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ANTINOCICEPTIVE ACTIVITY OF MUSCARINOMIMETIC AGENTS

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Summary. - *The effect of the agents with muscarinomimetic activity on the nociceptive transmission in the spinal cord was studied in spinal rats. Oxotremorine (5-20 μg/kg, i.v.), arecoline (0.25-1.0 mg/kg, i.v.), pilocarpine (5-20 mg/kg, i.v.) and aceclidine (0.25-1.0 mg/kg, i.v.) inhibited the nociceptive flexor reflex induced by intraarterial injection of bradykinin. Muscarinomimetics in the same doses and anticholinesterase agents physostigmine (1-4 μg, intrathecally) and galanthamine (25-100 μg, intrathecally) inhibited bradykinin-induced bioelectric activity in the spinal ventrolateral tracts. Atropine(1 mg/kg, i.v.) abolished the inhibitory effect of the agents tested on the nociceptive flexor reflex and bioelectric activity.*

Riassunto (Attività antinocicettiva di agenti muscarinomimetici). - *Nel presente studio è stata valutata, nel ratto spinale, l'attività di agenti muscarinomimetici sulla transmissione nocicettiva spinale. L'ossitremorina (5-20 μg/kg, i.v.), l'arecolina (0,25-1 mg/kg, i.v.), la pilocarpina (5-20 mg/kg, i.v.) e l'aceclidina (0,25-1 mg/kg, i.v.) inibiscono il reflesso flessore nocicettivo indotto dalla somministrazione intraarteriosa di bradichinina. I suddetti muscarinomimetici e farmaci anticolinesterasici, quali la fisostigmina (1-4 μg, intratecale) e la galantamina (25-100 μg/kg, intratecale), inibiscono l'attività bioelettrica indotta dalla bradichinina a livello del tratto spinale ventrolaterale. L'effetto inibitorio esplicato dai suddetti farmaci sul riflesso flessorio nocicettivo e sull'attività bioelettrica è antagonizzato dall'atropina (1 mg/kg, i.v.).*

Introduction

Some compounds with muscarinomimetic activity were reported by many authors to abolish nociception-induced reactions in animals and humans [1-6]. The antinociceptive effect appears after systemic administration of muscarinomimetics as well as after administration into the ventricles [5, 7-9] and some brain structures [10, 11, 5].

The purpose of the present paper has been to assess whether there is a spinal component in the antinociceptive action of agents stimulating muscarinic receptors. This hypothesis was based on autoradiographic studies in which muscarinic cholinoceptors were detected in the spinal cord in the neurons of *substantia gelatinosa* [12, 13] which plays an important role in the transmission of afferent nociceptive information. Moreover, Taylor *et al.* [14] report that intrathecally injected carbachol can inhibit tail flick in rats in response to thermal stimulation. This effect was eliminated by atropine. The effect of the muscarinomimetic agents on the transmission of nociceptive stimuli in the spinal cord was studied in spinal animals. Their efficiency was assessed according to the action on: *a*) flexor reflex, *b*) spontaneous and *c*) evoked bioelectric activity in the spinal ventrolateral tracts. For nociceptive stimulation bradykinin was used.

Methods

The experiments were carried out in male albino rats weighing 300 to 350 g. In the first series of experiments the action of muscarinomimetics on reflex nociceptive reaction was studied. The animals were anesthetized by halothane. A thin polyethylene catheter was introduced into the right femoral artery to reach aortal bifurcation. The spinal cord was cut at C_{VII} level and the rat was suspended in a hammock. The experiment was started 90 min after discontinuation of halothane. Bradykinin triacetate was injected intraarterially (1 to 5 μg, 0.2 ml). Flexor response to bradykinin was recorded. Depending on the dose of bradykinin the interval between the injections varied from 10 to 20 min. The agents

tested were administered after a constant response was achieved. Antinociceptive effect was evaluated according to the ability of the agents to inhibit the reflex flexion of the limb.

In the second series of experiments the bioelectric activity of afferent pathways was recorded in the spinal ventrolateral tracts. A catheter was introduced into the artery of halothane-anesthetized rats as described above and the neuromuscular blocking agent dipyronium was injected. The animals were artificially ventilated. The cerebrospinal canal was opened at T_{III} - T_{IV} level and the spinal cord was cut at C_{VII} level. For direct application of the agents to the spinal cord the catheter (external diameter 0.2 mm) was passed in the caudal direction through an additional incision in the meninx fibrosa as far as to reach the last thoracic vertebra. In this way the agent reached directly the presumable site of action. Bioelectric activity was recorded with a glass microelectrode (tip diameter about 1 μm, resistance about 5 Mohm) at the T_{IV} level from ventrolateral tracts of the right-hand portion of the spinal cord. The recorded signals were applied to the computing device of the frequency meter. The latter was connected to a pen recorder which enabled graphic registration of the frequency variations of neuronal discharges. The frequency of spontaneous and evoked activities after administration of the agents was compared with the control and expressed as a percentage. The arterial pressure was recorded in the carotid artery. The rectal temperature was maintained at 37 °C level. Bradykinin was injected in the same way as in the tests of the flexor reflex.

Muscarinomimetics (oxotremorine, arecoline, pilocarpine, aceclidine (3-acetoxyquinuclidine salicylate) and anticholinesterase agents (physostigmine, galanthamine) were tested. Anticholinesterase agents were injected intrathecally. Other agents were injected intravenously. The antimuscarinic agent metacine (1-2 mg/kg) which does not penetrate through the blood-brain barrier was injected to prevent the peripheral effects of systemically administered muscarinomimetics.

Results

The action of muscarinomimetics on the nociceptive flexor reflex

The results are presented in Table 1 and Fig. 1. Oxotremorine (5-20 μg/kg) arecoline (0.25-1.0 mg/kg), pilocarpine (5-20 mg/kg) and aceclicine (0.25-1.0 mg/kg) inhibit dose-dependently the nociceptive flexor reflex evoked by intraarterially injected bradykinin in spinal rats. All the agents tested produce an inhibitory action on the bradykinin-induced flexor reflex. The action of all the agents tested was prevented or abolished by atropine (1 mg/kg).

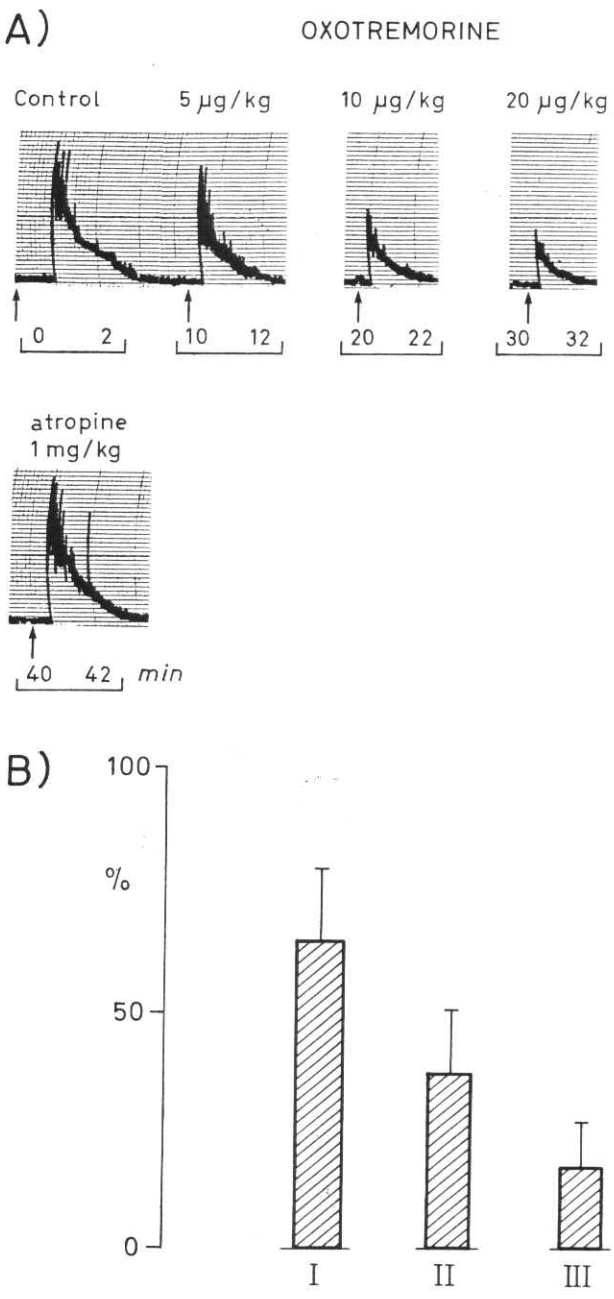

Fig. 1. - The effect of oxotremorine and atropine on the nociceptive flexor response in spinal rats. *A*) effect of oxotremorine and atropine on bradykinin-induced (1 μg) flexor response (the data of one experiment) ↑ - bradykinin (intraarterial injection); *B*) effect of oxotremorine 5 μg/kg (I), 10 μg/kg (II), and 20 μg/kg (III). (Mean ± SEM, no. = 6).

The action of muscarinomimetics and anticholinesterase agents on the bioelectric activity in spinal ventrolateral tracts

Three to 5 s after intraarterial injection of bradykinin the frequency of discharges in the spinal ventrolateral tracts appeared to increase abruptly. The muscarinomimetics tested inhibit in a dose-dependent manner both spontaneous and bradykinin-evoked

Table 1. - *The action of muscarinomimetics on nociceptive flexor reflex and bioelectric activity in the spinal ventrolateral tracts of spinal rats*

Agents	Doses (i.v.)	Amplitude of flexor reflex (percentage of the baseline level) Mean ± SEM, no. = 6	Bioelectric activity (frequency of neuronal discharges as a percentage of the baseline level) Mean ± SEM, no. = 6	
			spontaneous	evoked
Oxotremorine	5 µg/kg	68 ± 12	80 ± 10	87 ± 13
	10 µg/kg	42 ± 15	67 ± 5	69 ± 7
	20 µg/kg	19 ± 10	52 ± 8	51 ± 9
Arecoline	0.25 mg/kg	75 ± 11	91 ± 7	88 ± 7
	0.5 mg/kg	55 ± 10	73 ± 11	74 ± 9
	1.0 mg/kg	26 ± 12	55 ± 9	52 ± 12
Pilocarpine	5 mg/kg	92 ± 11	89 ± 7	95 ± 6
	10 mg/kg	65 ± 12	66 ± 7	82 ± 7
	20 mg/kg	38 ± 12	48 ± 12	62 ± 11
Aceclidine	0.25 mg/kg	62 ± 21	83 ± 11	77 ± 12
	0.5 mg/kg	30 ± 17	66 ± 7	57 ± 10
	1.0 mg/kg	7 ± 5	46 ± 13	41 ± 10

no.: number of rats in each group.

bioelectric activity in the ascending spinal pathways (Table 1). Atropine (1 mg/kg) enhanced both the spontaneous activity and that evoked by bradykinin. Atropine abolished the inhibitory effect of oxotremorine on bioelectric activity; both spontaneous and evoked activity were higher than the baseline level (Fig. 2). The action of arecoline, pilocarpine and aceclidine on the interneuronal nociceptive transmission in the spinal cord was similar to that of oxotremorine. The spontaneous and evoked activities in the ventrolateral tracts were inhibited by all the agents tested. Atropine abolished their inhibitory action.

For the assessment of the action of anticholinesterase agents physostigmine and galanthamine they were directly applied to the spinal cord through an intrathecal catheter. This route was chosen because after systemic administration these agents act upon both muscarinic and nicotinic peripheral cholinoceptors. To abolish the action on nicotinic cholinoceptors ganglion blocking agents were used in control experiments which led to a significant decrease of arterial pressure. This was followed by a dramatic fall of bioelectric activity in the ventrolateral tracts. Therefore, it was impossible to assess the action of the agents on the bioelectric activity in the ascending pathways under these conditions. For these reasons local application of anticholinesterase agents was used. Marked peripheral effects were prevented without the use of peripheral cholinoblockers.

The experiments have shown that physostigmine and galanthamine inhibit both the spontaneous and bradykinin-evoked activity in the ascending ven-

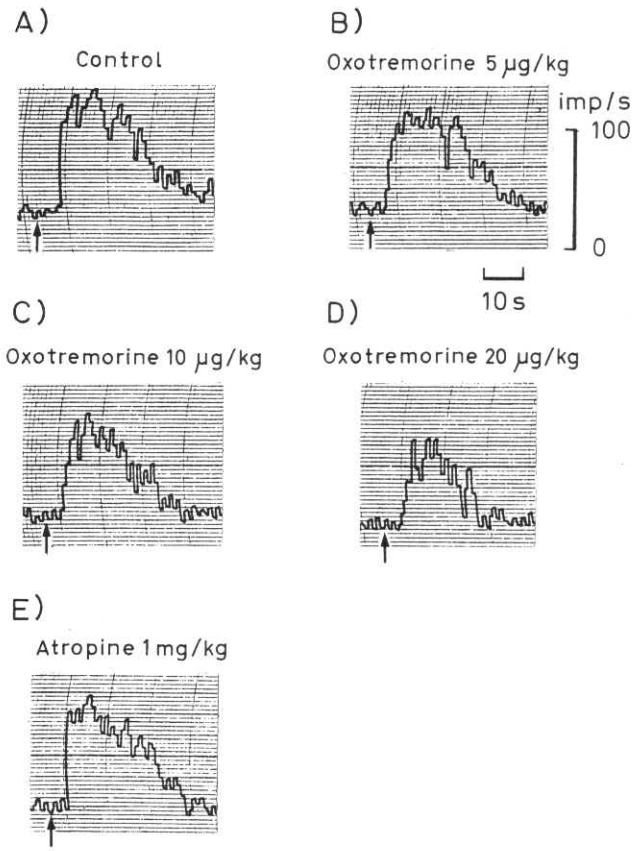

Fig. 2. - The effect of oxotremorine and atropine on the frequency of discharges in the spinal ventrolateral tracts. Bradykinin (↑) was injected every 10 min (1 µg) *A*) control; *B*) 10 min after oxotremorine 5 µg/kg, intravenously; *C*) 10 min after oxotremorine, 10 µg/kg, intravenously; *D*) 10 min after oxotremorine 20 µg/kg, intravenously; *E*) 10 min after atropine 1 mg/kg, intravenously.

Table 2. - *The action of anticholinesterase agents on the bioelectric activity in the spinal ventrolateral tracts*

Agents	Doses (intrathecally)	Biolectric activity (frequency of neuronal discharges as a percentage of the baseline level) Mean ± SEM no. = 6	
		spontaneous	evoked
Physostigmine	1 μg	87 ± 7	83 ± 11
	2 μg	71 ± 8	65 ± 9
	4 μg	46 ± 12	40 ± 10
Galanthamine	25 μg	86 ± 6	79 ± 7
	50 μg	75 ± 6	62 ± 7
	100 μg	53 ± 11	50 ± 6

no.: number of rats in each group.

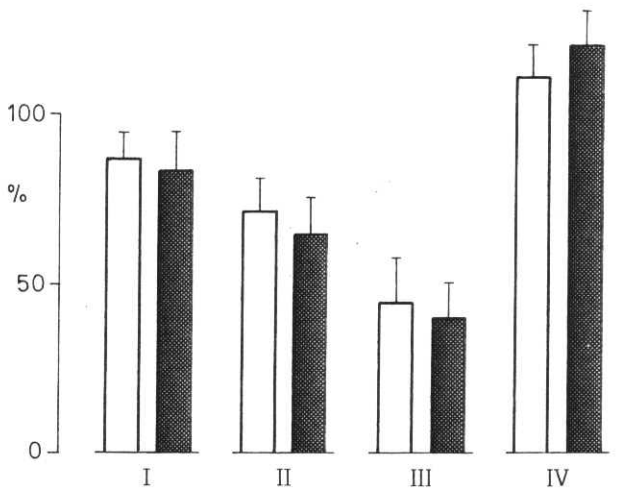

Fig. 3. - The effect of physostigmine and atropine on the bioelectric activity in the spinal ventrolateral tracts. The frequency of spontaneous discharges (light columns) and bradykinin-induced activity (dark columns) in percentage of the baseline level (Mean ± SEM, no. = 6)

I - physostigmine 1 μg, intrathecally

II - physostigmine 2 μg intrathecally

III - physostigmine 4 μg intrathecally

IV - atropine (1/mg/kg, intravenously) injected after physostigmine (2 μg intrathecally).

trolateral tracts (Table 2 and Fig. 3). Atropine (1 mg/kg) abolished the inhibitory effect of physostigmine and galanthamine on the bioelectric activity. The administration of atropine after anticholinesterase agents resulted in an increase in spontaneous and evoked activity as compared to the baseline level.

Discussion

The data obtained in different laboratories [15, 16] suggest that the stimulation of spinal muscarinic cholinoceptors in intact animals inhibits motor nociceptive responses. In the present study in order to abolish the descending supraspinal influences the experiments were carried out in the animals with dissected spinal cord. The inhibition of the flexor reflex and bioelectric activity in the spinal afferent tracts by muscarinomimetics under these conditions indicate a direct inhibitory action of these agents on the segmental transmission of nociceptive impulses [17]. The inhibitory effect was eliminated by atropine and, consequently, was expressed by the stimulation of muscarinic cholinoceptors located in the spinal cord. The data obtained suggest that the inhibition of interneuronal transmission in the spinal afferent pathways can play an important role in the mechanisms of antinociceptive action of muscarinomimetics.

Anticholinesterase agents physostigmine and galanthamine also inhibit the interneuronal nociceptive transmission at the spinal level. The two anticholinesterase agents inhibit the spontaneous bioelectric activity as well as the activity evoked by bradykinin. This effect was eliminated by atropine [18]. The experiments provided additional evidence for the participation of the agents stimulating central muscarinic cholinoceptors in the regulation of nociceptive transmission in the spinal cord. This constitutes one of the components of the antinociceptive mode of action of muscarinomimetics.

Autoradiographic studies of central muscarinic cholinoceptors carried out by Yamamura *et al.* [13] using [H^3] quinuclidinyl benzilate and [H^3] pirenzepine have shown that spinal muscarinic cholinoceptors are heterogeneous. [H^3] quinuclidinyl benzilate equally binds to muscarinic cholinoceptors located in the dorsal horn and in the lateral portion of spinal gray matter of the ventral horn, whereas [H^3] pirenzepine only binds to muscarinic cholinoceptors located in the former. Furthermore, Hartvig *et al.* (1987, personal communication) have found that antinociceptive effect induced by intrathecally administered carbachol is abolished by an M_1 receptor antagonist, pirenzepine, but not by M_2 receptor antagonist, AFDX 116. The authors concluded that the inhibition of nociceptive responses caused by intrathecal injection of muscarinomimetics may be related to the stimulation of M_1-cholinoceptors located in *substantia gelatinosa*. We believed it to be appropriate, therefore, to study the action of the selective M_1-cholinoceptor agonist McN-A 343 directly applied to the spinal cord on the nociceptive responses of laboratory animals. In our experiments McN-A (0.05-250 μg) was intrathecally injected to mice, and tail flick to thermal stimulation was tested. No changes of latent period of nociceptive response, however, were observed (unpublished data). Thus, possible role of one of the subtypes of spinal muscarinic cholinoceptors in the antinociceptive action of muscarinomimetics has yet to be established.

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