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DIFFERENCES IN EXCITATORY ACTION OF ACETYLCHOLINE AND METHACHOLINE TO HIGH-THRESHOLD C-FIBER MECHANOSENSITIVE CUTANEOUS SENSORY UNITS IN CATS

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Acetylcholine (ACh) and acetyl-β-methylcholine (methacholine, MCh), introduced through the blood stream into the intercellular space of the cat small intestine, excite the interoceptor of that organ, and, in concentrations as low as 1-10 ng/ml, induce relatively small and slowing rising pressor reflexes [3, 11]. An increase in the MCh concentration, even by 10^5-10^6 times, does not change the character of the reflexes. By contrast, if the ACh concentration is increased up to 10 μg/ml, it begins to act as a nocigenic stimulus: the pressor reflexes acquire the features characterizing the circulatory components of the nocifensive (defensive) response [2, 4, 11]. Beginning with a concentration of 5-50 μg/ml ACh behaves as an algogenic substance: if applied to the human skin denuded of the epidermis it elicits pain [10], MCh does not have this property even in a concentration of 1 mg/ml [10]. The information given above prompted us to compare responses of the same cutaneous sensory units (SU) when excited by ACh and MCh. We considered that such a comparison would reveal the differences between the character of responses of SU induced by ACh within the concentration range in which it exhibits its action of a nocigenic and algogenic (for man) stimulus. We shall consider responses only of those SU whose excitation threshold by a mechanical stimulus is high, and which therefore may be accepted as nociceptors [5, 12].

EXPERIMENTAL METHOD

Two segments of a subcutaneous nerve were isolated in nine cats, anesthetized with chloralose (40 mg/kg) and urethane (600 mg/kg): at the level of the knee joint, where the nerve was placed on a bipolar stimulating electrode, and in the proximal third of the thigh, where microbundles of fibers were isolated from the nerve. The nerve was tightly ligated proximally. Impulses of single C-fibers were derived by a monopolar technique by a platinum

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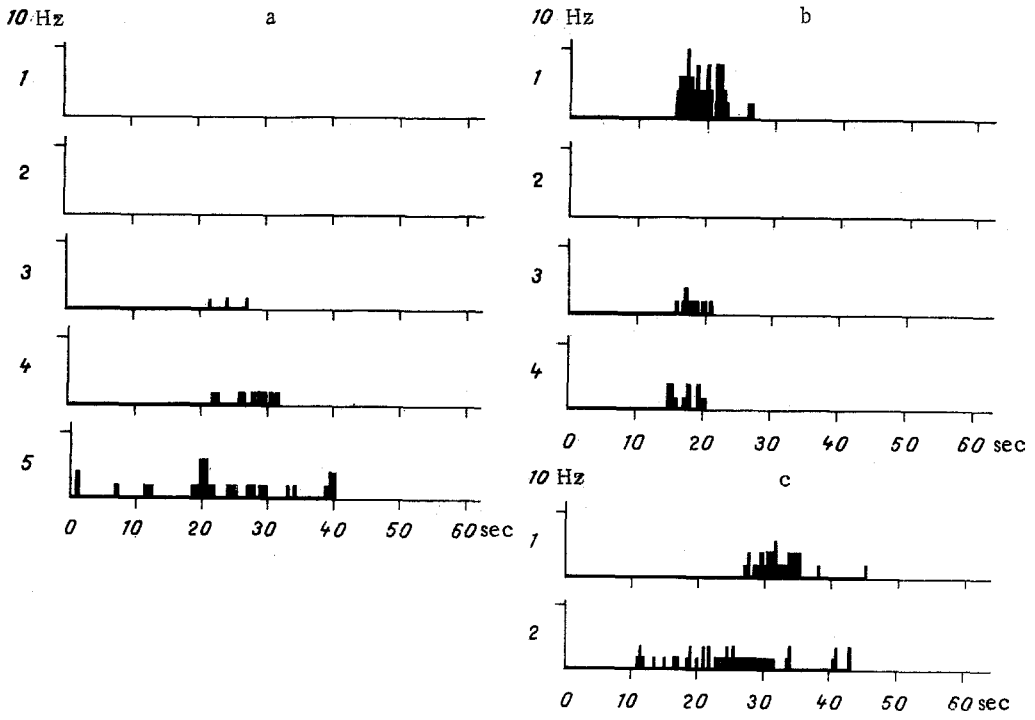

Fig. 1. Temporal histograms of discharges induced by ACh and MCh in three different SU. a: 1) Control injection of Ringer's solution; 2, 3, 4, 5) of ACh in concentrations of 0.02, 0.2, 2, and 10 μg/ml; b: 1 and 4) ACh in concentrations of 1 and 2 μg/ml; 2 and 3) MCh in concentrations of 0.2 and 2 μg/ml; c: 1 and 2) MCh in concentrations of 10 and 100 μg/ml.

electrode, introduced into a cavity formed from the skin and filled with mineral oil. Signals from the fibers were amplified within the 85-2000 Hz band and recorded on magnetic tape. Time and frequency histograms of spike trains of SU were plotted by computer. The fact that the SU axons belonged to the C class was confirmed by selective suppression of signals of C-fibers in the microbundle [1]. The velocity of conduction of action potentials along axons of SU was 0.7-2.1 m/sec. Mechanosensitive endings were located on the medial part of the skin of the leg, using Frey's bristles, 0.3-0.4 mm in diameter. Only high-threshold SU, which responded to a pressure of 16 g/mm^2 by not more than 1 or 2 spikes, and a well-marked discharge, were selected. The chosen SU were not excited at rest and were insensitive to subnocigenic heating of the skin (42°C). To ensure that ACH and MCh were placed mainly in the vascular bed of the skin region innervated by the subcutaneous nerve, small muscular and articular branches of the femoral artery were ligated. Ligatures were placed around segments of the femoral and popliteal arteries mobilized from the tissues, and these were drawn tight 30 sec before injection of ACh and MCh, arresting the blood flow for 2 min. Ringer's solution of the following composition (in mM): NaCl 154, KCl 5.6, $CaCl_2$ 2.2, Tris-HCl (pH 7.4) 5.0, containing ACh and MCh chloride, heated to 37°C, was injected in a volume of 1 ml at a constant rate over a period of 15-20 sec through a cannula introduced into the artery to the gracilis muscle. Solutions of ACh and MCh were injected in increasing concentrations alternately, with intervals of 10-15 min between injections. The animal's blood pressure and temperature were recorded continuously and maintained at the physiological level.

EXPERIMENTAL RESULTS

Of the nine SU tested, eight responded to ACh in concentrations of under 10 μg/ml (Table 1), which are lower than the threshold of its nocigenic action [3, 11]. Responses to ACh in minimally effective concentrations consisted of 3-9 low-frequency spikes (0.5-1.5 Hz), appearing after a latent period (LP) of 12-25 sec for different SU (Fig. 1a). When the ACh concentration reached nocigenic values, the structure of the responses usually changed (Fig. 2a; Fig. 3, 1). A few seconds after the appearance of discharges their frequency increasd up to a maximum of 4-12 Hz, after which it decreased. For some SU it decreased to zero (Fig. 2b, 1, 2), whereas for others it was replaced by a train of low-frequency discharges (Fig.

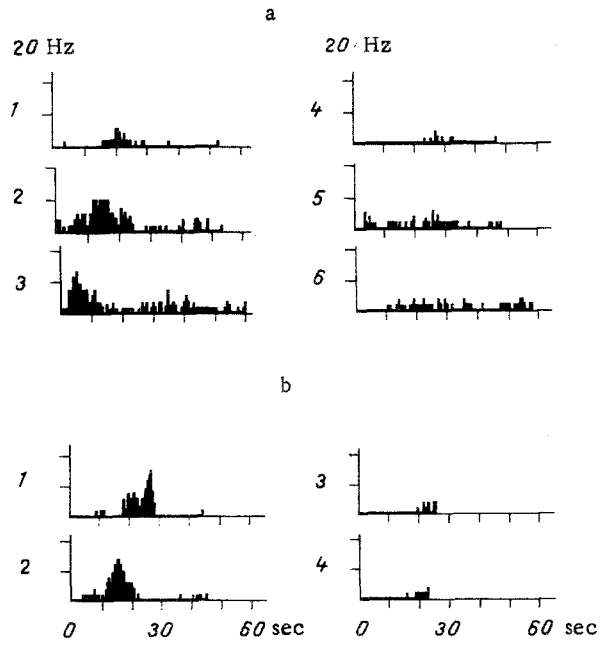

Fig. 2

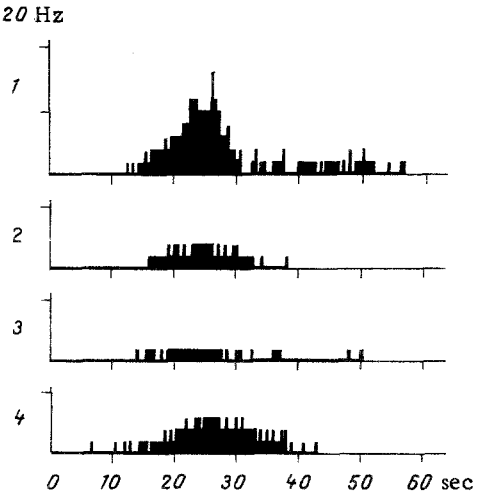

Fig. 3

Fig. 2. Changes in structure of responses of SU to an increase in ACh concentration (left) and absence of change in their structure during increase in MCh concentration (right). Temporal histograms of discharges of two different SU induced by ACh and MCh in concentrations of: a) 1 μg/ml (1 and 4), 10 μg/ml (2 and 5), 100 μg/ml (3 and 6); b) 10 μg/ml (1 and 3), 20 μg/ml (2 and 4).

Fig. 3. Inhibitory action of MCh on excitation of sensory units by MCh and ACh. Temporal histograms of discharges induced by ACh (10 μg/ml — 1, 20 μg/ml — 4) and by MCh (10 μg/ml — 2, 20 μg/ml — 3). Interval between successive injections 1-4 was 15 min.

2a, 2, 3; Fig. 3, 1). The higher the ACh concentration in the nocigenic region, the steeper the initial rise of discharge frequency, and the higher its value at the maximum of the burst (Fig. 12a, 2, 3; b, 1, 2). An increase in the ACh concentration from 10 to 20 μg/ml led to stepwise reduction of LP of the response in five SU: on average from 14 ± 6 to 6 ± 2 sec. A similar jump of LP took place in two SU between ACh concentrations of 2 and 10 μg/ml, but in another two SU the reduction of LP between concentrations of 1 and 100 mg/ml was smoothed.

MCh in a concentration of 1 μg/ml or higher excited all SU (Table 1). However, it will be noted that the action of MCh was tested after ACh in concentrations of 1-2 μg/ml (Fig. 1b), in which ACh can reduce responses of SU to its own repeated action (this effect was found in two SU). Conjecturally, this could be the reason for the ineffectiveness of MCh in concentrations below 1 μg/ml. With an increase in the MCh concentration the number of discharges in the response and its duration in the response and its duration usually increased (Fig. 1c, a, 2; see Fig. 2a, 4-6). However, the structure of the responses was unchanged. Even with the highest of the concentrations tested (100 μg/ml), the response to MCh consisted of a spike train with average frequency of not more than 3.5 Hz (see Fig. 2a, 6). The response of seven SU was over within 1 min, but discharges of two SU induced by MCh in concentrations of 10-100 μg/ml lasted 15 min. A stepwise shortening of LP of the responses was found in only two SU (Fig. 1c, 1, 2). In the rest, LP of the responses fell only with an increase in MCh concentrations, or showed no significant change (Fig. 3: 2,3). MCh could also have an inhibitory action of MU, weakening discharge generation in response to subsequent stimulation both by MCh itself and also by ACh. For example, one MU responded to ACh with a discharge of 45 spikes (Fig. 1b, 1), but after two applications of MCh the response of the same SU to ACh, even in twice the concentration, consisted of only 12 spikes (Fig. 1b, 4). Another SU, subjected to the action of MCh (Fig. 3), ceased to respond to ACh with a high-frequency discharge and reduced the total number of spikes, although in this case also the ACh concentration was doubled (Fig. 3: 1, 4). Finally, the response to MCh in a higher dose could be weaker than the response to a lower dose (Fig. 3: 2, 3). The cause of these effects must be

TABLE 1. Distribution of SU by Threshold of Sensitivity to ACh and MCh

Number of units responding to	Concentration, μg/ml						
	0,02	0,2	0,2—1	1	2	2—10	10
ACh	1	1	1	2	2	1	1
MCh	0	0	0	2	2	2	3

sought in later experiments. The long duration of the inhibitory action of MCh may be partly due to the fact that it is hydrolyzed much more slowly than ACh [9].

The fundamental difference between the action of ACh and MCh is that only the first compound can induce a high-frequency discharge. The beginning of formation of such a discharge in the form of a dense burst of spikes was found in two SU in response to their excitation by ACh in a concentration of 1 μg/ml (Fig. 1b, 1; Fig. 2a, 1), and in another SU — in a concentration of 2 μg/ml. Starting from these concentrations, ACh induces nocifensive reflexes, by exciting the spinal afferent system of the cat's heart [5]. However, a high-frequency discharge usually appeared in response to ACh in a concentration of 10 μg/ml (Fig. 2a, 2 and b, 1; Fig. 3: 1), corresponding to the threshold of its algogenic action [10].

What lies at the basis of the ability of ACh to induce a high-frequency discharge? We know that agonists of both nicotinic (N) and muscarinic (M) acetylcholine receptors excite c-axons of SU of another type, responding to light touch in the hairy skin [7, 8, 13]; the action of agonists of each of these classes, moreover, is prevented by their specific blockers [7, 13]. The essential point is that these SU are excited as a result of activation of their own acetylcholine receptors, or sympathetic fibers [7, 13]. This conclusion also is valid for the SU investigated in the present experiments: they were significantly less sensitive to mechanical stimulation. It may also be noted that ACh is equally an agonist for N and M receptors, whereas MCh is a powerful agonist of M receptors but a very weak agonist of N receptors [9]. Since only ACh, and not MCh, generates the high-frequency discharge of SU, it is reasonable to conclude that this discharge is determined by activation of N acetylcholine and arises as a result of binding of ACh with a certain critical number of them.

The importance of this conclusion is increased if it is considered that nocifensive reflexes in animals [5, 11] and pain in man [10] are caused by agonists of N, but not of M acetylcholine receptors. It can therefore be tentatively suggested that ACh evokes these reflexes and pain by virtue of its ability to generate a high-frequency discharge in SU of the type which we studied. If other agonists of N acetylcholine receptors are found to have the same property, the high-frequency part of the discharge of these SU may perhaps be regarded as a signal specific for nociceptive stimuli.

On the other hand, it is important to note that SU with high thresholds for mechanical stimulation, the number of which may reach about 50% of all C-axon SU in the hairy skin of the cat [12], and which are classed as nociceptors [6, 12], are excited by MCh, which is not a nocigenic or algogenic stimulus, and also by ACh and by K^+ ions in subnocigenic concentrations [3]. By responding to such stimulation by low-frequency discharges these SU exhibit their ability to give warning of chemical changes in the intercellular medium, i.e., to function as chemoreceptors of that medium.

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EFFECT OF PRAZOCIN ON TISSUE OF THE HUMAN PROSTATE WITH BENIGN HYPERTROPHY

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KEY WORDS: benign hypertrophy of the prostate gland; prazocin; adrenomimetics.

Recent investigations have shown the presence of an adrenergic innervation in tissue of the human prostate [2, 6]. A pharmacological study of the tissue of the prostatic capsule and of benign hypertropy of the prostate (BHP), taken during prostatectomy, showed the presence of humerous α-adrenoreceptors in objects studied in vitro [4]. It is considered that α-adrenoreceptor stimulation may increase the ureteric obstruction caused by HBP [3]. The beneficial effect of certain α-adrenoblockers (prazocin, phentolamine, phenoxybenzamine) on the parameters of micturition in patients with BHP has been described (diminution of the barrier pressure in the prostatic urethra, improvement of the stream of urine, and so on) [1, 3, 5, 7]. Investigations in vitro have confirmed the hypothesis regarding the α-adrenoblocking mechanism of action of prazocin [8] and phentolamine [4] on the tissue of BHP and the prostatic capsule.

The Soviet preparation prazocin, synthesized at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, has recently been approved for use as an antihypertensive agent. Information on the use of prazocin in urologic practice is not to be found in the Soviet literature. We have undertaken an experimental study of the effect of prazocin on tissue of BHP.

EXPERIMENTAL METHOD

BHP tissue was obtained during prostatectomy. Immediately after the operation the tissue was placed in Krebs' solution. A strip of BHP 2 cm long, 0.5 cm wide, and 0.2 cm thick, was excised and suspended in a cuvette for isolated organs (a thermostatically controlled bath for Hugo Sachs Elektronik, West Germany). The nutrient solution (37°C) was aerated with carbogen. The period of equilibrium was 60 min. Contractions were recorded under isometric conditions by means of a transducer from Ugo Basile (Italy). The duration of action of prozocin was 3 min.

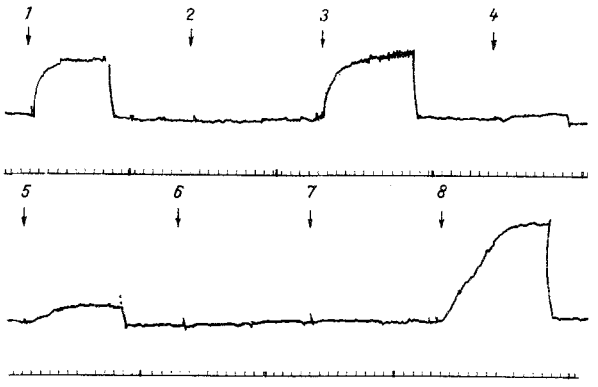

Fig. 1. Effect of different agonists on human BHP tissue. Here and in Figs. 2 and 3: arrows indicate addition of substances to cuvette containing BHP tissue. 1) Adrenalin $1\cdot10^{-5}$ g/ml; 2) acetylcholine $1\cdot10^{-5}$ g/ml; 3) noradrenalin, $2\cdot10^{-5}$ g/ml; 4) histamine, $1\cdot10^{-5}$ g/ml; 5) phenylephrine, $2\cdot10^{-5}$ g/ml; 6) ephedrine, $2\cdot10^{-5}$ g/ml; 7) serotonin, $2\cdot10^{-5}$ g/ml; 8) KCl, $1\cdot10^{-2}$ g/ml.

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