BLOOD PRESSURE REFLEXES EVOKED BY EXCITATION OF SCIATIC NERVE A- AND C-AFFERENTS IN ANESTHETIZED AND UNANESTHETIZED FROGS

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Information on the evolutionary development of reflex regulation of the circulation is extremely scarce [3, 7]. For amphibians, in particular, information about reflex connections of spinal afferents with the vasomotor system is limited to the statement that the arterial blood pressure (BP) in frogs rises in response to stimulation of certain unidentified sensory fibers of the scientic nerve (SN) [4, 6].

The object of the present investigation was, by studying reflex responses of BP to impulses traveling along different groups of spinal afferents, to determine which of these afferents are connected with the vasomotor system and to obtain some idea of the effect of general anesthesia on vasomotor reflexes.

EXPERIMENTAL METHOD

Under general ether anesthesia a polyethylene cannula was tied into the abdominal vein of a frog (*Rana temporaria*) and either viadril (0.13 mg/g body weight — anesthetized animals) or flaxedil (20 μg/g body weight — unanesthetized, immobilized animals) was injected through it. In the first case, to prevent possible reflex movements the animals also received flaxedil, but it was not given until immediately before responses to stimulation began to be studied. BP was recorded in the dorsal aorta through a cannula connected to a highly sensitive electromanometer, the signals of which were recorded on a KSP-4 instrument.

The central end of the sciatic nerve was stimulated by square pulses with a duration τ = 0.1 msec from an ESL-1 stimulator (through an isolating transformer). To determine the thresholds of excitation and conduction velocities of the afferent fibers of SN, the compound action potential (CAP) was recorded from the peripheral ends of the dorsal roots VIII-X, divided close to the spinal cord. To record discharges of postganglionic sympathetic nerves, either the thickest nerve trunks running along the common visceral artery (the visceral nerve), or one of the trunks running from the sympathetic chain to the kidney (renal nerve) were isolated. The trunk was divided and its central end placed on bipolar electrodes (diameter 0.3 mm). To average the reflex responses and detect low-amplitude waves of CAP in the dorsal root, the ATAS-201 instrument was used. Usually responses to 20 consecutive stimuli were averaged. Signals from the recording electrodes were led through a UBP 1-02 amplifier to a UMF 2-01 recorder with motion picture camera.

BP reflexes were studied in 11 anesthetized and 7 unanesthetized frogs, thresholds of excitation and conduction velocities of afferent fibers were studied in 5 anesthetized frogs (in 15 dorsal roots), and somatosympathetic responses were studied in 3 anesthetized frogs. The BP level in the anesthetized frogs was between 20 and 30 cm water, and in the unanesthetized frogs between 30 and 40 cm water.

EXPERIMENTAL RESULTS

The relationship between the character and magnitude of reflex changes in BP and stimulus amplitude — the $\Delta P(U)$ curve — typical of anesthetized frogs is illustrated in Fig. 1a. At a frequency of 10 Hz stimuli of relatively low amplitude in all experiments evoke depressor re-

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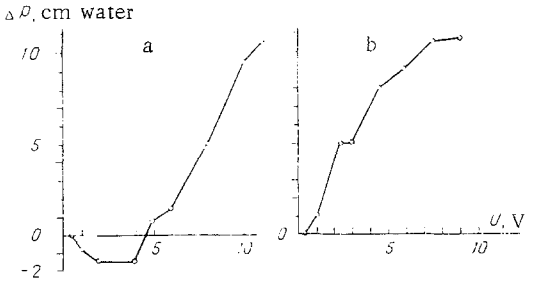

Fig. 1. Character and magnitude of reflex changes in BP (ordinate) as a function of amplitude of stimuli applied to SN (abscissa): a) anesthetized frogs; b) unanesthetized frogs. Frequency of stimuli 10 Hz, duration 1 msec.

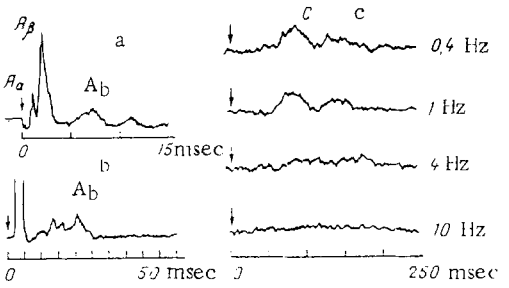

Fig. 2. CAP of afferent fibers of SN. a, b) Supramaximal stimulation of A fibers (single stimuli), c) supramaximal stimulation of C fibers (frequency of stimuli shown on right of traces). Each trace obtained by averaging 20 realizations. Arrow indicates moment of stimulation.

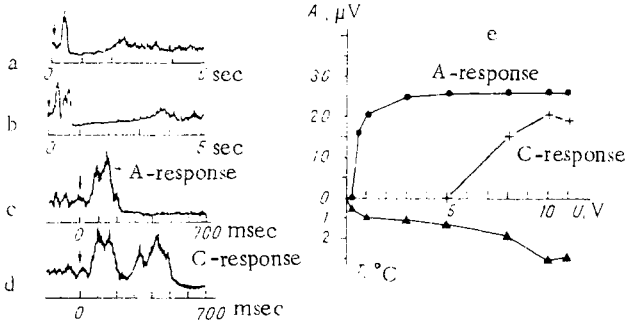

Fig. 3. Reflex discharges and inhibition of tonic activity in visceral nerve in response to single stimulation of SN. a, c) Responses to stimulation of A fibers; b, d) responses to stimulation of A and C fibers. Averaging of 20 realizations; e) dependence of amplitude of A and C responses and also of duration of inhibition on stimulus amplitude. Filled circles on graph represent amplitudes of A responses, crosses — of C responses, triangles — duration of inhibition.

flexes (threshold 0.2-0.5 V at τ = 1 msec). With an increase in stimulus amplitude these reflexes increased initially, to reach their highest value (3.0 ± 0.4 cm water; n = 6) at 1-2 V, after which they decreased. Starting from 3-6 V pressor reflexes developed, and they usually reached their highest value (13 ± 2.2 cm water; n = 8) at 7-12 V; in two experiments the pressor reflexes were exceptionally high, namely 21 and 24 cm water (66 and 70% of the initial BP level).

At τ = 0.1 msec the threshold of the depressor reflexes was 0.8-1.0 V and they reached their highest values at 3-5 V, after which they decreased, and pressor reflexes developed at 6-9 V.

A typical $\Delta P(U)$ curve for unanesthetized frogs is shown in Fig. 1b. No depressor reflexes appeared in such frogs. The threshold of the pressor reflexes in three experiments was 0.3-0.5 V at τ = 1 msec and 0.6-0.8 V at τ = 0.1 msec, in the rest it was 1-3 V (τ = 1 msec) and 3-4 V (τ = 0.1 msec). With an increase in the strength of stimulation the magnitude of the reflexes rose to reach the highest values at 7-9 V (τ = 1 msec).

During stimulation with higher frequencies (up to 40 Hz) the $\Delta P(U)$ curves differed only very little from those shown in Fig. 1, a and b, but the highest value of the reflexes was often smaller than with stimulation at a frequency of 10 Hz.

The CAP of A-fibers consists of three waves: A_α, A_β, and A_δ [5]. The A_α wave was presented in 8 roots, in 6 of them in the form of a separate wave, whereas in two it was merged with the A_β wave. An A_β wave was found in 10 roots and A_δ waves in 7 roots (Fig. 2).

The threshold of excitation of the A_α fibers at τ = 1 msec was 0.2-0.4 V and at τ = 0.1 msec it was 0.5-0.8 V; the conduction velocity (v) was 45-25 m/sec. The threshold of excitation of the A_β fibers (v = 25 to 10 m/sec) was 0.1-0.2 V higher than for A_α fibers. The A_δ fibers (v from 8 to 1.5 m/sec) had an excitation threshold of 1.2-1.4 V (at τ = 0.1 msec) and 0.5-0.8 V (at τ = 1 msec). Impulses in fibers of the C group (v < 1.5 m/sec) formed a small, often multiphasic wave, in CAP. Their excitation threshold at τ = 1 msec was 3-5 V, but at τ = 0.1 msec it was 6-8 V.

With an increase in stimulus frequency up to 40 Hz no change was found in the amplitude of the A_α, A_β, and A_δ waves. However, the amplitude of the C waves fell to 10-50% of its initial value at a frequency of 4 Hz, and at frequencies of 7-10 Hz it was virtually no higher than the noise level (Fig. 2c).

The threshold of onset of depressor reflexes in the anesthetized animals thus actually coincided with the threshold of excitation of the A_β fibers. During excitation of A_β + A_δ fibers these reflexes increased. However, impulses of the relatively high-threshold A_δ fibers and of the most excitable C fibers reduced the depressor reflexes. The threshold of appearance of pressor reflexes was a little above the threshold of the C wave. Depressor reflexes were thus due to impulses in A_β fibers and, partly in A_δ fibers, whereas pressor reflexes were due to impulses in C fibers. This conclusion is in agreement with data for warm-blooded animals [2], although the range of conduction velocities of A fibers differs substantially in amphibians and mammals [5].

A volley of A_β afferents of SN evoked inhibition of tonic activity lasting 0.6-0.8 sec in the renal and visceral nerves, whereas a volley in A_β + A_δ afferents evoked a series of discharges (latent period 60-70 msec, duration 0.2-0.25 sec) — an A-response (Fig. 3a, c). Meanwhile impulses in A_δ fibers lengthened the period of inhibition of tonic discharges to 1-1.3 sec (Fig. 3a, e). During stimulation of A + C afferents, a two-component C response appeared immediately after the A response (Fig. 3b, d). With a further increase in stimulus amplitude the components of the C response merged, but the duration of inhibition increased, to reach 2-2.5 sec in the case of stimulation of supramaximal strength for C-afferents (Fig. 3e).

If pairs of stimuli were used to stimulate A afferents, a small response to the second stimulus appeared only if it was applied 1-1.3 sec after the first. With rather smaller intervals the duration of inhibition was increased to 1.5-2 sec, i.e., the second stimulus had an inhibitory action. Summation of inhibition evidently also led to the appearance of depressor reflexes. The mechanisms of formation of pressor reflexes require further study.

The appearance of reflex changes of BP in unanesthetized frogs can be linked with impulses in A_β fibers only in certain cases. Threshold pressor reflexes appeared most frequently in response to stimuli with amplitudes sufficient to excite A_δ fibers. Impulses in fibers of the C group increased the pressor reflexes.

Possibly the qualitative difference between responses of the vasoconstrictor system to impluses in A afferents of SN in anesthetized and unanesthetized frogs may be attributable, just as in mammals, to depression of the A-response, the one with the longest latency, the so-called very late response, under anesthesia [1].

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LONG-LASTING POST-TETANIC POTENTIATION IN HIPPOCAMPAL NEURONS IN TISSUE CULTRUE

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Long-lasting post-tetanic potentiation (LLPTP) is a characteristic functional property of hippocampal neurons which reflects their plastic properties [2, 7, 10]. LLPTP is known to consist of the long preservation of the focal potential, in response to a single stimulus, enhanced by tetanization. According to some workers [2, 7], LLPTP is one of the phenomena that reflect plastic changes in the structure of synaptic connections that lie at the basis of memory. At the present time an important role in the formation of long-lasting trace processes is ascribed to the monoaminergic systems of the brain [3, 9], which are considered [2, 8] to participate also in the formation of LLPTP. It is evident that hippocampal afferent fibers (including monoaminergic) may become involved in the process of excitation of neurons during tetanization of intrahippocampal systems of interneuronal connections (Schaffer's collaterals, for example), leading to the appearance of LLPTP, for they run in the same bundle with these connections and terminate on the same neurons. During the development of LLPTP, studied on a model of short-limbed hippocampal slices [1, 10], segments of these afferents which evidently function for a certain time *in vitro* may participate in excitation of the neurons. Meanwhile explantation of slices for a longer period under tissue culture conditions inevitably leads to degeneration of the axon terminals belonging to neurons of other brain structures and divided during explantation. Consequently, the appearance of LLPTP in explants in culture may be evidence that it is an internal functional property of the hippocampus and arises without the direct participation of its afferent connections. The object of the present investigation was to study the ability of neurons to undergo LLPTP and to study it in explants of the hippocampus surviving for several days in culture.

EXPERIMENTAL METHOD

Experiments were carried out on 11 explants obtained from C57BL mice aged 9-14 days and cultured for 4-7 days by a method similar to that used to obtain and culture hippocampal explants from newborn mice [4]. The technique for the electrophysiological experiments was the same as that described by the writers previously [6]. Testing stimuli were applied in series of ten pulses with an interval of 10 sec, and the amplitude of the responses evoked by them was averaged on an NTA-1024 analyzer. Intervals between series measured 15 min. Tetanization was carried out with a frequency of 20 Hz for 7 sec.

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