metabolism to a rise or fall of the functional activity of the endogenous opioid system may depend on the functional state of the body. Nevertheless, the mechanisms of the modulating action of enkephalins on the various components of regulation of carbohydrate metabolism at the cellular level are evidently universal and consists of inhibition of adenylate cyclase activity and lowering of the intracellular cAMP concentration.

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# NEUROGENIC MUSCARINIC VASODILATATION OF THE FELINE CAUDAL FEMORAL ARTERY *IN VITRO*


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KEY WORDS: feline caudal femoral artery; transmural stimulation; guanethidine; atropine; quinacrine


The adrenergic (vascoconstrictor) innervation of the blood vessels of the cardiovascular system of most animals has been sufficiently well studied [3, 16], whereas there are few data in the literature on their neurogenic dilatation. The neurogenic cholinergic innervation of the lungs [8], heart [7], uterus, and kidneys [2] has been demonstrated. Most investigators consider that these cholinergic influences are mediated by fibers belonging to the parasympathetic system [3]. However, there are physiological data on the existence of cholinergic dilator fibers of sympathetic nature. They innervate blood vessels of skeletal muscles of some species of mammals [14]. It has been shown that cholinergic influences of sympathetic nature are realized in certain behavioral responses [1]. Morphological data on the localization of fibers of this type are few in number and are based on determination of cholinesterase, an enzyme which is found in other types of fibers also [6]. The mechanism of realization of sympathetic cholinergic influences in blood vessels of skeletal muscles remains unclear and requires further investigation.

The aim of this investigation was to study whether neurogenic cholinergic dilatation can be demonstrated in a vascular preparation isolated from feline skeletal muscles.

## EXPERIMENTAL METHOD

The caudal femoral artery, which runs between the medial and lateral heads of the gastrocnemius muscle of the hind limb, was dissected in cats weighing 2.5-3 kg, anesthetized with urethane (0.9 g/kg, intervenously). A segment (internal diameter 0.6-0.4 mm) 8-10 mm long was excised, freed from fat and connective-tissue membranes, and incubated for 40 min at 8-10°C in modified Krebs' solution: NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.52 mM, $MgSO_4$ 1.64 mM, $NaHCO_3$ 24.88 mM, $KH_2PO_4$ 1.18 mM, and glucose 10 mM [4]. The vessel was then placed in a


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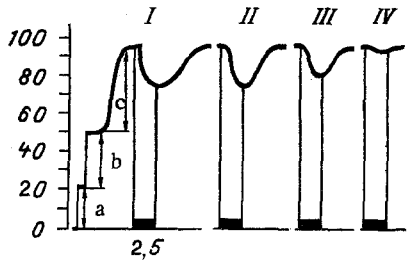


Fig. 1

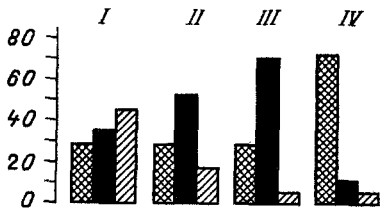


Fig. 2

Fig. 1. Trace of change in perfusion pressure in caudal femoral artery of cat, in response to transmural stimulation, recorded in one experiment. Abscissa, time (in min); ordinate, perfusion pressure (in mm Hg); a) pressure created by perfusion system; b) pressure created by vessel with constant flow rate (passive, see: Experimental Method ); c) pressure created by action of NA ($10^{-5}$-$10^{-6}$ g/ml). I) Dilator effect in response to transmural stimulation; II) the same effect after addition of quinacrine $5 \cdot 10^{-5}$ g/ml); III) after removal of endothelium; IV) after action of atropine ($10^{-6}$ g/ml). Parameters of stimulation: frequency 6 Hz, pulse duration 0.1 msec, voltage 45 V, current 0.6 A. Duration of each stimulation 2.5 min.

Fig. 2. Histogram of incidence of different types of responses in perfused feline caudal femoral artery depending on frequency of transmural stimulation of preparation. Abscissa, frequency of stimulation (in Hz): I) 2; II) 4; III) 8; IV) 16; ordinate, frequency of effect (in %, total number of all types of responses or their absence at a given frequency of stimulation taken at 100%). Types of responses: cross-hatching — constriction, black columns — dilatation, oblique shading — no response. Parameters of stimulation: voltage 50 V, stimulus duration 0.1-0.2 msec, current 0.4-0.6 A.

thermostatically controlled chamber (37°C), aerated with carbogen (95% $O_2$ + 5% $CO_2$), and allowed to stand for 60 min to stabilize the preparation. For perfusion, the proximal end of the vessel was fitted on a stainless steel cannula (outer diameter 0.4-0.6 mm). The distal end of the vessel was left free and through it the solution entered the incubation chamber. Perfusion was carried out at constant flow of 0.7-1 ml/min, produced by a roller pump (LKB, Sweden). The perfusion system, which consisted of a polyethylene tubes connected in series and a cannula, when fluid passed through it at constant rate, exerted a certain resistance to this flow, which created a definite perfusion pressure, not exceeding on average 21 ± 0.5 mm Hg (Fig. 1a). This value depended on the perfusion rate: it increased with an increase in the velocity flow of the fluid and was unchanged if the flow rate was constant. After the vessel had been fitted on the cannula, the recorded perfusion pressure at a given perfusion speed rose on average by 30 ± 1.2 mm Hg (Fig. 1b). Thus the initial perfusion pressure (Fig. 1a, b) averaged 51 ± 1 mm Hg. The vessel, when subjected to this pressure, did not react to papaverine ($10^{-5}$ g/ml), which means that the vessel was in the maximally relaxed state. The perfusion pressure was measured by an electromanometer (Statham, USA). Transmural stimulation of the nerve fibers in the vessel wall was carried out by means of a pair of electrodes, one of which was inserted inside the vessel (this acted simultaneously as the perfusion cannula), whereas the other, in the form of a closed ring (583° gold), was fitted over the vessel. The preparation was stimulated by ac pulses with the following parameters: voltage 50 V, duration 0.1-0.2 msec, frequency 2-16 Hz, strength of current 0.4-0.6 A. The time interval between successive stimulations of the preparation was 10 min. The endothelium inside the vessel was removed by passing a jet of air through it for 30 sec under the same pressure as that under which the vessel was perfused during the experiment [12]. Removal of the endothelium was verified physiologically (no response to acetylcholine) and histologically. The following drugs were used: noradrenalin bitartrate (NA), atropine sulfate (both from Sigma, USA), guanethidine and quinacrine (both from Serva, Sweeden). The experimental results were subjected to statistical analysis by Student's t test. The mean data are given in the form M ± m, for a number of vessels n corresponding to the number of animals.

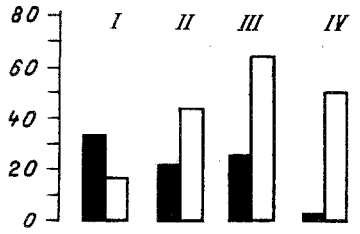


Fig. 3. Effect of guanethidine ($5 \cdot 10^{-5}$ g/ml) on manifestation of dilator response to transmural stimulation at different frequencies. Abscissa: the same as to Fig. 2. Black columns — frequency of dilator response before guanethidine, unshaded — after guanethidine.

## EXPERIMENTAL RESULTS

To create the initial vascular tone the preparation was perfused with NA solution in a concentration of $10^{-5}$–$10^{-6}$ g/ml, which caused the perfusion pressure to rise to 60-80 mm Hg. During prolonged perfusion with NA solution, the pressure in the vessel remained at the chosen level unchanged for several hours. Transmural stimulation of the vascular segment, previously constricted by the action of NA, had both a constrictor and a dilator effect. A histogram of the frequency of constrictor and dilator responses or of their absence, depending on the frequency of stimulation, is given in Fig. 2 (n = 21). The results of those experiments for which both dilator and constrictor responses were given by the same preparation are included in the analysis. It will be clear from Fig. 2 that manifestation of dilator responses was most probable at frequencies of 4 to 8 Hz, and of constrictor responses at 16 Hz. Phentolamine in a concentration of $10^{-6}$ g/ml blocked the constrictor effect obtained by stimulation with frequencies of between 4 and 16 Hz virtually completely, and in a few cases it actually reversed the effect, but did not affect dilation. On average for 10 experiments in which constriction was observed (an increase of perfusion pressure by 53 ± 19% of the initial level), constrictor effect was reduced by 98 ± 2%. Atropine in a concentration of $10^{-6}$ g/ml blocked dilatation virtually completely, but did not affect the constrictor effect. On average for six experiments in which dilatation was recorded in response to stimulation with frequencies of between 4 and 16 Hz (the fall of perfusion pressure amounted to 17 ± 3% of its initial level) the reduction of amplitude of the effect was 93 ± 1%. These data indicate that the vascular effects observed differed in nature: constrictor responses are mediated through α-adrenoreceptors, whereas dilator responses are mediated through muscarinic acetylcholine receptors.

The dilator response observed was thus cholinergic in nature. In the modern view there are several mechanisms by which dilator reactions involving the muscarinic acetylcholine receptors are realized. First, we know that muscarinic acetylcholine receptors are found on presynaptic endings of sympathetic fibers, and that they reduce the release of NA from those endings, which may lead to reduction of the constrictor response and be expressed as vasodilatation [15]. Second, the muscarinic acetylcholine receptors may be found on the vascular endothelium and may mediate endothelium-dependent dilator responses [9]. The third possible variant is the onset of dilatation due to activation of cholinergic dilator fibers, the mediator of which acts directly on the vascular smooth muscle.

To determine what type of cholinergic dilatation was realized in the present experiments, the probability of appearance of dilator cholinergic responses to transmural stimulation was studied before and after addition of guanethidine in a concentration of $5 \cdot 10^{-5}$ g/ml, sufficient to block NA release from sympathetic endings. It will be clear from Fig. 3 that against the background of this blockade of constrictor effects the probability of obtaining dilator responses was doubled at a frequency of 4 Hz (n = 9) and increased by 2.5 times at 8 Hz (n = 8). At a frequency of 1 Hz, before addition of guanethidine they virtually never appeared, whereas after guanethidine the probability of their appearance reached 50%. These data are evidence that the neurogenic cholinergic effect observed in the present investigation was potentiated by blocking the adrenergic innervation of the vessel, i.e., in this case the effect was not mediated by presynaptic muscarinic acetylcholine receptors, and adrenergic constrictor influences evidently masked the appearance of cholinergic dilatation.

To determine the role of endothelial muscarinic acetylcholine receptors in the realization of this dilatation experiments were carried out with quinacrine, a blocker of this type of dilatation [9], and on vessels from which the endothelium had been removed. Quinacrine was added to the perfusion solution in a concentration of $5 \cdot 10^{-5}$ g/ml, which is sufficient for manifestation of its blocking action [11]. According to the results of six experiments, no significant changes in neurogenic cholinergic dilatation were observed after addition of quinacrine. Before its addition the dilator effect was 63.4 ± 10.3%, falling to 59.2 ± 10.3% after addition. The effect of quanacrine on the neurogenic dilator effect in one of these

experiments is shown in Fig. 1, II. Removal of the endothelium likewise did not significantly change the dilator effect, although it reduces it a little. Before removal of the endothelium, the average dilator effect, based on the results of four experiments, was 76 ± 16%, compared with 53 ± 10% after removal (Fig. 1, III; $p > 0.05$).

In response to transmural stimulation of a perfused segment of the feline caudal femoral artery, besides adrenergic constrictor responses, dilator responses of cholinergic nature may also be observed. These dilator responses do not depend on muscarinic acetylcholine receptors located on the vascular endothelium and on presynaptic adrenergic endings. In this case the dilator effect is evidently the manifestation of activity of nerve fibers of a cholinergic nature. To reach a firm conclusion regarding the presence or absence of a cholinergic innervation of the feline caudal femoral artery, morphological investigations must be undertaken, but the physiological data described above are evidence that this type of innervation of the arteries of feline skeletal muscles may exist. Similar data on the existence of a cholinergic dilator innervation in experiments on isolated preparations have been obtained for the cerebral arteries [10] and the posterior auricular artery of the cat [5]. In the last case, just as in the present experiments, neurogenic dilatation was shown to be independent of the presence of the endothelium. The question of the functional role of these cholinergic dilator influences arises. It has been suggested in relation to certain superficial vessels of the cat's head that these fibers may participate in temperature regulation [5]. However, the vessels which we have studied play virtually no part in temperature regulation, and these fibers may play some other role. We know that cholinergic dilatation can be observed in the skeletal muscle vessels of conscious and anesthetized cats during stimulation of the sympathetic system [14].

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