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SELECTIVE ELECTRICAL INHIBITION OF C-FIBER SPIKES IN FINE NERVE STRANDS AS A METHOD OF IDENTIFYING A AND C AFFERENTS

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KEY WORDS: fine nerve strands; C fibers; direct current.

When the properties of receptor endings of an afferent fiber, present in a fine strand teased from a nerve, are studied, it is necessary to determine whether the fiber belongs to type A or to type C. If there are several active fibers in the strand, either the colliding impulses method [2] or electrical stimulation of the afferent fiber close to its ending in the tissues [3] can be used for this purpose. The use of the colliding impulses method requires matching of the times of excitation of the afferent endings and of electrical stimulation of the nerve trunk within a narrow time interval [2]. It can therefore be used in order to study mainly mechanoreceptors, whose time of excitation can be assigned reasonably accurately. The use of the second method requires a high voltage (hundreds of volts), in order to excite C afferents remaining undetected in the tissues [3]. Both these traditional methods are difficult to use in order to investigate afferent chemoreceptive endings in the tissues (tissue chemoreceptors) [1]. In this case it is virtually impossible either to localize the receptor endings precisely or to match the times of excitation of the receptor and of electrical stimulation of the nerve trunk.

In this paper we suggest a simple method of identifying the type of nerve fibers, based on selective inhibition of C-afferent spike generation in a fine bundle of nerve fibers containing A afferents also.

EXPERIMENTAL METHOD

Two regions of the saphenous nerve were isolated in 30 cats anesthetized with chloralose (40 mg/kg) and urethane (600 mg/kg): at the level of the knee, where the nerve was placed on a bipolar stimulating electrode, and in the proximal third of the thigh, where fine strands of fibers were teased from the nerve (Fig. 1). The nerve was tightly ligated proximally. At the site of teasing of the strands the nerve was placed on a platform with grounding electrode and bathed with oxygenated Krebs–Henseleit solution under a layer of mineral oil. Strands containing 2-5 myelinated fibers, distinguishable under the microscope (magnification: 20-40), were isolated by teasing with dissection needles, placed on a polished platinum electrode applied from above, and lifted into the layer of oil. The electrode was connected either to a Park-113 low-interference amplifier (for recording) or to an ÉSU-2 electrical stimulator (to inhibit the fibers selectively). Signals recorded from the fibers were amplified in the 85-2000 Hz band and recorded on magnetic tape for subsequent computer analysis.

EXPERIMENTAL RESULTS

In response to stimulation of the nerve trunk with square pulses 1 msec in duration and 12-15 V in amplitude (3 times higher than the threshold of excitation of C nerve fibers) a

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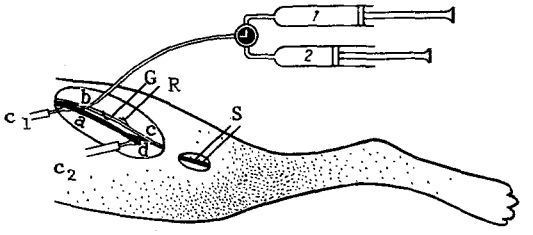

Fig. 1. Scheme of experiment. R) Recording electrode used also to apply pulse of direct current, G) grounding, S) stimulating electrodes, C) device for compressing artery. a) a. femoralis, b) a. gracilis, c) a. saphena, d) a. poplitea. 1 and 2) Syringes containing standard and testing Ringer–Locke solution. Region of sensory endings of mechanosensory C fibers indicated by dots.

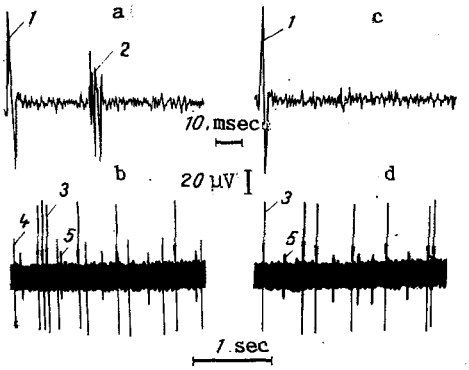

Fig. 2. Inhibitory action of direct electric current passed through strand of nerve fibers on generation of C-fiber spikes. Component action potential of strand (a, c) and spike trains in fibers of strand in response to stimulation of nerve endings (b, d) by injection of a solution containing K^+ in a concentration of 44 mM into a. saphena. a, b) Before, c, d) after passage of direct current (2.2 μA, 10 sec) through strand. 1, 2) Waves of CAP (A and C fibers respectively), 3, 4, 5) spikes in A (3, 5) and C (4) fibers.

C wave of the component action potential (CAP) of the strand was recorded (Fig. 2a), which showed that the strand contained functioning C fibers. The number of C fibers in the strand (usually not more than 10) was estimated from the number of spikes in the C wave of the CAP. In experiments with 122 strands, the minimal magnitude of the current pulse (duration 10 sec), sufficient to cause total inhibition of spikes in all C fibers in the strand, was determined: it was 1-3 μA. No appreciable effect of the direction of the current on inhibition of spikes in the nerve fibers could be found.

The effect of the electric current on A fibers was studied in 30 strands. Altogether they contained 53 fibers of this type, excited by mechanical stimulation of the skin. That these fibers belong to type A was shown either by the absence of a C wave of the CAP or by the ability of the fibers to conduct inpulses with a frequency higher (up to 400 Hz) than is possible for C fibers (150 Hz [4]). Microbundles containing A fibers were subjected to repeated stimulation by pulses of current of increasing strength. It was found that with a current of 3 μA the amplitude of the spike in 80% of fibers was virtually not reduced at all, whereas with a current of 9-13 μA the amplitude fell by 50-75%. The amplitude of spikes in the remaining A fibers, in response to a current of 3 μA, was reduced by not more than two-thirds, and the shape of the signal was unchanged. A current of 6-9 μA led to complete suppression.

Thus a pulse of current, 10 sec in duration and 3 μA in amplitude, selectively inhibit spikes in all C fibers, while leaving A-fiber spikes unaffected. To reduce the action of the

current on A fibers to the minimum, identification of the type of fibers always began by passing a weak (1 μA) current, and the stimulation was repeated, by increasing the current until complete disappearance of the C wave (as a rule a current of 2 μA was sufficient for this purpose).

The use of the method described above is illustrated by Fig. 2. CAP of the strand (Fig. 2a) indicate that it contains active C fibers (the wave in the center of the trace). As a result of the action of the current (2.2 μA for 10 sec) the C wave in the CAP disappeared (Fig. 2c). Meanwhile, the spike (4) on the trace recorded during chemical stimulation of the receptor zone with K^+ ions also disappeared (Fig. 2b, d). Consequently, the two spikes which remained (3 and 5) were A spikes, and the one which disappeared (4) was a C spike.

Differences in the resistance of A and C fibers to the action of a direct current can thus be used to identify afferents of each type. Since C spikes were not restored after being inhibited, the identification must be carried out at the end of any investigations on the particular strand of nerve fibers.

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ANGIOTENSIN II IN THE ORGANIZATION OF FEEDING BEHAVIOR OF RATS

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The renin-angiotensin system plays an important role in the formation of food motivation [2-5]. In most animals, including rats, a close interlinking between drinking and feeding behavior has been discovered [2, 7]. With an increase in thirst, food intake of animals is reduced [8]. However, the neurochemical mechanisms of the interlinking of these forms of natural biological motivations have received little study.

In the investigation described below the role of the dipsogenic neuropeptide angiotensin II in the realization of feeding behavior of animals was studied, with particular reference to the time course of food and water consumption by hungry rats receiving central and peripheral injections of angiotensin II.

EXPERIMENTAL METHOD

Experiments were carried out on 75 noninbred male rats weighing initially 200-300 g. As a first step, all the animals were deprived of food for 48 h. The hungry rats received injections of angiotensin II or saralasin 10 min, and in some experiments 60 min also, before receiving food and water, and in other experiments, angiotensin II was injected after preliminary administration of saralasin. Asp^1-Val^5-angiotensin II diacetate ("Berlin Chemie," East Germany), dissolved in physiological saline, was injected into the lateral ventricles (in a volume of 5 μl) or intraperitoneally (in a volume of 0.5 ml) of the rats in concentrations of 100 and 10 ng/kg body weight respectively. Saralasin (Sar^1-Val^5-Ala^8-angiotension II;

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