SEPARATION OF AFFERENT AND EFFERENT IMPULSE FLOWS IN SYMPATHETIC NERVE FIBERS IN CHRONIC EXPERIMENTS BY LOCAL REVERSIBLE COOLING

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Selective blocking of various brain structures by cold has been used for the experimental analysis of physiological mechanisms [10, 11] and clinically during surgical procedures on the brain [11, 13, 14]. Local cooling of the spinal cord has also been found possible [6, 9]. However, there is no information in the literature regarding blocking of conduction in somatic and autonomic nerves in chronic experimental conditions. The need for such a procedure is determined by the fact that several authors have recently suggested methods of recording electrical activity in these nerve fibers in chronic experimental conditions in waking animals [1, 2, 4, 7, 8].

In these cases, however, the recorded impulses were mixed, i.e., they were not separated into afferent and efferent. In the present article a method of reversible blocking of conduction is described, by means of which, depending on the purpose of the investigation, the two types of impulses can be separated, and it also describes results obtained by means of this method.

EXPERIMENTAL METHOD

Experiments were conducted on five waking dogs with permanently implanted electrodes and on three animals anesthetized with ether. The apparatus used for local cooling of the nerve consisted of thin U tubes made of stainless steel (diameter 2 mm) to which colored polyvinyl chloride tubes were connected (the insulating covers from electric wires). The metal tubes were applied to the nerve proximally and distally to the capsule with the electrode so that the nerve trunk was in the middle of the bend (Fig. 1B). In this way optimal conditions were created for contact between the nerve and the cooling surface. The tubes were placed not less than 5 mm from the recording electrodes, because if they were too near part of the afferent flow of impulses could be blocked as well as the efferent. The polyvinyl chloride tubes were brought out through special holes in the collector of the electrodes. One of them (the inflow) was connected by means of an adapter with a thermally insulated bottle, preliminarily filled with cold water and lumps of ice. A thermometer and a device for injecting air were inserted into the stopper of the bottle. To the other (outflow) tube of the cooling system a rubber hose was attached, the free end of which was placed inside an empty bottle. When the pressure in the first bottle was increased, cold water was forced along the system of tubes into the empty vessel (Fig. 1A). The most intensive cooling of the tissue took place at the point where the metal U tube was in contact with the perineural cellular tissue.

Electrodes were implanted by the method described above [4, 5] into the greater splanchnic, the inferior mesenteric and the hypogastric nerves, and also into the preganglionic branches of the inferior mesenteric ganglion.

The dog was fixed in a frame. The electrical potentials were amplified by a type UBP1-01 amplifier and recorded on a type N-102 loop oscillograph. The sensitivity of the apparatus was 17 μV/cm. The intrinsic noise did not exceed 3-4 μV. Simultaneously visual observations were made on the S1-4 cathode-ray oscillograph. Parallel with the recording of the neurograms, recordings were made of the electrocardiogram (ECG) and respiration. The apparatus also included a device [5] by means of which a simultaneous recording could be made on the film of the amplitude of the incoming flow of impulses and their frequency could be estimated quantitatively.

The temperature in the zone of cooling was measured by means of a thermocouple. The error of measurements was 0.1°. Readings were taken every 15 sec.

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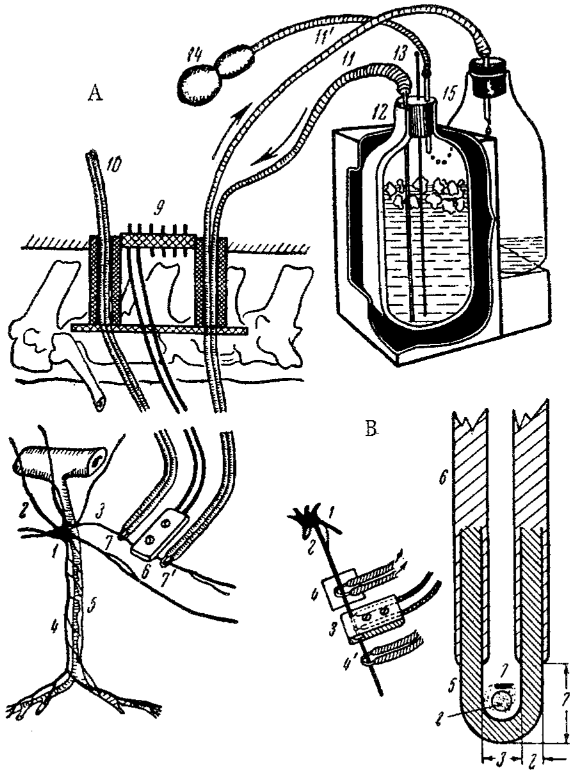

Fig. 1. Scheme of apparatus for local cooling of a nerve. A) General appearance: 1) inferior mesenteric ganglion; 2) three ganglionic branches; 3) hypogastric nerve; 4) inferior mesenteric nerve; 5) inferior mesenteric artery; 6) capsule with implanted electrodes; 7-7') proximal and distal tubes; 8) colored polyvinyl chloride tubes; 9) collector of electrodes; 10) outer ends of proximal polyvinyl chloride tubes; 11-11') outer ends of inflow and outflow distal tubes; 12) vessel with cooling fluid; 13) thermometer; 14) bulbs for raising pressure in bottle; 15) empty bottle for collecting cooling fluid. B) Scheme of cooling system applied to a nerve: 1) inferior mesenteric ganglion; 2) hypogastric nerve; 3) capsule with implanted electrodes; 4 and 5) proximal and distal cooling metal tubes; 6) polyvinyl chloride tube; 7) position of thermocouple when recording temperature in zone of cooling.

EXPERIMENTAL RESULTS

At the beginning of the investigation, acute experiments were carried out to select the most suitable method of cooling the nerve fiber and of determining if it could be used in chronic conditions. In a paper by Ri and coworkers [15] special semiconductor thermodes for cooling a nerve are described. However, they are cumbersome and unsuitable for chronic experiments. In addition, an apparatus of this type is an antenna system, and thus is a source of induction and interference, preventing recording of the electrical activity during the cooling. The method of cooling nerves by means of dry ice [12] and gases [1] is also unsuitable. For this reason, the simplest method was chosen in which water is the cooling fluid, at freezing point, and is passed through metal tubes applied to the nerve.

Thermophysical calculations were required to determine the distribution of temperatures at a point corresponding to the position of the nerve trunk between the branches of the cooling tube (the point A on Fig. 2A). No solutions were available corresponding directly to the case in question, so that the existing formulas were used for calculating the temperatures at point B [3]. It follows from calculations using the formula:*

$$t = t_T + (t_0 - t_T)\frac{\lg\frac{r}{a}}{0{,}4 + 0{,}46\lg\frac{K\tau}{a^2}},$$

where t is the temperature at point B created by one tube, t_T the temperature of the fluid in the tube, t_0 the initial temperature at point B, r the distance of point B along the axis of the tube, a the radius of the tube, τ the time of cooling, and K the coefficient of thermal conductivity of the medium, that the temperature at point B 3 min after the liquid began to flow was 5.7°, and 5 and 10 min after it was 5.3 and 4.8°, respectively. In this case, of course, allowance is made for the fact that the temperature changes at point B will be less marked than at point A, for the latter is in contact with the cooling surface on three sides, whereas point B is in contact only two sides (Fig. 2A). Nevertheless, despite these conventional assumptions, it was to be expected that even at this arbitrary point B, a level of temperature would be created adequate for plotting the transmission of a nerve impulse.

It was then necessary to measure experimentally the temperature at the point A. To do this, in acute experiments a thermocouple was fixed to the nerve trunk situated between the branches of the cooling tube. When the cooling fluid flowed at a speed of 60-80 ml/min and the length of the thermally insulated outer outflow tubes did not exceed 50 cm, the temperature at the point where the cooling apparatus was in contact with the nerve gradually changed, to become 7° after 3 min and 4.5-4.0° after 5 min, and 3.0-2.5° after 10 min. With subsequent cooling the temperature remained stable at 2.5-2.0°, and the scatter of the points in these circumstances was very

* All thermophysical calculations were made by I. A. Ioffe.

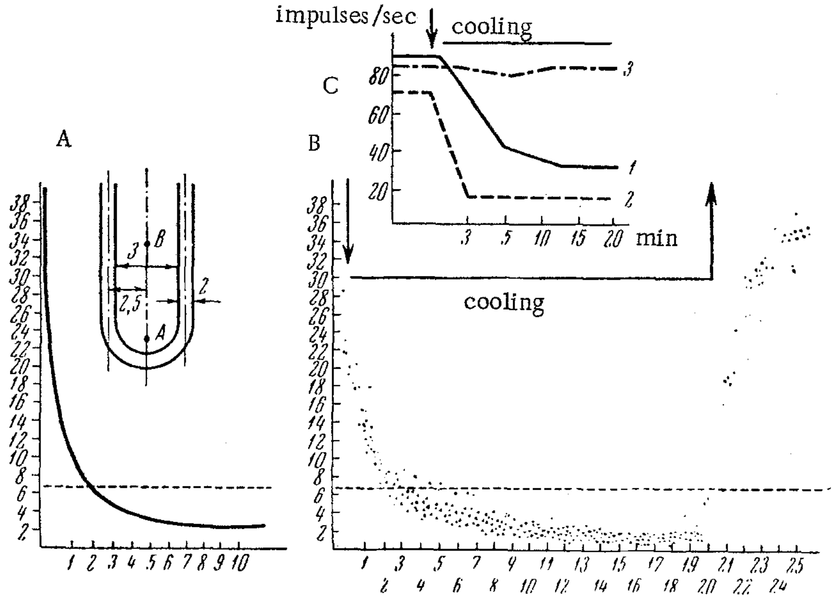

Fig. 2. Calculated distribution of temperatures (A) at the point B (the position of the point B in relation to the cooling surface is shown above) and actual distribution of temperatures in the zone of cooling determined by means of a thermoelectrical method (B). C) Number of impulses in sympathetic trunks recorded during separation of the afferent flow of impulses. Along the axis of ordinates in A and B) temperature (in degrees), along the axis of abscissas – time (in minutes).

small (Fig. 2B). As might be expected, the temperature curve for the point A was steeper and its fall was deeper than for the calculated point B. When the supply of cooling fluid ceased the temperature in the zone of cooling returned to the temperature of the surrounding tissues after 5 min.

To determine the time required for the temperature level in the zone of cooling to produce a reliable block of nervous conduction, in chronic experiments on waking dogs the unseparated "spontaneous" impulses were first recorded. Next, to separate the afferent flow, local cooling was applied to the nerve trunk above the implanted electrodes. It can be seen in Fig. 2C that with the development of the cold block, the number of impulses recorded in the combined flow of oscillations fell gradually, and only the afferent impulses could be distinguished. After 3 min their number in the hypogastric nerve reached 15-16/sec, and was subsequently held at this level. In the greater splanchnic nerve the development of the cold block took rather longer. Evidently the thickness of the nerve trunk was responsible for this, i.e., the actual mass of nerve fibers to be cooled. For comparison, in the same animal the impulses in the preganglionic branch to which the cooling tube was not applied were recorded. During simultaneous recording the impulses in this trunk remained relatively constant (Fig. 2C, curve 3).

To verify that the block was complete, the nerve trunk was simultaneously cooled proximally and distally to the capsule with the electrodes, thus blocking both afferent and efferent impulses. In this case, 5 min after the beginning of cooling the oscillations completely disappeared, demonstrating the effectiveness of the cold block. Conductivity was restored 5-7 min after cooling ceased, and the development of impulses could again be observed (Fig. 3I).

The blocking of nervous conduction by means of cold was completely reversible and reproducible. In chronic experiments, to study the character of the electrical activity in the sympathetic nerves, the block was repeated 50 or 60 times in the course of 6 months; in these circumstances, judging by the state of the electrical activity, no injury to or death of the nerve trunks was observed. As previously established [11], during local cooling to 0° no cell damage was found, and significant irreversible anatomical changes appeared only if cooling took place below 0°.

It follows from a comparison of the temperature readings in the zone of cooling and the changes in the number of impulses during the development of a cold block at the blocking of nervous conduction in the sympathetic trunks developed over a period of 3-5 min, when the number of impulses recorded became stabilized and the temperature in the zone of cooling was 4-7°. This is in agreement with the results of investigations [11] showing that nervous conduction is blocked by a temperature below 8°, while synaptic transmission is blocked at 20°. In the present case

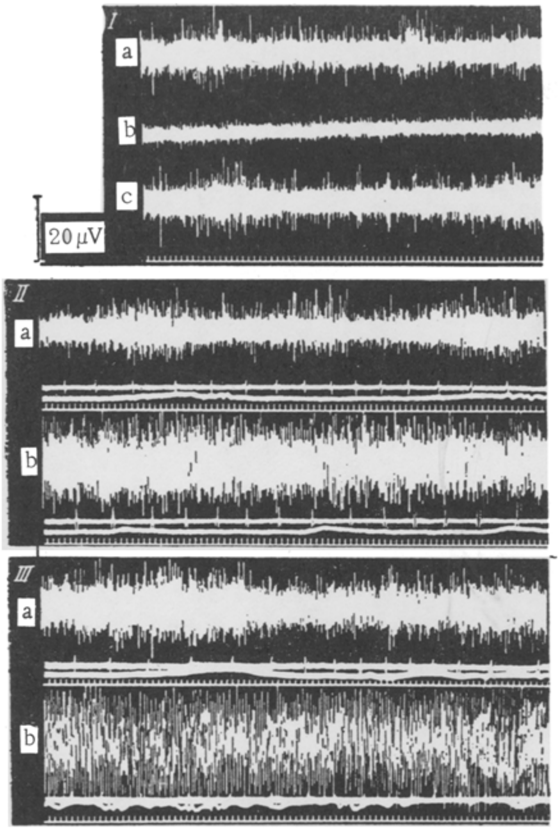

Fig. 3. Afferent and efferent impulses in the autonomic nerves of a dog (waking animal, chronic experiment), obtained by means of a cold block. I) Interruption of conductivity in the greater splanchnic nerve: a) afferent (efferent blocked) impulses; b) absence of oscillations during simultaneous blocking of afferent and efferent impulses; c) restoration of afferent conduction. II) Changes in afferent (efferent blocked) impulses in the inferior mesenteric nerve after injection of 50 μg acetylcholine into a metatarsal vein: a) initial spontaneous impulses; b) 0.5 min after injection. On each oscillogram, here and later: neurogram, ECG, pneumogram, time marker (10 cps). III) changes in efferent (afferent blocked) impulses in the greater splanchnic nerve after stimulation of the skin of the paw by an electric current: a) initial spontaneous impulses; b) immediately after stimulation.

the block developed gradually. Evidently the more superficial fibers, situated nearer to the cooling source, were blocked first; the number of blocked fibers increased as the whole thickness of the trunk was cooled.

The blocking of nervous conduction has been used to separate the afferent and efferent impulses in chronic experiments to study electrical activity in the sympathetic nerves in response to the action of interoceptive and exteroceptive stimuli (Fig. 3, II and III).

The results of this investigation thus show that a block of nervous conduction can be produced by local reversible cooling in sympathetic nerves. The block takes 3-5 min to develop when the temperature in the zone of colling is 4-7°.

SUMMARY

A special device based on local reversible cooling of the nerve is used for separating afferent and efferent impulses. Experimental measurements of the temperature with a thermocouple have shown that the temperature at the site of contact between cooler and nerve was 7° in 3 min time, 4.5-4° in 5 min, and 3-2.5° in 10 min. Chronic experiments on conscious dogs were used to register unseparated "spontaneous" impulses, after which local cooling of the nerve trunk above implanted electrodes was undertaken to separate the afferent flux. With the onset of the cooling block the number of registered impulses in the general flux of oscillations gradually decreased and only afferent impulses could now be separated. In 3 min time their number became constant. In simultaneous cooling of the nerve above and below the implanted electrodes oscillations were totally absent, which was evidence of the effectiveness of the cooling block.

The research shows that under conditions of a chronic experiment a block of nerve conduction can be induced in the sympathetic conductors with the aid of local reversible cooling. The block sets in 3-5 min when the temperature in the cooled zone is 4-7°.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
