PHARMACOLOGY

CORRELATION OF THE CHANGES IN THE FUNCTION OF THE ADRENAL CORTEX AND CHOLINESTERASE ACTIVITY OF BLOOD AND BRAIN UNDER THE ACTION OF PROSERINE

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The role of the cholinoreactive structures in the regulation of the activity of the pituitary-adrenal system has been little studied. The use of acetylcholine for this purpose is inconvenient on account of its rapid inactivation in the organism; hence it is more expedient to use anticholinesterase substances.

The purpose of this work was to determine the influence and certain aspects of the mechanism of the effect of proserine on the function of the cortical layer of the adrenals in guinea pigs.

Preliminary experiments indicated that proserine penetrates rather rapidly through the hematoencephalic barrier in this species of animals, inducing inhibition of the cholinesterase activity not only of the blood, but also of the brain. In this respect, the action of proserine on guinea pigs differs from its influence on other animals, in particular, cats and rabbits, in which it passes very poorly through the blood-brain barrier [1, 3]. Hence, the administration of proserine to guinea pigs permits the use of this preparation for the analysis of the role not only of the peripheral, but also of the central cholinergic mechanisms in the regulation of the function of the pituitary-adrenal system.

EXPERIMENTAL PROCEDURE

The experiments were conducted on 104 guinea pigs of both sexes. Proserine was injected subcutaneously in a dose of 0.0033-0.1 mg per kg of weight in 1 ml of distilled water.

The central and peripheral effects of proserine were judged by the inhibition of the cholinesterase activity of the blood and brain, which was determined according to G. A. Panosyan's method [4], and also by electroencephalographic and electrocardiographic investigations (on the "Kaiser" and "Orion" electroencephalographs). Implanted needle electrodes with a difference between electrodes of 2 mm were used. The potentials were taken off from the sensomotor and parietal regions of the cerebral cortex. The ECG was taken in the second standard takeoff.

In order to differentiate the central effect of proserine from its peripheral influence, in part of the guinea pigs the brain stem was served with a spatula under brief ether narcosis. The plane of the section passed behind the posterior corpora quadrigemina or along them and the anterior third of the pons Varolii (pretrigeminal section) or between the corpora quadrigemina and anterior to the pons ("cerveau isolé") [6].

To determine the functional activity of the hypothalamo-hypophysary-adrenal system against a backgorund of severing of the brain stem, the control animals recieved rapid injections of 0.3 ml of cold physiological saline with a 5% glucose solution through a cannula implanted in the lateral ventricle of the brain an hour after the severing, and the content of 17-hydroxycorticosteroids in the blood was determined. The intactness of the vessels of the base of the brain after the severing was monitored at the end of the experiment with the aid of methylene blue, injected

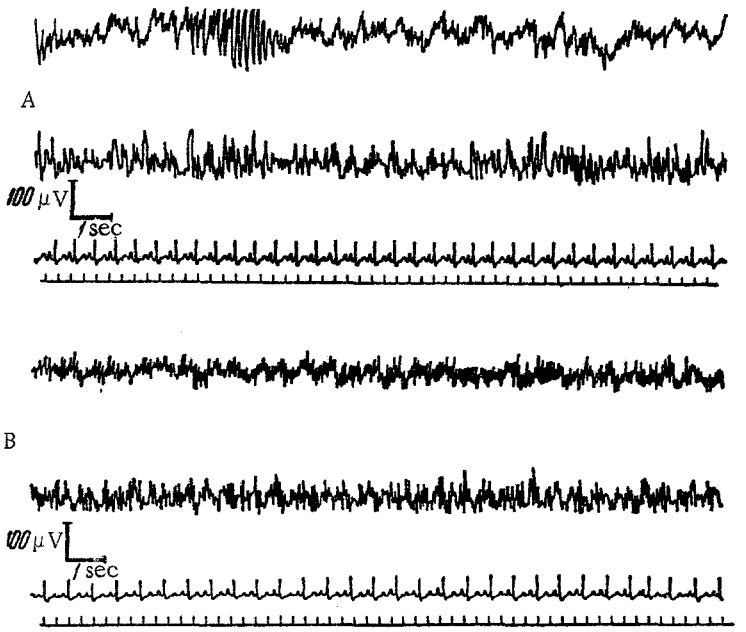

Fig. 1. EEG and ECG of guinea pigs with intact brain (A) and an hour after subcutaneous injection of 0.1 mg/kg proserine (B). From top to bottom: EEG of left sensomotor and parietal regions of the cerebral cortex; ECG.

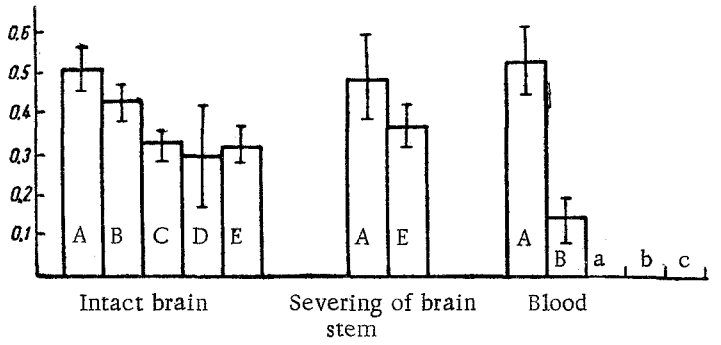

Fig. 2. Cholinesterase activity of the brain and blood an hour after injection of proserine. a, b, c) Rectangles of zero height, corresponding to zero activity of cholinesterase after injection of proserine in doses of 0.005, 0.05, and 0.1 mg/kg, respectively. A) norm; B) 0.033 mg/kg; C) 0.005 mg/kg; D) 0.05 mg/kg; E) 0.1 mg/kg. Along Y-axis—cholinesterase activity (expressed in 0.001 M acetic acid solution per 0.2 ml of brain homogenate and 0.1 ml of blood serum).

centrally through the carotid artery. The ability of the vessels of the brain to be stained above the site of the section and the level of the cross section were established after the animals were killed. The change in the functional state of the adrenal cortex was judged by the level of 17-hydroxycorticosteroids in the peripheral blood plasma of the guinea pigs [5]. In animals with intact brains, blood was taken an hour after the injection of proserine or distilled water. In the experiments with severing of the brain stem, the first blood sample was collected an hour after the operation, and the preparation or distilled water was injected immediately. The second blood sample was examined two hours after the severing (an hour after the injection of proserine or distilled water).

EXPERIMENTAL RESULTS

Subcutaneous injection of proserine into guinea pigs is accompanied by a change in the bioelectric activity of the cerebral cortex and heart, the cholinesterase activity of the brain and blood, as well as the function of the adrenal

TABLE 1. Content of 17-Hydroxycorticosteroids in the Blood of Guinea Pigs with Intact Brain an Hour After Injection of Proserine (in μg %). M ±m

Series of experiments	17-Hydroxycorticosteroids		No. of animals
Distilled water Proserine (0.0033 mg/kg)	57.77 ±4.24 51.96 ±4.23	(P > 0.1)	10
Distilled water Proserine (0.005 mg/kg)	36.69 ±4.43* 55.99 ±10.36	(P > 0.1)	8
Distilled water Proserine (0.05 mg/kg)	26.6> ±2.81* 69.49 ±5.98	(P < 0.001)	12
Distilled water Proserine (0.1 mg/kg)	33.38 ±3.93* 112.17 ±7.10	(P < 0.001)	15

*The relatively low initial level of corticosteroid content in the blood is evidently due to the fact that the experiments were conducted in the autumn to winter period, when the secretion of corticosteroids is at a minimum [7].

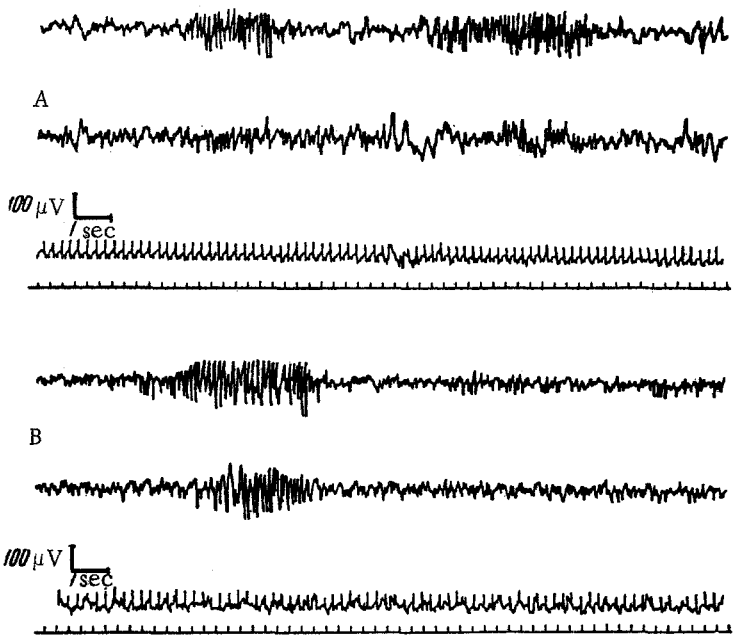

Fig. 3. EEG and ECG of guinea pigs with "cerveau isolé" before (A) and an hour after subcutaneous injection of 0.1 mg/kg proserine (B). From top to bottom: EEG of left sensomotor and parietal regions of the cerebral cortex; ECG.

cortex. As was indicated by electroencephalographic investigations, the introduction of proserine leads to activation of the EEG (Fig. 1). In the sensomotor and parietal regions of the cerebral cortex, the high-amplitude (60-70 μV) and slow (3 to 4 vibrations per second) rhythm is replaced by a low-amplitude (35-40 μV) and rapid (15-20 vibrations per second) rhythm. These changes develop gradually, the effect being manifested only by the thirtieth to fortieth minute; by an hour it becomes pronounced; during the following few hours, a low-amplitude, high-frequency rhythm predominates in the background bioelectric activity; however, it is noted that a "spindle" is retained in most of the animals. Determination of the cholinesterase activity of the brain and blood an hour after the injection of proserine indicated its inhibition even when the preparation is used in a dose of 0.0033 mg/kg. Further increasing the dose of the preparation administered is accompanied by a drop in the acetylcholinesterase activity of the brain by more than 1.5-fold. The blood cholinesterase activity is considerably more strongly reduced: an hour after the injection of proserine in a dose of 0.005 mg/kg and above, its activity in the blood drops to zero (Fig. 2).

TABLE 2. Content of 17-Hydroxycorticosteroids in the Blood of Guinea Pigs Against a Background of Severing of the Brain Stem (in μg %). M ±m

Series of experiments	17-Hydroxycorticosteroids		No. of animals
Control	One hour after section	65.75 ±7.33	
	Two hours after section	70.63 ±6.68 (P> 0.1)	10
Proserine (0.1 mg/kg)	One hour after section	76.57 ±4.45	
	Two hours after section (one hour after injection of proserine)	(P> 0.1)	15
		83.65 ±8.02	

A study of the content of 17-hydroxycorticosteroids in the peripheral blood plasma of the guinea pigs indicated that proserine in doses of 0.0033 and 0.005 mg/kg does not induce any statistically reliable change in the function of the adrenal cortex. The use of proserine in larger doses is accompanied by a substantial increase in the corticosteroid content in the blood, the level of which is higher, the larger the dose of the preparation (Table 1).

Thus, the data obtained indicate that at a definite degree of inhibition of the cholinesterase activity of the blood and brain, there is a change in the functional state of the adrenal cortex. However, the results of our experiments do not give an answer of what cholinoreactive structures (central or peripheral) are responsible for this action. To clarify this question, we conducted a series of experiments with different levels of severing of the brain stem. Such sections permit the exclusion of the entire peripheral impulsation, except for the impulsation transmitted through the olfactory and optical nerves.

Severing of the brain stem itself does not exert any significant effect upon the function of the adrenal cortex during a period of two hours after the operation. The hypothalamo-hypophysary-adrenal system remains functionally full valued under such experimental conditions, which is evidenced by the results of experiments with intragastric administration of cold physiological saline to the guinea pigs after severing of the brain stem. Such an influence leads to a substantial increase in the content of corticosteroids in the blood in comparison with the original level ($P < 0.001$).

Subcutaneous injection of 0.1 mg/kg proserine against a background of severing of the brain stem, in contrast to its administration to animals with intact brain, is not accompanied by any increase in the level of 17-hydroxycorticosteroids in the blood (Table 2). At the same time, in such guinea pigs the acetylcholinesterase activity of the brain above the site of section (see Fig. 2) is distinctly inhibited to approximately the same degree as in animals with an intact brain, while when the bioelectric activity of the cerebral cortex is recorded, activation of the EEG is noted (Fig. 3).

The experimental results give evidence that the cholinoreactive structures, situated above the level of the severing of the brain stem, evidently do not take part in the regulation of the function of the hypothalamo-hypophysary-adrenal system. Although our data also cannot entirely exclude the role of the cholinoreactive systems of the hindbrain, they still force us to assume that the main role in the mechanism of the influences of proserine on the function of the adrenal cortex is played by its peripheral anticholinesterase effect.

The change in the bioelectric activity of the brain upon the administration of proserine to guinea pigs is evidently accomplished by another mechanism: while in rabbits and cats the removal of the gasserian ganglion against a background of "l'encephale isolé" or of curarization of the animals [2, 3] prevents activation of the EEG under the action of proserine, in guinea pigs an activating effect of proserine is noted—in animals with pretrigeminal sections and "cerveau isolé," when the flux of impulses in the presence of muscular fibrillation is entirely stopped. Hence, the activation of the EEG observed after the administration of proserine to this species of animals probably depends upon inhibition of the acetylcholinesterase activity of the brain.

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