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PARTICIPATION OF AN UNUSUAL GANGLIONIC PATHWAY IN THE MEDIATION OF THE PRESSOR EFFECT OF PHYSOSTIGMINE IN THE RAT

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In spinal rats physostigmine failed to produce a pressor response even after treatment of the animals with hexamethonium, whereas noradrenaline, McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) and AHR602 (3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide) all produced large pressor effects. In rats anaesthetized with urethane, hexamethonium completely abolished the pressor effect of dimethylphenylpiperazinium but only partially blocked the pressor response to physostigmine; pressor effects of noradrenaline, McN-A-343 and AHR602 were potentiated. Combined treatment with hexamethonium and atropine and with hexamethonium and cocaine, however, completely abolished the pressor effect of physostigmine; simultaneously the pressor effects of McN-A-343 and AHR602 as well as of dimethylphenylpiperazinium were also blocked. P-286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea) produced an early and a late block of the pressor effect of physostigmine; the initial block was due to an adrenergic blocking action while the late block was probably due to a dual action of the drug in abolishing the effects of both the nicotinic and non-nicotinic ganglion stimulants. Pressor responses to physostigmine, McN-A-343, AHR602 and dimethylphenylpiperazinium were abolished immediately after ganglion-blocking doses of nicotine. It is suggested that an unusual ganglionic pathway participates in the mediation of the pressor response to physostigmine in the rat, especially when the established ganglionic pathways are blocked.

The pressor response to physostigmine in the rat arises from an activation of central nervous sympathetic mechanisms (Varagić, 1955; Lešić & Varagić, 1961; Varagić & Vojvodić, 1962). It is usually assumed that this response is mediated by way of the established peripheral sympathetic pathways (Varagić, 1955; Dirnhuber & Cullumbine, 1955). In an earlier paper (Gokhale, Gulati & Joshi, 1963) we reported that hexamethonium, a highly selective ganglion-blocking agent but used in doses (100 mg/kg) far above those usually effective in blocking autonomic ganglia and which completely blocked the pressor responses to dimethylphenylpiperazinium, only partially blocked the pressor effect of physostigmine; this result suggested that the central sympathetic excitation initiated by physostigmine may be partly mediated by a ganglionic pathway insensitive both to block by hexamethonium and to stimulation by dimethylphenylpiperazinium. On the other hand, it is also possible that in the presence of hexamethonium

physostigmine raises blood pressure through a direct excitation of ganglionic receptor sites which are pharmacologically different from those usually associated with transmission through sympathetic ganglia. A similar mechanism has been suggested for the pressor effect of physostigmine in dogs after block of ganglia by hexamethonium (Hilton, 1961).

Our paper describes experiments which were designed to examine these possibilities.

METHODS

Albino rats of either sex weighing between 200 and 300 g were anaesthetized with urethane (1.5 g/kg, intraperitoneally, in a 20% w/v solution). Arterial blood pressure was recorded from a polyethylene cannula tied into a common carotid artery and connected to a Sanborn electromanometer (Model 121C) and a Sanborn Twin-Viso recorder (Model 60-1300). Injections were made through a polyethylene cannula in an external jugular vein. Drugs were injected in a constant volume of 0.1 ml., and washed in by the same volume, of 0.9% saline. Spinal rats were prepared by dividing the spinal cord at the level of the second cervical vertebra and destroying the brain by pushing a metal probe through the foramen magnum. Ether anaesthesia was used in the preparation of spinal rats. Artificial ventilation was given by means of a miniature "Ideal" pump (Palmer).

Drugs. Physostigmine salicylate, hexamethonium chloride, dimethylphenylpiperazinium iodide, atropine sulphate, cocaine hydrochloride, (±)-noradrenaline hydrochloride, *N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea (P-286), 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide (AHR602) were used; the doses refer to the salts. Adrenaline base and nicotine base were dissolved in 0.9% saline immediately before injection and the doses refer to the bases. Heparin (1,000 U/kg) was injected intravenously as an anticoagulant.

Design of experiments

Pressor responses to adrenaline or noradrenaline were used to test vascular reactivity. Dimethylphenylpiperazinium was used as a classical or nicotinic ganglion stimulant (Levy & Ahlquist, 1962). McN-A-343 and AHR602 were used as unusual or non-nicotinic ganglion stimulants (Roszkowski, 1961; Franko, Ward & Alphin, 1963). Hexamethonium was used as a conventional ganglion-blocking agent. Atropine and cocaine were used as drugs which selectively block the action of the non-nicotinic ganglion stimulating agents (Roszkowski, 1961; Jones, Gomez Alonso de la Sierra & Trendelenburg, 1963). P-286 and nicotine antagonize ganglion stimulants of both categories (Levy & Ahlquist, 1962; Jones *et al.*, 1963), and were used in this capacity.

A fixed dose (60 μg/kg) of physostigmine, injected intravenously at 30 to 40 min intervals, produced a rise of blood pressure, which was remarkably constant in magnitude for a period of 5 to 6 hr during individual control experiments, although the value varied from rat to rat (50 to 75 mm Hg). After two equipressor responses to physostigmine had been obtained, the following "test drugs" were injected at 15 min intervals: noradrenaline (1 to 1.5 μg/kg), or adrenaline (1 to 1.5 μg/kg), dimethylphenylpiperazinium (60 to 100 μg/kg), McN-A-343 (100 to 200 μg/kg) and AHR602 (300 μg/kg). This sequence was followed by the administration of the blocking drugs; after a specified interval the responses to physostigmine and the "test drugs" were again determined.

RESULTS

Spinal rats

In each of five spinal rats, physostigmine (60 μg/kg), administered intravenously 40 min after cutting the spinal cord, did not increase blood pressure. However,

responses to all the " test drugs " were intact. In six other experiments hexamethonium (10 to 15 mg/kg, in divided doses) was administered 40 min after cutting the spinal cord, and physostigmine, even in doses two- to three-times the usual effective dose, then failed to increase blood pressure. In these animals noradrenaline, McN-A-343 and AHR602 all produced large pressor effects (Fig. 1).

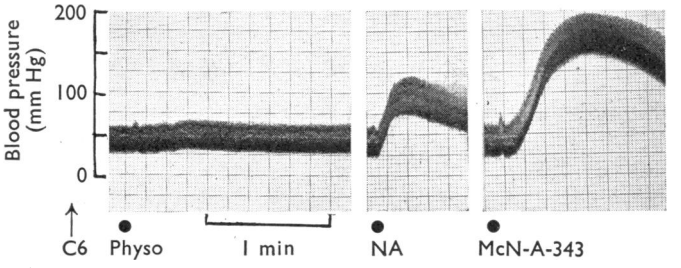

Fig. 1. Spinal rat, 260 g, artificial ventilation. Record of carotid arterial blood pressure. Responses to physostigmine (120 μg/kg at Physo), to noradrenaline (1 μg/kg at NA) and to McN-A-343 (150 μg/kg at McN-A-343). Hexamethonium (C6, 15 mg/kg in three divided doses) was injected 10 min before physostigmine was given. Time mark, 1 min. Injections were intravenous.

Effects of combined treatments with hexamethonium and atropine and with hexamethonium and cocaine

In recent years investigations of the unusual ganglion stimulant properties of McN-A-343 and AHR602 have shown that these drugs discharge ganglionic receptor sites which are pharmacologically different from those blocked by hexamethonium, and that their action is specifically blocked by atropine and cocaine (Roszkowski, 1961 ; Franko *et al.*, 1963 ; Jones, 1963). The effects of combinations of hexamethonium and atropine and of hexamethonium and cocaine on the pressor response to physostigmine were studied to determine whether these " muscarinic " ganglionic receptors participate in the mediation of this response.

Hexamethonium and atropine. In nine experiments hexamethonium (20 mg/kg) produced a small (15 to 20 mm Hg) but sustained fall of blood pressure ; 15 min later the pressor effect of physostigmine had been reduced by about half. Simultaneously the pressor effects of noradrenaline, McN-A-343 and AHR602 were considerably potentiated, whereas the response to dimethylphenylpiperazinium was completely abolished. A small dose (20 μg/kg) of atropine was administered at this stage, and 15 min later the pressor responses to physostigmine, McN-A-343 and AHR602 were totally blocked, but the response to noradrenaline remained (Fig. 2).

In six other experiments atropine (20 to 30 μg/kg) by itself produced only a small reduction of the pressor effect of physostigmine but totally blocked the effects of McN-A-343 and AHR602. Subsequent administration of hexamethonium (20 mg/kg) in the same animal, however, completely blocked the pressor response to physostigmine.

Hexamethonium and cocaine. In six experiments, cocaine (2 to 4 mg/kg) was injected intravenously 10 min after hexamethonium (20 mg/kg) had partially blocked

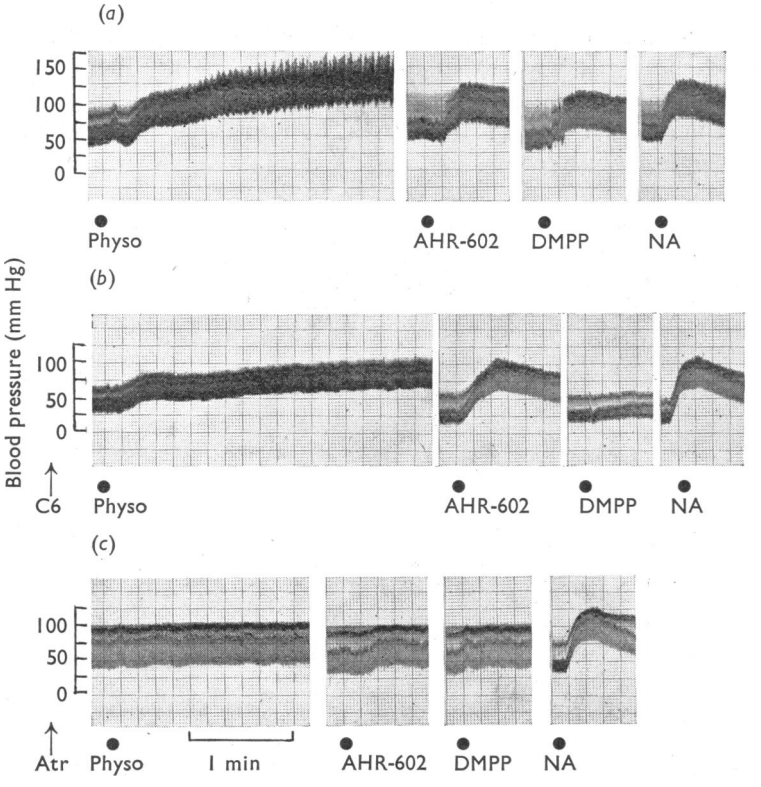

Fig. 2. Rat, 243 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to AHR-602 (300 μg/kg at AHR-602), to dimethylphenylpiperazinium (60 μg/kg at DMPP) and to noradrenaline (1·5 μg/kg at NA). Between (*a*) and (*b*), 20 mg/kg of hexamethonium (C6) were injected 15 min before (b) ; between (*b*) and (*c*), 20 μg/kg of atropine (Atr) were injected 15 min before (*c*). Time mark, 1 min. Injections were intravenous.

the pressor effect of physostigmine ; 10 to 15 min later physostigmine had no pressor action. The pressor responses to McN-A-343 and AHR602 were also totally blocked, whereas the pressor effect of noradrenaline remained potentiated.

Cocaine (2 to 4 mg/kg) by itself had a rather variable effect on the pressor response to physostigmine but invariably blocked the effects of McN-A-343 in each of three rats and of AHR602 in each of three. Out of six experiments only in three was the pressor effect of physostigmine reduced after the administration of cocaine ; in the remaining three cocaine either slightly potentiated (in two) or had no effect (in one) on the response. Subsequent administration of hexamethonium in the same animal, however, always completely abolished the pressor effect of physostigmine (Fig. 3).

Effect of P-286

P-286 (5 to 10 mg/kg, given slowly intravenously during 5 min) produced a fall (20 to 40 mm Hg) of arterial blood pressure. The drug also induced a conspicuous bradycardia ; in twelve experiments the heart rate was reduced by an average of

56% ; 10 to 15 min after the administration of P-286 the pressor effect of physostigmine as well as responses to all the " test drugs " were completely abolished (Fig. 4). Over the next 40 min the blood pressure and the heart rate gradually returned to control levels ; the pressor effects of physostigmine and the " test drugs " were now completely restored.

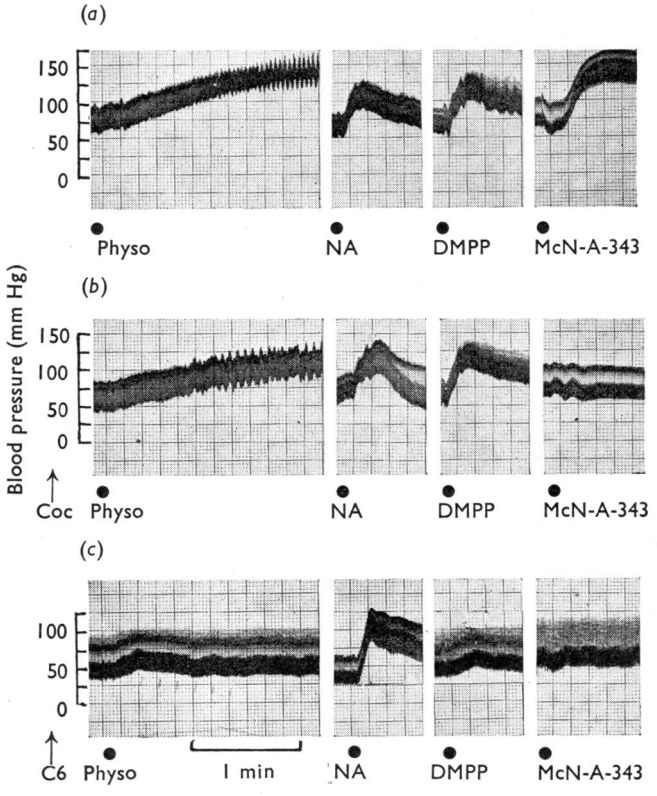

Fig. 3. Rat, 288 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to noradrenaline (1 μg/kg at NA), to dimethylphenylpiperazinium (80 μg/kg at DMPP) and to McN-A-343 (100 μg/kg at McN-A-343). Between (*a*) and (*b*), 2 mg/kg of cocaine (Coc) was injected 10 min before (*b*) ; between (*b*) and (*c*), 20 mg/kg of hexamethonium (C6) was injected 15 min before (*c*). Time mark, 1 min. Injections were intravenous.

At 1 to 1.5 hrs after the cumulative administration of 15 to 20 mg/kg of P-286, the blood pressure had fallen by 10 to 20 mm Hg but there was no bradycardia (eight experiments). Now the pressor responses to physostigmine, dimethylphenylpiperazinium, McN-A-343 and AHR602 were totally blocked but pressor effects of adrenaline and noradrenaline were potentiated (Fig. 4).

Effect of nicotine

Increasing doses (0.2, 0.4, 0.6 and 1 mg/kg ; 1.2 to 2.2 mg/kg total dose) of nicotine were injected intravenously in rapid succession until the drug no longer

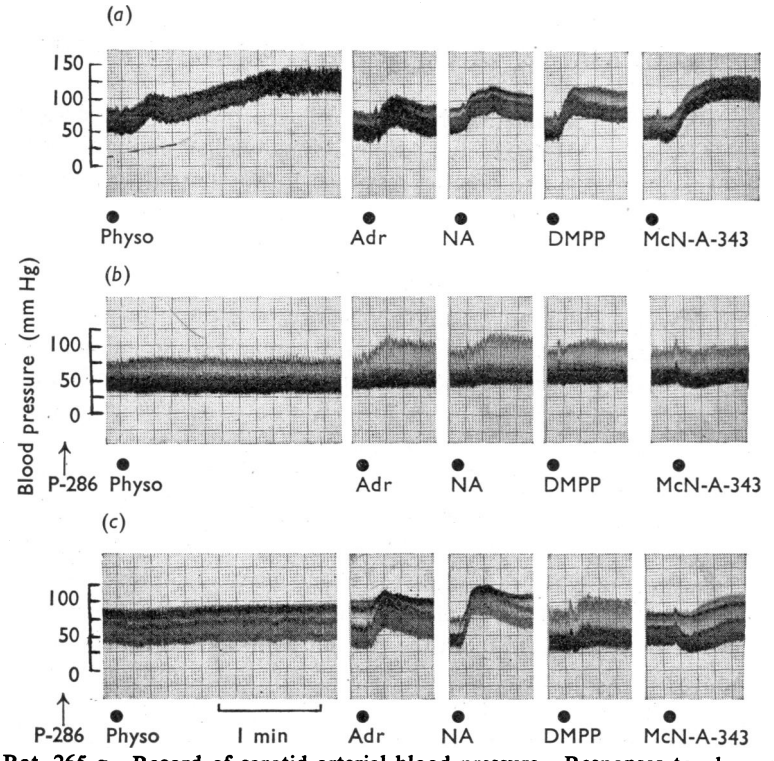

Fig. 4. Rat, 265 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to adrenaline (1 μg/kg at Adr), to noradrenaline (1 μg/kg at NA), to dimethylphenylpiperazinium (80 μg/kg at DMPP) and to McN-A-343 (150 μg/kg at McN-A-343). Between (*a*) and (*b*), 5 mg/kg of P-286 was injected 10 min before (*b*). Responses shown in (*c*) were obtained 1·5 hr. after cumulative administration of 15 mg/kg of P-286. Time mark, 1 min. Injections were intravenous.

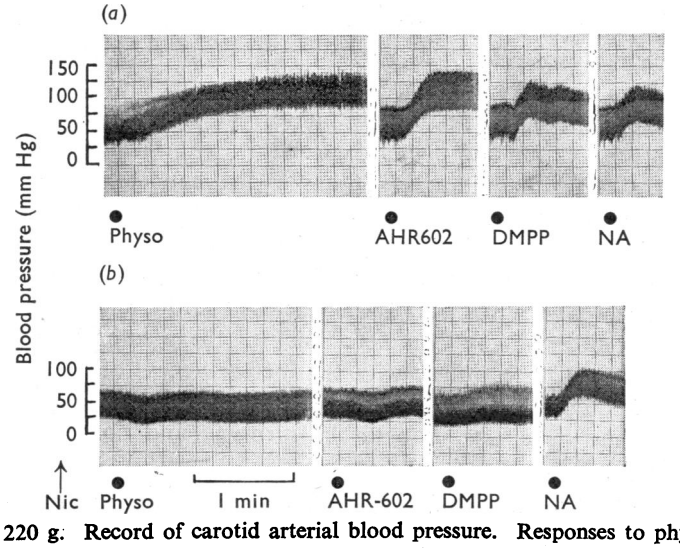

Fig. 5. Rat, 220 g. Record of carotid arterial blood pressure. Responses to physostigmine (60 μg/kg at Physo), to AHR602 (300 μg/kg at AHR602), to dimethylphenylpiperazinium (80 μg/kg at DMPP) and to noradrenaline (1 μg/kg at NA). Between (*a*) and (*b*), nicotine (Nic, 0·2, 0·4 and 0·6 mg/kg) was injected at 2 min intervals. The last injection of nicotine, which failed to produce a pressor effect, was given 2 min before (*b*). Time mark, 1 min. Injections were intravenous.

produced a pressor effect; immediately afterwards the pressor responses to physostigmine, McN-A-343, AHR602 and dimethylphenylpiperazinium were totally blocked (six experiments). The pressor response to noradrenaline was potentiated (Fig. 5).

DISCUSSION

In spinal rats, physostigmine failed to produce a pressor response even after treatment of the animals with hexamethonium, whereas noradrenaline, McN-A-343 and AHR602 all produced large pressor effects. This observation indicated that the pressor effect of physostigmine is due exclusively to a central stimulant action and also excluded the possibility of a peripheral action of physostigmine accounting for the failure of hexamethonium to block completely the response to physostigmine.

Soon after the administration of P-286 in a dose of 5 to 10 mg/kg, the pressor effects of physostigmine, McN-A-343, AHR602 and dimethylphenylpiperazinium were totally blocked. Pressor responses to adrenaline and noradrenaline were also simultaneously abolished. This result was somewhat unexpected in view of the finding of Levy & Ahlquist (1962) that the pressor and intestinal-inhibitory effects of adrenaline and phenylephrine were not reduced by P-286 (15 mg/kg). Gardier, Abreu, Richards & Herrlich (1960) also reported that P-286 had no adrenergic blocking action on the dog blood pressure and nictitating membrane. Nevertheless, in our experiments P-286 (5 to 10 mg/kg) invariably blocked the pressor effects of adrenaline and noradrenaline and this could adequately account for the abolition of the pressor effect of physostigmine by P-286 in these conditions, especially as the time course of the two effects was very similar.

Though originally described as a blocking agent specific for the adrenal medulla (Gardier et al., 1960), there is enough evidence in the recent literature to show that P-286 in sufficient doses (15 to 20 mg/kg) acts as a classical ganglion-blocking agent with ganglionic effects rather hard to distinguish from those of hexamethonium. Thus P-286 blocks blood pressure responses to dimethylphenylpiperazinium, peripheral vagal stimulation and bilateral carotid artery occlusion (Levy & Ahlquist, 1962; McLean, 1962). In addition, P-286 has an atropine-like action at sympathetic ganglia (Levy & Ahlquist, 1962). In the present experiments P-286 (1.5 to 2 hr after administration; 15 to 20 mg/kg, cumulative dose) produced a sustained fall of blood pressure, abolished the pressor effects of physostigmine, dimethylphenylpiperazinium, McN-A-343 and AHR602, but enhanced the pressor responses to adrenaline and noradrenaline. Complete block of the pressor response to physostigmine by P-286, which contrasts with the ineffectiveness of even large doses of hexamethonium, could clearly be due to a dual action of the agent on sympathetic ganglia in blocking the effects of both the nicotinic and non-nicotinic ganglion-stimulating agents.

Paton & Perry (1953) found that the ganglion block caused by nicotine is biphasic, the initial block by "depolarization" being followed by a later phase of "non-depolarizing block" which is similar to the ganglion block produced by hexamethonium. In our experiments nicotine, during the early depolarizing phase of its blocking action, completely abolished the pressor effects of dimethylphenylpiper-

azinium as well as those of McN-A-343 and AHR602 and at the same time totally blocked the pressor effect of physostigmine. Combined treatment with hexamethonium and atropine or with hexamethonium and cocaine also resulted in a total block of the pressor response to physostigmine.

From the evidence presented, it would be reasonable to infer that during block of the established ganglionic pathways (as indicated by a complete abolition of the pressor response to dimethylphenylpiperazinium) the pressor effect of physostigmine is mediated by way of an unusual ganglionic pathway resistant to block by hexamethonium but sensitive to block by atropine, cocaine, P-286 or nicotine. In fact there is good electrophysiological evidence for the existence of functionally distinct neural pathways through the cat superior cervical ganglion (Bishop & Heinbecker, 1932 ; Eccles, 1935).

Moreover, in recent years, the results of a number of studies have indicated that pharmacologically distinctive cholinoceptive sites are present in sympathetic ganglia (Ambache, Perry & Robertson, 1956 ; Eccles & Libet, 1961 ; Roszkowski, 1961 ; Takeshige & Volle, 1962, 1963). Thus, in addition to the well-established nicotinic receptors, atropine-sensitive muscarinic ganglion receptors appear to be fairly strongly established (Jones, 1963). Our results accord well with this concept of pharmacological heterogeneity of ganglionic cholinoceptive sites and indicate that the muscarinic ganglion receptors might subserve transmission in the suggested unusual ganglionic pathways.

Both atropine and cocaine (in some experiments) by themselves produced a small but appreciable inhibition of the pressor effect of physostigmine. This observation indicates that the muscarinic ganglion receptors might even ordinarily participate in the mediation of this response. This proposal is consistent with the observations of Takeshige & Volle (1962, 1963) that physostigmine unmasks the " late " component of the postganglionic discharge evoked by acetylcholine and that this " late " response is blocked by atropine.

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