INFLUENCE OF COOLING ON THE CONTENT OF PHOSPHORUS FRACTIONS IN THE SUPERIOR CERVICAL GANGLION

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In our previous investigations [2], the effect of gangliolytic agents on the exchange of energy-rich phosphorus fractions and on the conduction of neural excitation in the superior cervical ganglion was studied. In line with the fact that hypothermia has acquired great importance in current surgical clinical practice, we dedicated ourselves to the task of studying the effect of cooling on the content of the energy-rich phosphorus fractions and on the transmission of the neural stimulus in the superior cervical ganglion.

EXPERIMENTAL METHODS

The experiments were conducted on urethane-narcotized cats weighing from 1.5 to 3.5 kg. The functional state of the ganglion was checked on the basis of the contraction of the nictitating membrane in response to stimulation of the preganglionic trunk of the sympathetic nerve by electrical current. An ASM-3 type stimulator, generating square- wave impulses, served as the source of the stimulus. The impulses had a frequency of 40 cps, the duration of each individual stimulus was 3-8 millisec, the excitation being extended over a period of 10 seconds. The contractile actions of the nictitating membrane were registered on the smoked tape of a kymograph.

Artificial hypothermia was achieved by sandwiching the animal between dry and conventional ice. The animal was placed on a double-walled examination table. The hollow formed by the table was filled with ice water. The animal's temperature was measured with a mercury thermometer placed in the rectum. The cooling varied, in different experiments, from 28 to 15 deg.

After the required cooling temperature had been reached, the supetior cervical ganglion was quickly removed and immediately frozen in liquid nitrogen. In its frozen state, the ganglion was weighed on a torsion balance and then ground for 10-15 minutes with ice-chilled 5% trichloroacetic acid. The precipitate was then removed by centrifugation. The filtrate so obtained served for the determination of the phosphorus fractions. Inorganic phosphorus was precipitated by a magnesial mixture. In the centrifugate, after removal of the inorganic phosphorus, phosphocreatine was determined on the basis of inorganic phosphoric acid present, formed in acid medium in the presence of ammonium molybdate, on standing in a constant-temperature oven for 40 minutes at 37 deg. The content of adenosinetriphosphate (ATP) with its adenosinediphosphate (ADP) impurity, was taken as the difference between the phosphate content in the sample subjected to a 7-minute hydrolysis and phosphocreatine. Phosphorus was determined using the microchemical technique of Kuttner and Cohen. The experiment and control were usually carried out on the same animal, since it had been established by us earlier that the right and left ganglion of one and the same animal differed slightly in the content of the phosphorus fractions [2]. The experiments were usually performed in the following order: after suitable preparations had been made, the normal reaction of the nictitating membrane in response to irritation of the preganglionic nerve fiber of the sympathetic nerve was measured by electrical current, was measured. In some experiments, the respiration and pulse were also registered. The control ganglion was then quickly excised and frozen. The animal was then cooled

to the required temperature (23-26-20-18-15 deg), after which the experimental ganglion was taken for investigation (just as in the case of the control, respiration and pulse were registered in individual experiments).

EXPERIMENTAL RESULTS

Three series of experiments were carried out in the present work. In the first series of experiments, the influence of the temperature on the transmission of nerve excitation in the superior cervical ganglion and on the general state of the animal were studied. In the second series of experiments, the effect of cooling on the content of the phosphorus fractions present in the superior cervical ganglion was studied. In the third series of experiments, the influence of ATP on the transfer of the neural stimulus in the superior cervical ganglion under conditions of blockage of synaptic conduction was clarified through the technique of cooling.

First series of experiments. In this series of experiments, the animal was cooled to a temperature of 15-17 deg (i.e. to provide a possibility of carrying out the observations in all phases of the cooling). The beast was then warmed. The animal was sometimes recooled, after warming, to the assigned temperature (17-15 deg). The experiments demonstrated that cooling from 37-35 deg (the normal rectal temperature) to 17 deg by the method indicated could be achieved in $1\frac{1}{2}$ to 2 hours, while the recooling (after warming) took place in much less time. In 2 experiments, we had the possibility of comparing the periods required for the onset of cooling in decerebrate and narcotized cats. The onset of cooling in decerebrate cats occurred much sooner (in 1 hour to an hour and ten minutes) than was the case in narcotized cats. At the onset of cooling, i.e. at a 30-26° temperature, a stepping-up of the respiration and pulse rates was observed, while in some of the experiments, an enhancement of the tonus of the nicititating membrane in response to excitation of the preganglionic trunk of the sympathetic nerve by electrical current was noted. Further cooling to 25-23° resulted in a dropoff in the reaction of the nictitating membrane, and a slowing down of respiration and pulse. At 18-15°, the reaction of the nictitating membrane ceased entirely, breathing stopped, and then the heart followed suit. Survival of the animals without controlled respiration was then out of the question. However, after cooling to 18-15°, it was possible to restore the vital activity of the animal by warming it. The cold water in the reservoir was replaced with hot water to bring about warming. Hot water bottles were applied to the animal. In 5-10 minutes (after warming was initiated) the respiration and pulse rates increased, the reaction of the nictitating membrane improved, and, in 30-40 minutes the original level was reattained (this corresponded to a temperature of 36-34 deg). When the beast was warmed to a temperature above 40 deg, sharp dyspnea and blocking of ganglionic transmission were observed.

Second series of experiments. In this series of experiments, the animals were cooled to 28-25-22-20-18-15 deg. On cooling to 28-26 deg, most of the experiments showed an increase in ATP and phosphocreatine content, with essentially no change recorded in inorganic phosphorus content. The reaction of the nictitating membrane in response to irritation of the preganglionic trunk of the sympathetic nerve was enhanced. Cooling to below 25 deg caused a diminution of the reaction of the nictitating membrane, although ATP content retained its high levels. On cooling to below 22-20 deg, a drop in ATP and phosphocreatine content was observed, along with an increase in the content of inorganic phosphorus. The reaction of the nictitating membrane dropped sharply in the process. At a 18-15 deg temperature, the content of ATP and phosphocreatine dropped off sharply, while inorganic phosphorus increased; the reaction of the nictitating membrane disappeared entirely (cf. Table below).

Third series of experiments. In this series of experiments, a solution of ATP in a 1-2 mg/kg dose was administered via intravenous route, on a background of blockage of conduction caused by cooling down to 25-20 deg. The ATP dose was taken equal to a dosage which in our previous experiments facilitated the conduction of excitation in the superior cervical ganglion. The experiments showed that the administration of ATP, under this experimental arrangement, failed to facilitate the conduction of the neural stimulus, on the contrary hindering its transmission in a majority of the experiments.

DISCUSSION

Our data stand in agreement with the viewpoint expressed in the literature to the effect that, at different degrees of cooling, dissimilar functional states are to be observed. For example, A. A. Izbinsky, A. A. Kalikhman, J. W. Buzen [5,6,1] and others conditionally divide the duration of hypothermia into 4 phases. The first phase

Influence of Cooling on Conduction of Neural Stimulus and on Content of Phosphorus Fractions in Superior Cervical Ganglion (averaged data)

Temperature	Reaction of nictitating membrane	ATP + ADP content, as % of control	Phosphocreatine content as % of control	Content inorganic phosphorus as % of control	Breathing, % of control	Pulse rate, % of control
36°	Normal	100	100	100	100	100
28—26°	Elevated	271	122	95.5—116	160	120
22—20°	Depressed	52.7	84	155.5	60	76
18—15°	Depressed to point of block	12.4	38.2	122	25	40

involved cooling from 35 deg down to 29 deg: the second phase, cooling from 29 to 27 deg, the third, cooling from 21 to 19 deg, and the fourth, cooling to below 19 deg. All physiological functions are compensated in the first and second phases, and total decompensation of all physiological functions takes place in the third and fourth phases.

In the compensated cooling phase (i.e. the first and second phases), we observed an increase in ATP and phosphocreatine content and a facilitation of ganglionic conduction in the superior cervical ganglion. During this cooling period, according to data in the literature, there occurs an enhancement of all metabolic processes [1], excitation of the vegetative centers, facilitation of transmission in the central nervous system and in the ganglionic synapses, etc. [4,7]. It is difficult to find an explanation for the accumulation of ATP which takes place during this period in hypothermia. There are 2 possible variants: it takes place either 1) because of intensified ATP formation, or 2) because of blocking of ATP breakdown.

A. E. Gurvich and M. V. Kirzon [3], by cooling an isolated fatigued rabbit muscle, obtained a temporary intensification of individual muscle contractions. In connection with this, they advanced the suggestion that the cold brings about a delay in the breakdown of some substance, which appears upon the transfer of the stimulus from nerve to muscle. The accumulation of a store of this substance thereupon becomes a possibility. Juxtaposing our data to the results of the work performed by A. E. Gurvich and M. V. Kirzon, it may be inferred that ATP was accumulated in both investigations, possibly due to a hindrance to ATP breakdown. At a temperature of 25 deg, when a blockage of ganglionic conduction is observed and the content of phosphorus fractions remains at a high level, we suggest that the excess accumulation of ATP acts as a hindrance to synaptic conduction, since it is known that ATP, in small doses, facilitates, and in large doses hinders, ganglionic transmission of stimuli.

The third series of experiments brought confirmation to the suggestion advanced, since the administration of ATP in doses which, under normal conditions, would facilitate ganglionic transmission not only failed to facilitate it, but served as a powerful hindrance.

An explanation of the lowering of the content of the phosphorus fractions at temperatures below 20 deg presents no great difficulty, since this stage of hypothermia is characterized by a lowering of all vital functions: the mechanism governing body heat gets out of commission, as a result of which a passive drop in temperature ensues, along with inhibition of the activity of the vegetative centers, and a diminution of all forms of metabolism. It is apparent that the reduction in ATP content during this phase may be explained by an impairment in the processes involved in phosphorylation, as a consequence of the total decompensation of all vital functions.

On the basis of the experiments carried out, it may be concluded that, on cooling to 28 deg, there were observed a facilitation of ganglionic transmission and an increase in ATP and phosphocreatine content in the superior cervical ganglion. The inorganic phosphorus content remained essentially unchanged.

On cooling to 25 deg, blocking of ganglionic transmission was observed, although ATP and phosphocreatine content increased, while inorganic phosphorus either dropped or showed no change when compared to normal conditions. When ATP was administered in a 1-2 mg/kg dose, under conditions of cooling ganglionic transmission was obstructed.

When the animal was cooled to a 20-15 deg temperature, the content of the phosphorus fractions in the superior cervical ganglion showed a decrease, with ganglionic transmission being hampered, at the same time, up to the point of complete block.

SUMMARY

Experiments were performed on cats under urethane anesthesia. The effect of cooling on the content of the phosphorus fractions and transmission of nervous excitation in the superior cervical ganglion was studied. Experiments showed that ganglionic transmission is facilitated in cooling to 28°C. There is likewise increased content of adenosinetriphosphate (ATP) and phosphocreatine (PC) in the superior cervical ganglion, while the content of inorganic phosphorus remains almost the same. The ganglionic transmission is hindered in cooling to 25°C. However, the content of ATP and PC is increased in comparison with normal conditions, while the content of inorganic phosphorus is decreased or remains unchanged. The ganglionic transmission is hindered in introduction of ATP in the dose of 1 to 2 mg/kg of the body weight in conditions of cooling. Cooling of animals down to the temperature of 15 to 20°C is connected with decrease of phosphorus fractions and inhibition of ganglionic transmission up to complete block.

LITERATURE CITED

[1] J. W. Buzen, Polski Medyczny Zurnal, 43, 1416-1421 (1955).

[2] N. B. Vysotskaya, "Effect of gangliolytic preparations on the content of phosphorus fractions in the superior cervical ganglion," Farmakol. i Toksikol., 18, 2,12-15 (1957). *

[3] A. E. Gurvich and M. V. Kirzon, Byull. Eksptl. Biol. i Med., 21, 5, 34 (1946).

[4] V. S. Delov and E. G. Petrova, Transactions of Leningrad Branch of VIEM, 185 (1946).

[5] A. A. Izbinsky, Voprosy Kriopatologil, edit. by Girgolav (1953).* *

[6] A. A. Kalikhman, Voprosy Kriopatologii, edit. by Girgolav, (1953).* *

[7] B. V. Petrovsky, S. L. Babichev and O. D. Kolyutskaya, Khirurgiya, 9, 6-14 (1955).

* Original Russian pagination. See C. B. Translation.

** In Russian.