THE EFFECT OF INSULIN HYPOGLYCEMIA ON THE CONTRACTION OF THE NICTITATING MEMBRANE OF THE CAT DURING STIMULATION OF PREGANGLIONIC SYMPATHETIC NERVE FIBERS

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The working of the nervous system, and in particular of its autonomic division, at different levels of the blood sugar is one of great interest and a large number of investigations have been devoted to it (see the summary by Hellhorn [12]. In recent years several investigations on this subject have been carried out in our own laboratory [1-7]. The majority of workers point out that during slight hypoglycemia excitation of the sympathetic division of the nervous system predominates, but as hypoglycemia increases the parasympathetic division becomes more excited. However there is an almost complete absence of a differential analysis of the part played in these reactions by the cells of the various levels of the sympathetic division; in particular little work has been done on the study of the function of the sympathetic ganglia during hypoglycemia.

McIntosh [14] discovered that during perfusion of the superior cervical ganglion of the cat with Ringer's solution not containing glucose, stimulation of the preganglionic nerve prevents the transmission of impulses (as shown by diminution of the contraction of the nictitating membrane) and causes a fall in the acetylcholine content; during perfusion with the same solution containing glucose, according to Brown and Feldberg [11] even prolonged stimulation causes no change in the acetylcholine content of the ganglion tissue.

Kahlson and McIntosh [13] showed that during perfusion of the superior cervical ganglion with a solution not containing glucose, with prolonged stimulation a more rapid suppression of the transmission process appears (tested by the contraction of the nictitating membrane) than when glucose is present in the perfusion fluid; however the reaction of the ganglion to actylcholine injected from outside is not affected. Perry and Reinert [15] found that there is a falling off in the reaction to injected acetylcholine.

Conditions of perfusion and total deprivation of glucose are far from natural; for this reason it is of interest to investigate the function of the superior cervical ganglion in the intact animal in a state of insulin hypoglycemia.

The aim of this investigation was to trace the changes during insulin hypoglycemia of the threshold of excitation of the preganglionic nerve fibers and also the transmission of submaximal stimuli. The indicator used was the contraction of the nictitating membrane of the cat.

EXPERIMENTAL METHOD

Experiments were carried out on cats which were starved for 24 hours. The first group of experiments was performed under chloralose anesthesia. Ringer's solution, containing 0.8% chloralose, was injected (after induction with ether) into the femoral vein in a dose of 100 mg of anesthetic per kg body weight of the animal. The cervical sympathetic nerve was dissected out, ligated and divided; the peripheral end was laid on

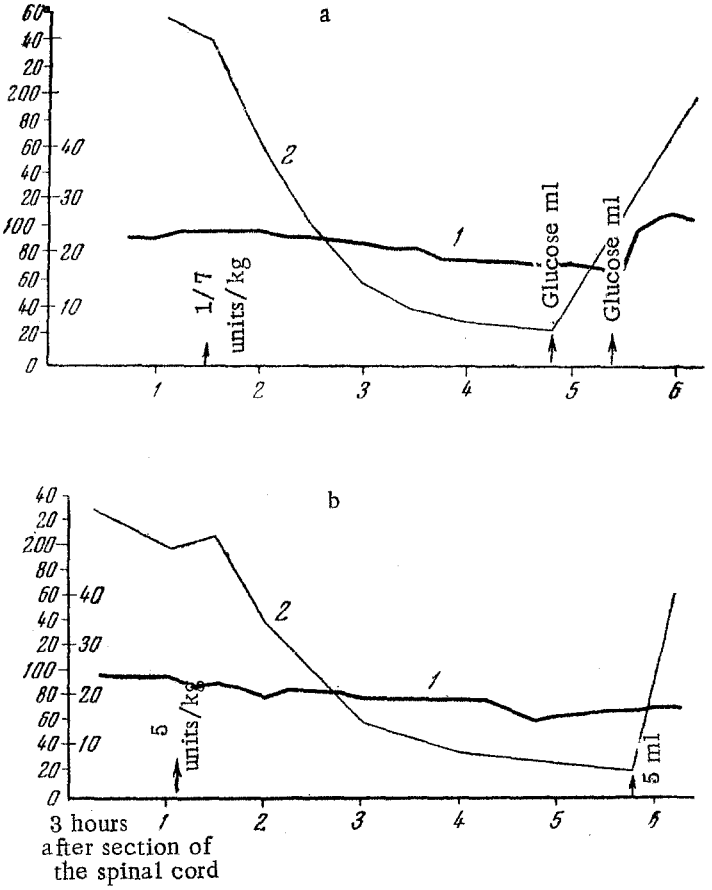

Fig. 1. The effect of insulin hypoglycemia on the threshold of stimulation of the preganglionic cervical sympathetic nerve, as indicated by contraction of the nictitating membrane of the cat. a) During chloralose anesthesia (experiment on January 15, 1957), b) during section of the spinal cord (experiment on April 13, 1957); 1) value of the threshold of stimulation; 2) level of the blood sugar in mg %. Along the axis of the abscissae – time in hours; along the ordinate axis: figures on the left – blood sugar level in mg %, figures on the right – value of the threshold of stimulation in divisions of the potentiometer scale. The first arrow indicates the moment of injection of insulin, the second and third – the moments of injection of glucose.

immersible electrodes. Gauze drainage strips were placed in the wound, soaked in Ringer's solution. To prevent displacement, the electrodes were fixed with a clamp attached to the stand. As a result of many hours of exposure on the electrodes, despite great care in some of the experiments the nerves were nevertheless damaged; these experiments were not considered. The nictitating membranes were grasped in clips and their contractions recorded on the kymograph by means of Swedish levers. The animal was kept warm throughout the experiment. When preparations were completed an interval of 1-2 hours was allowed, and then measurement of the thresholds of stimulation of the nerve was begun. Stimulation was carried out by means of an induction coil with an alternating current of a frequency of 50 cps. Into the secondary circuit of the coil was introduced a potentiometer with a scale graduated in 100 divisions. In the records and on the graphs the voltage of the stimulating current is expressed not in absolute values but in divisions of the potentiometer scale. When the threshold was being determined the stimulus lasted 3 seconds, with an interval of not less than 3 minutes between stimuli. The submaximal stimulation lasted 15 seconds and was given not more often than once in 15 minutes. In all the experiments blood was taken at definite intervals for estimation of the blood sugar content by the Hagedorn-Jensen method. After the threshold of stimulation of the nerve had remained

constant for not less than an hour, insulin in a dose of 5 units per 1 kg body weight of the animal was injected into the femoral vein.

In the second group of experiments, after ether induction the spinal cord was divided below the medulla; no other conditions were altered.

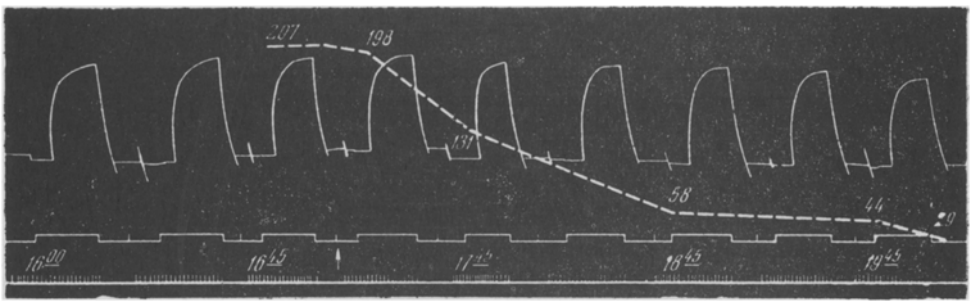

Fig. 2. Contraction of the nictitating membrane of the cat during submaximal stimulation of the preganglionic nerve during a state of insulin hypoglycemia (experiment on April 19, 1957). Significance of the curves: continuous lines (from above downwards) – record of the contraction of the nictitating membrane, marker of stimulation of the nerve (a rise in the line), time marker (1 second); broken line – curve of the blood sugar level in mg % (the zero line for measurement is the line of the time marker). The arrow indicates the moment of injection of insulin (5 units/kg).

In the third group of experiments, in order to ascertain the influence of hypoglycemia on the nictitating membrane, stimulation of the sympathetic nerve was replaced by intravenous injection of adrenalin. In order to increase the sensitivity of the nictitating membrane to adrenalin the right superior cervical ganglion was excised from 3-8 days before the experiment. The doses of adrenalin in different experiments were altered in accordance with the individual susceptibility of the animal (from 0.5 ml of a 1:20,000 solution to 1 ml of a 1:10,000 solution).

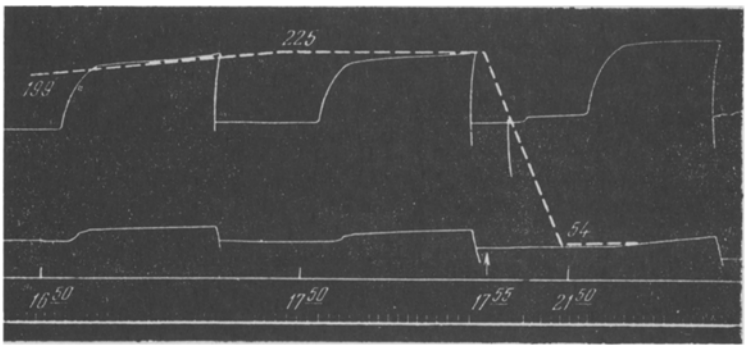

Fig. 3. The effect of insulin hypoglycemia on the contraction of the nictitating membrane of the cat under the influence of injection of adrenalin (experiment on May 21, 1957). Significance of the curves: continuous lines (from above downwards) – contraction of the right nictitating membrane (denervated), contraction of the left nictitating membrane (normal), marker of injection of adrenalin (0.5 ml of 0.02%), time marker (5 seconds); broken line – curve of the blood sugar level in mg %. The arrow indicates the moment of injection of insulin (5 units/kg).

EXPERIMENTAL RESULTS

The effect of insulin hypoglycemia on the threshold of stimulation of the preganglionic nerve fibers, recorded by the contraction of the nictitating membrane, was studied in 17 experiments (12 experiments under chloralose anesthesia and 5 experiments with section of the spinal cord).

In 8 of the experiments under chloralose anesthesia and in all the experiments in which the spinal cord was divided, during severe changes in the level of the blood sugar (from 250 to 20 mg %) no changes in the threshold of stimulation were observed, as may be seen in Fig. 1, a and b). In 3 experiments under chloralose anesthesia variations were noted in the threshold of stimulation, both a rise and fall. However, as shown by control experiments, in both the anesthetized and the spinal animals with a relatively stable high level of the blood sugar even more significant variations in the threshold are possible; only in one experiment under chloralose anesthesia did a slight hypoglycemia (61 mg %) give rise to an increase in the threshold of stimulation, but injection of glucose restored it to normal.

In some experiments in addition to determination of the threshold the contraction of the nictitating membrane was recorded in response to a submaximal stimulus (1.5 – 2 rheobases). In these conditions also it was not possible to detect any changes in the function of the ganglion during hypoglycemia (see Fig. 2).

The experiments with injection of adrenalin showed that insulin hypoglycemia does not cause changes in the reaction of the nictitating membrane to injection of adrenalin. The variations observed in the magnitude of the contractions were no greater than the same variations with the blood sugar at a high level (Fig. 3).

In the conditions which we adopted a fall in the level of the blood sugar caused no detectable effect on the function of the superior cervical ganglion.

Hellhorn [12] points out the significantly greater resistence to hypoglycemia of the sympathetic nerve cells as compared with the somatic. He considers this to be the result of the low metabolism of the sympathetic cells. Grounds in favor of this explanation in respect of the superior cervical ganglion may be found in the work by Bargeton [9], who showed that the ganglion functions even as long as 10-15 minutes after total cessation of its blood supply, and by Bronk [10], who found that even after anemia lasting 6 hours restoration of the blood flow leads to recovery of the function of the ganglion.

In the investigations of McIntosh [14], Kahlson and McIntosh [13], Perry and Reinert [15] in the first place the ganglion was completely deprived of glucose, which does not happed during a hypoglycemic state in the intact animal. Kahlson and McIntosh point out even negligible doses of glucose (starting with 5.6 μ g) restore the function of the ganglion. In the second place these authors cited used stimuli which were prolonged (for several hours), exhausting and supermaximal in the strength. Kahlson and McIntosh found that the effect of depriving the ganglion of glucose begins to show itself only after 10-15 minutes of maximum stimulation and that short interruptions lead to the restoration of transmission, but with short (1-2 minutes) periodic stimuli even complete exclusion of glucose from the perfusion fluid does not affect the magnitude of the contraction of the nictitating membrane. In the third place the investigations cited were carried out by perfusion of the ganglion. In these circumstances the humoral influence of the animal on the ganglion is excluded and the blood is replaced by saline solution. Perry and Reinert [15] point out that glutamate, for example, normalized the abnormal action of hexamethonium on the denervated ganglion only if it was given at the very beginning of perfusion. After perfusion had proceeded for a few minutes the effect of glutamate was lost. These authors refer to the work of Raaflaub [16] who showed that in a saline medium it is possible for enzymes to be washed out of the mitochondria, and consequently for severe disturbances of metabolism to take place.

Thus our findings do not contradict those of the authors cited, although in the latter case the changes in the function of the superior cervical ganglion were observed only in experimental conditions far removed from natural ones.

A. L. Shabadash [8], using a method similar to our own, found that glucose (especially glucose with insulin) suppresses the process of transmission in the ganglion, and accounts for this effect by overfilling of the preganglionic end plates with glycogen. We never observed suppression of this sort. This contradiction may possibly be explained by the fact that A. L. Shabadash obtained his results during mixed barbiturate anesthesia, and barbiturates are known to act on the endings of sympathetic nerve fibers. In two of our own experiments the use of pentothal sodium as an anesthetic caused a sharp reduction in the contraction of the nictitating membrane, in consequence of which we no longer used this anesthetic.

Our results demonstrate that during insulin hypoglycemia, under acute experimental conditions, the threshold of stimulation of the preganglionic nerve fibers leading to the superior cervical ganglion of the cat, as shown by contraction of the nictitating membrane, showed no appreciable change; contraction of the nictitating membrane during subminimal stimulation of the preganglionic nerve fibers and also under the influence of injection of adrenalin is also unaffected.

SUMMARY

Acute experiments were performed on cats with insulin hypoglycemia. It was established that the threshold of stimulation of preganglionic nerve fibers passing to the superior cervical ganglion (tested by the contraction of the nictitating membrane) does not show any significant change.

The contraction of the nictitating membrane remains unchanged in submaximal stimulation of the preganglionic nerve fibers. Adrenalin injection also has no effect on its contraction.

LITERATURE CITED

[1] N. Iu. Belenkov, Fiziol. Zhur. SSSR, 30, 704-710 (1941).

[2] N. Iu. Belenkov, Fiziol. Zhur. SSSR, 31, 3-4, 211-216 (1945).

[3] N. Iu. Belenkov, Fiziol. Zhur. SSSR, 31, 3-4, 218-227 (1945).

[4] N. Iu. Belenkov and E. N. Speranskaia, Fiziol. Zhur. SSSR, 34, 2, 285-291 (1948).

[5] M. I. Mitiumov, Biull. Eksptl. Biol. i Med. 7, 35-38 (1950).

[6] M. I. Mitiumov, Biull. Eksptl. Biol. i Med. 4, 48 (1952).

[7] M. I. Mitiumov, Biull. Eksptl. Biol. i Med. 6, 3-7 (1952).

[8] A. L. Shabadash, The Histochemical Investigation of Glycogen in the Normal Nervous System, Moscow, 1949.*

[9] D. Bargeton, Am. J. Physiol. 1938, v. 121, pp. 261-269.

[10] D. W. Bronk, J. Neurophysiol. 1939, v. 2, pp. 380-401.

[11] G. L. Brown and W. J. Feldberg, J. Physiol. 1936, v. 88, p. 265.

[12] E. Hellhorn, The Regulatory Functions of the Autonomic Nervous System, Moscow, IL, 1948 (Russian Translation).

[13] G. Kahlson and F. C. McIntosh, J. Physiol. 1939, v. 96, pp. 277-292.

[14] F. C. McIntosh, J. Physiol. 1938, v. 93, p. 47, P.

[15] W. L. Perry and H. Reinert, J. Physiol. 1955, v. 130, pp. 156-175.

[16] Raaflaub cited by W. L. Perry and H. Reinert.

*In Russian.