimulation (the LPR). A small depressor response is finally observed. (b) After repeated periods of stimulation the LPR reaches a constant magnitude of about 15 mmHg. The depressor response is not apparent. (c) After atropine (25 μg/kg at arrow) only the small rise in blood pressure produced during the stimulation period is apparent. The LPR is abolished, leaving the depressor response which occurs immediately following cessation of stimulation.

(2) an increase in blood pressure (3-15 mmHg) which occurred shortly *after* cessation of stimulation and lasted for about 15-30 seconds. In view of the latency of this response it was termed the 'delayed pressor response' (late pressor response; LPR). In some cases the LPR was accompanied by an increase in the heart rate. The magnitude and duration of both effects were related to the voltage strength and stimulation frequency.

(3) a lowering of the blood pressure (4-8 mmHg) which succeeded the LPR and lasted some 3-4 minutes. These features are illustrated in Figure 1.

Successive periods of sympathetic outflow stimulation increased and prolonged the LPR and reduced the subsequent depressor response (Figure 1b). The optimal stimulation parameters required to produce the LPR were 5 s periods of stimulation at 50 V and 50 Hz. These parameters were used in all subsequent experiments.

The LPR was abolished by bretylium (5-10 mg/kg) and phenoxybenzamine (2-5 mg/kg). In contrast to the responses produced during sympathetic outflow stimulation in the absence of chlorisondamine (3-12 Hz; 20 V), the LPR was abolished by small doses of atropine (25-50 μg/kg, i.v.). Smaller doses of atropine (10-15 μg/kg) had no discernible effect on the LPR.

Following abolition of the LPR by atropine (25-50 μg/kg) the depressor response was seen to occur almost immediately upon cessation of sympathetic outflow stimulation (Figure 1c). The depressor response was found to be insensitive to larger doses of atropine (up to 1 mg/kg), propranolol (2 mg/kg) or mepyramine (2 mg/kg).

The initial increase in blood pressure observed during the period of preganglionic nerve stimulation (in the presence of chlorisondamine) was not reduced following a ten-fold increase in the dose of chlorisondamine, although occasionally it could be reduced by doses of tubocurarine (5 or 10 mg/kg), additional to the dose given for reducing muscle responses during stimulation.

Effects of ganglion stimulants on the blood pressure of the pithed rat

DMPP was chosen as an example of a 'pure' nicotinic ganglion stimulant, since it has little ganglion blocking activity subsequent to stimulation (Leach, 1957). DMPP (40-320 μg/kg) produced dose-dependent pressor responses accompanied by an initial decrease and subsequent increase in the heart rate.

AHR-602 (0.2-3.2 mg/kg), a muscarinic ganglion stimulant (Franko, Ward & Alphin, 1963), produced small, transient depressor responses followed by prolonged dose-dependent increases in blood pressure and heart rate.

Tubocurarine (1 mg/kg) had no effect on the dose-response relationship obtained with either DMPP or AHR-602. Atropine (10 μg-1 mg/kg) did not alter the dose-response relationship of DMPP. However, responses to AHR-602 (0.2-3.2 mg/kg) were slightly reduced (about 20%) by atropine 10-15 μg/kg and abolished by atropine 25 μg/kg or higher doses.

Responses to noradrenaline (250 and 500 ng/kg) were unaffected by either atropine or tubocurarine in doses up to 1 mg/kg.

Effect of anticholinesterases on the blood pressure of the pithed rat

The i.a. injection of physostigmine (100 μg-2.0 mg/kg) produced an initial, rapid increase in blood pressure (10-15 mmHg) commencing almost immediately after injection, and lasting 60-90 seconds. No change in heart rate accompanied this response. As the blood pressure returned a secondary, slowly developing increase in blood pressure (5-10 mmHg), accompanied by an initial increase and a subsequent, prolonged decrease in the heart rate, was seen to occur. This effect lasted some 4-5 minutes.

Neostigmine (100-400 μg/kg, i.a.) produced a monophasic pressor response (10-40 mmHg) commencing 30-60 s after injection, and lasting 5-10 minutes. Though increased during the first 1-2 min after neostigmine injection, the heart rate progressively declined to 100-150 beats/min and was accompanied by an increase in pulse pressure. Similar, though smaller, changes were observed following injection of Ro2-0683 (100-800 μg/kg).

BW 284C51 (10-400 μg/kg i.a.) did not alter the resting blood pressure or heart rate. Larger doses (0.4-2.0 mg/kg) produced a small and transient fall in blood pressure which recovered within 10-20 seconds. No change occurred in heart rate.

At the dose levels described above, physostigmine, neostigmine and Ro 02-0683 all produced salivation, micturition and defecation concurrent with the reduction in the heart rate. These peripheral muscarinic effects were prevented by atropine at a dose level (10-15 μg/kg) smaller than that (25-50 μg/kg) required to depress the LPR following electrical stimulation.

A dose level of 25-50 μg/kg of atropine abolished the secondary slow pressor response produced by physostigmine. However, doses of atropine as large as 250 μg/kg failed to depress the initial rapid response to physostigmine.

Administration of neostigmine (200-400 μg/kg) after atropine (10-50 μg/kg) still caused some pressor and heart rate increases which exhibited tachyphylaxis. Pretreatment with large doses of atropine (250 μg/kg) completely prevented cardiovascular responses to neostigmine.

Ro 02-0683 produced effects similar to those described for neostigmine but all the responses were abolished by atropine at a dose level of 50 μg/kg.

Atropine (up to 250 μg/kg) failed to influence responses to BW 284C51 (0.4-2.0 mg/kg).

The cardiovascular responses to physostigmine, Ro 02-0683 or BW 284C51 were unaltered by chlorisondamine (0.5 mg/kg) either before or after administration of atropine. However, the pressor and heart rate responses to neostigmine appeared to be slightly reduced by chlorisondamine (three preparations only).

Anticholinesterases and responses to ganglionic stimulation

After the attainment of LPRs of constant magnitude, the effects of the anticholinesterases on them were examined. Despite initial enhancement, physostigmine (100 μg-1.0 mg/kg), neostigmine (25 μg-1.5 mg/kg) and Ro 02-0683 (100 μg-1.0 mg/kg) each depressed the magnitude of the LPRs within 1-11 min after their administration, this effect being temporally associated with the onset of the peripheral muscarinic effects of the anticholinesterases. Cardiovascular disturbances thus hindered accurate measurement of the LPRs under these conditions.

The administration of atropine at a dose less than that required to depress the LPR (10-15 μg/kg), 12-13 min after the administration of the anticholinesterases, immediately restored the heart rate to pre-injection levels. Under these conditions it was found that physostigmine, neostigmine and Ro 02-0683 all enhanced, both in magnitude and duration, the LPRs subsequently produced 16, 21 and 26 min after anticholinesterase administration. The extent of the enhancement was related to the dose of anticholinesterase administered. The optimal LPR-potentiating doses of physostigmine, neostigmine and Ro 02-0683 were found to be 0.4, 0.2 and 0.2 mg/kg, respectively. Larger doses produced less enhancement or actually reduced the LPR.

The enhanced LPRs were generally accompanied by an increase in heart rate, even if no such response had been present prior to anticholinesterase administration. If previously present, the

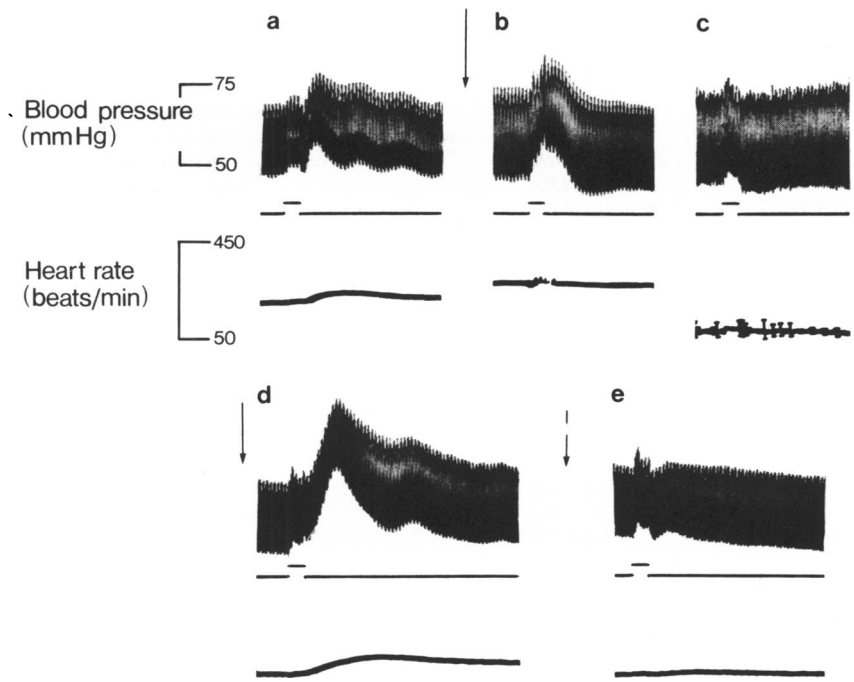

Fig. 2 The effects of Ro 02-0683 (0.2 mg/kg) on the late pressor response (LPR), produced by stimulation of the spinal sympathetic outflow at 50 V and 50 Hz for 5 s in the presence of chlorisondamine (0.5 mg/kg). (a) Control LPR. (b) Slight enhancement of LPR 60 s after administration of Ro 02-0683 (0.2 mg/kg). (c) Eleven min after injection of Ro 02-0683 (0.2 mg/kg at arrow) the heart rate is reduced and the LPR is markedly depressed. (d) One to two min after the injection of atropine (15 μg/kg at second arrow) a marked potentiation of the LPR is revealed. The heart rate increase is also enhanced. (e) The administration of a further 35 μg/kg of atropine (at third arrow) virtually abolishes both the LPR and attendant heart rate response (total dose of atropine: 50 μg/kg). Time marker bar = 5 s during stimulation period.

heart rate increase was enhanced to a similar extent as the LPR.

Both the enhanced LPRs and attendant heart rate increases were subsequently abolished by larger doses of atropine (50-100 μg/kg) (Figure 2).

In contrast, BW 284C51 (0.04-0.2 mg/kg) did not produce any peripheral muscarinic actions and caused only a slight enhancement of the LPR. The administration of atropine (10-15 μg/kg) did not reveal any further enhancement; in fact a slight reduction was occasionally observed. Larger doses of BW 284C51 (0.8-2.0 mg/kg) caused some depression of the LPR, which was intensified by the administration of atropine in low doses (10-15 μg/kg). Thus the optimal dose of BW 284C51 was considered to be between 0.2 and 0.8 mg/kg, and in subsequent experiments 0.4 mg/kg was chosen as the optimal dose.

Table 1 shows the maximal potentiation of the LPRs produced by optimal doses of the four anticholinesterases in the presence of atropine (10-15 μg/kg). In addition the effects of sub-optimal doses of Ro 02-0683 and BW 284C51 are shown. The smaller dose of Ro 02-0683 (20 μg/kg), by itself, enhanced the LPR, an effect not accompanied by a slowing of the heart rate or other symptoms of muscarinic activity. The subsequent administration of atropine (10-15 μg/kg) produced slight further enhancement of the LPR.

The effects of anticholinesterases, at the dose levels maximally potentiating the LPR, were examined on other aspects of ganglionic stimulation.

In contrast to their effects on the LPR, the anticholinesterases had little effect on the pressor responses normally obtained during sympathetic outflow stimulation at 6 Hz (20 V; 15 s) in the absence of chlorisondamine; in these experiments atropine (250 μg/kg) was administered at a dose level sufficient to prevent any effect at the ganglionic muscarinic receptors. Indeed, responses

Table 1 Potentiation of the late pressor responses (LPR), by anticholinesterases (after administration of atropine 10-15 μg/kg) in the pithed adrenalectomized rat.

Anticholinesterase administered	*LPR control response (100%) (mmHg and s.e.)*	*Change in LPR magnitude, expressed as % control response (= 100%) at various times after anticholinesterase administration and in the presence of atropine (10-15 μg/kg)*		
		16 min	*21 min*	*26 min*
Physostigmine (0.4 mg/kg) (n = 6)	16.6 ± 3.1	249.5 ± 31.2	219.0 ± 26.6	240.0 ± 37.2
Neostigmine (0.2 mg/kg) (n = 6)	19.6 ± 2.9	257.0 ± 27.6	233.0 ± 27.0	200.3 ± 24.5
Ro 02-0683 (0.02 mg/kg) (n = 5)	16.1 ± 1.8	168.4 ± 9.0	174.8 ± 8.6	175.2 ± 9.4
(0.2 mg/kg) (n = 6)	12.4 ± 1.8	222.2 ± 12.7	225.2 ± 11.2	218.2 ± 10.7
BW 284C51 (0.08 mg/kg) (n = 6)	18.3 ± 2.4	120.8 ± 8.7*	130.3 ± 7.4	134.8 ± 7.0
(0.2 mg/kg) (n = 6)	16.3 ± 1.8	115.0 ± 14.5*	138.7 ± 17.9	148.3 ± 21.2
(0.8 mg/kg) (n = 6)	15.6 ± 1.7	95.2 ± 2.5*	134.0 ± 13.0	139.0 ± 11.1

* P = > 0.05

were depressed by BW 284C51 particularly at the 60 s interval after administration (Figure 3).

In the presence of atropine (250 μg/kg), DMPP (160 μg/kg) or noradrenaline (500 ng/kg) produced pressor responses of a similar magnitude to those seen during sympathetic outflow stimulation at 6 Hz (20 V; 15 s).

The responses to DMPP and noradrenaline were not affected by physostigmine, neostigmine or Ro 02-0683. BW 284C51 slightly reduced the initial response to both DMPP and noradrenaline but subsequent responses were unaffected.

In contrast, responses to AHR-602 (0.8 mg/kg) were markedly depressed after the anticholin-

Table 2 The reduction of the diastolic pressure increase to AHR-602 (0.8 mg/kg) by anticholinesterases in the pithed adrenalectomized rat.

Anticholinesterase administered	*Control response to AHR-602 (0.8 mg/kg) (mmHg and s.e.)*	*Reduction in responses to AHR-602 (0.8 mg/kg) at different times after anticholinesterase administration. (mmHg and s.e.)*		
		1 min	*8.5 min*	*16 min*
Physostigmine (0.4 mg/kg) (n = 6)	22.5 ± 1.6	18.3 ± 1.7	8.2 ± 1.4	9.3 ± 1.7
Neostigmine (0.2 mg/kg) (n = 6)	34.2 ± 3.1	12.6 ± 1.7	7.0 ± 2.1	8.8 ± 1.5
Ro 02-0683 (0.02 mg/kg) (n = 5)	26.0 ± 1.8	18.2 ± 2.4	14.6 ± 2.4	16.6 ± 2.0
(0.2 mg/kg) (n = 6)	27.0 ± 2.0	6.8 ± 0.8	6.2 ± 0.5	6.0 ± 1.7
BW 284C51 (0.04 mg/kg) (n = 6)	30.3 ± 3.3	15.0 ± 3.9	23.0 ± 3.4	24.2 ± 3.4*
(0.4 mg/kg)	30.5 ± 3.4	4.0 ± 0.6	8.8 ± 1.4	10.5 ± 1.7

* P = > 0.05

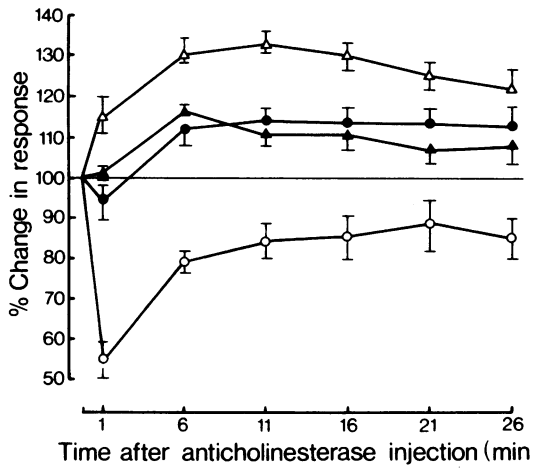

Fig. 3 The effect of anticholinesterases (given intra-arterially) on the magnitude of the pressor responses to sympathetic outflow stimulation at 6 Hz (20 V for 15 s) in the absence of chlorisondamine. Preparations were pretreated with atropine (250 μg/kg). Control responses = 100%. The figure shows means and vertical bars indicate s.e. mean. (▲) Physostigmine (0.4 mg/kg); (△) neostigmine (0.2 mg/kg) (●) Ro 02-0683 (0.2 mg/kg); (○) BW 284C51 (0.4 mg/kg).

esterase administration (Table 2). This effect was intensified, rather than reversed, by the subsequent administration of atropine (10-15 μg/kg).

Discussion

Stimulation of the sympathetic preganglionic nerves at high frequencies (25-100 Hz) for short periods, after blockade of the normal process of synaptic transmission by chlorisondamine, produced small increases in blood pressure both during and after the period of preganglionic nerve stimulation.

The sustained contraction of skeletal muscle seen during the period of high frequency stimulation, despite the presence of tubocurarine, probably restricted skeletal muscle blood flow, thus producing the initial small rise in blood pressure. Reactive hyperaemia may have caused the subsequent depressor response (Dusting & Rand, 1972).

The blood pressure increase which was seen after cessation of stimulation (the LPR) was adrenergic in origin, since it was abolished by phenoxybenzamine or bretylium. However, its abolition by small doses of atropine suggests that the LPR was a post-ganglionic consequence of activation of the ganglionic excitatory muscarinic receptor mechanism; that is, the slow excitatory postsynaptic potential (s.e.p.s.p.) or the 'LN' wave (Libet, 1964). The heart rate increase which often accompanied the LPR was probably of the same origin, since it too was abolished by atropine at the same dose level.

Similar effects have been observed following preganglionic sympathetic nerve stimulation of the nictitating membrane of the cat (Alkadhi & McIsaac, 1973) and dog (Chen, 1969, 1971).

The muscarinic effects (i.e. salivation and bradycardia) produced by anticholinesterase administration (presumably a consequence of cholinesterase inhibition) were prevented by doses of atropine smaller than those (10-15 μg/kg) required to reduce the LPR. It was subsequently found that atropine, at dose levels which abolished the LPR (50 μg/kg) also abolished the pressor responses to Ro 02-0683 and reduced those to neostigmine. In addition the slow secondary responses to physostigmine were abolished. The time course of these effects suggests that physostigmine, neostigmine and Ro 02-0683 stimulate the ganglionic muscarinic receptors directly (Takeshige & Volle, 1962, 1963; Hancock & Volle, 1970).

The initial, rapid pressor response following physostigmine administration was probably the result of direct stimulation of the vascular smooth muscle (McEwen, 1968), whilst non-muscarinic ganglion stimulating effects of neostigmine could have been responsible for the effects of this compound remaining after administration of atropine (50 μg/kg) (Garça, Hilton & Silva, 1970). BW 284C51 did not appear to stimulate ganglionic nicotinic or muscarinic receptors.

In the present experiments, after administration of a small dose of atropine (10-15 μg/kg), to prevent cardiovascular disturbances, the LPR following preganglionic nerve stimulation was seen to be markedly enhanced and prolonged by physostigmine, neostigmine and Ro 02-0683, whilst BW 284C51 caused only a moderate potentiation, as described by Libet (1967). The same doses of these anticholinesterases in the present experiments produced comparatively little enhancement of the pressor responses produced during sympathetic outflow stimulation in the absence of chlorisondamine when involvement of the ganglionic muscarinic receptor population was prevented by pretreatment with a large dose of atropine. Other authors have observed some potentiation of responses to low frequency, submaximal stimulation (Holaday, Kamijo & Koelle, 1954).

The reduction of the responses to DMPP and noradrenaline seen 60 s after administration of

BW 284C51 suggests a short lived depressant action of this compound. The more prolonged reduction in response to preganglionic nerve stimulation by this compound may be a result of a reduction in release of acetylcholine from the preganglionic nerve terminals.

Burn & Rand (1959) have suggested that anticholinesterases may enhance the release of noradrenaline from postganglionic adrenergic terminals. It seems unlikely that this mechanism accounts for the enhancement of either the LPR or the responses to preganglionic nerve stimulation, in the absence of chlorisondamine, since, in the present experiments, responses to DMPP remained unaffected.

Since the anticholinesterases did not influence responses to noradrenaline (in the presence of atropine) an action at the vascular α-adrenoceptor can also be excluded.

It has previously been suggested that anticholinesterases induce a persistent low level of depolarization of sympathetic ganglia, which results in a lowered threshold of muscarinic receptor activation (Takeshige & Volle, 1963). If such an action causes the enhancement of the LPR, then responses to AHR-602 should also have been enhanced. In fact, the contrary was found to be the case. Although the cardiovascular disturbances caused by some of the anticholinesterases almost certainly contributed to the depression of the responses to AHR-602, a more specific inhibitory or antagonistic action seems likely, in view of the fact that both BW 284C51 and Ro 02-0683 (0.02 mg/kg) depressed the responses to AHR-602 at dose levels which did not produce muscarinic side-effects. The effect may be related to a partial agonist effect of the anticholinesterases at the muscarinic receptor (Eccles, 1964). A similar, partial agonist, effect has been observed with pilocarpine (Roszkowski, Misekova, Volle, Hancock & Richards, 1971).

It is clear from the overall results that the anticholinesterases selectively enhanced the LPR. In male rats, Burgen (1949) reported that the *in vivo* potencies of physostigmine, neostigmine, Ro 02-0683 and 62C47 (similar to BW 284C51) in potentiating acetylcholine induced chromodacryorrhoea were 1, 8.6, 0.59 and 3.6 respectively. This effect was attributed to acetylcholinesterase inhibition. In the present experiments the potency ratios for the first three anticholinesterases in potentiating the LPR were 1, 2 and $2<1$, respectively.

The results obtained suggest that enhancement of the LPR may be a consequence of the inhibition of butyryl- rather than of acetylcholinesterase. It is considered unlikely that increased transmitter concentration at the ganglionic muscarinic receptors occurred as a consequence of overflow from neuromuscular junctions, since inhibition of acetylcholinesterase by BW 284C51 (Mendel & Rudney, 1943; Hawkins & Mendel, 1947) did not potentiate the LPR.

It is interesting to note that Kasa & Csernovszky (1967) observed that butyrylcholinesterase was associated with axo-somatic synapses in rat sympathetic ganglia. These observations lead one to speculate that ganglionic muscarinic receptors may be associated with axo-somatic rather than axo-dendritic synapses, at least in the sympathetic ganglia of the rat. The failure of atropine to depress the magnitude of the responses to sympathetic outflow stimulation at frequencies within the normal physiological range (3-12 Hz; 20 V; 15 s in the absence of chlorisondamine) suggests that the muscarinic receptors do not influence the extent of these responses, and so their contribution to ganglionic transmission in physiological circumstances remains unproven.

The authors wish to thank Organon and Roche for gifts of corticosterone and Ro 02-0683 respectively, and Mr C.J. Jones of A.H. Robins Co. Ltd. for the supply of AHR-602.

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(Received February 11, 1974)