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MORPHOLOGICAL AND HISTOCHEMICAL ANALYSIS OF THE CENTRAL EFFECT OF ANGIOTENSIN II ON RATS PREVIOUSLY EXPOSED TO EMOTIONAL STRESS

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Key Words: angiotensin-II; stress; drinking behavior; hypothalamus

Previous exposure to emotional stress significantly modifies drinking behavior induced by central administration of angiotensin II (A-II). The latent period of the drinking response was considered shortened and the response itself was intensified. Other forms of behavior accompanying the drinking reflex, namely grooming, moving around the cage, eating food, and so on, were not present in the animals subjected to previous emotional stress. It has been suggested that the changes mentioned above in drinking behavior are due to stress-induced structural and neurochemical damage.

This paper presents morphological and histochemical data on the state of several brain formations, responsible for the drinking reflex following intracentral administration of A-II. Comparison of intact animals and animals previously exposed to emotional stress can be used to identify the role of central structures of feeding behavior in stress-induced changes in the drinking reflex in rats.

EXPERIMENTAL RESULTS

Experiments were carried out on male Wistar rats weighing 250-300 g. Three of the five groups of animals (1st, 3rd, and 5th) were chosen for morphological and histochemical analysis. Rats of group 1 received an injection of A-II ("Serva") in a dose of 100 ng in 5 μl of physiological saline into the lateral ventricle. Animals of group 3 received an injection of A-II, after previous exposure to immobilization stress. Rats of group 5 were immobilized after having previously been scalped. Animals kept under the same conditions in the animal house served as the control. Five animals were studied in each group. After decapitation the brain was removed in the cold and fixed in Carnoy's mixture. Serial paraffin sections were stained by Einarson's method for determination of total nucleic acids (NA) [9]. Quantitative estimation of NA was carried out on the LYUMAN I-3 microscope with FMÉL-1 photometric attachment, and filter with wavelength of 570 nm. The density of the photic flux was measured in the cytoplasm and nucleoli of the neurons in the test structures, after which the optical density of basophilic granules in the cells was determined by the equation:

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TABLE 1. Results of Morphometric Measurements of Neurons of the Brain Structures Chosen for Testing ($M \pm m$)

	Area of cytoplasm (S_c)			Area of nucleus (S_n)			Nucleo-cytoplasmic ratio (S_c/S_n)		
	control	A-II	stress+A-II	control	A-II	stress+A-II	control	A-II	stress + A-II
PVN	0,384±0,04	0,396±0,04	0,304±0,05	0,132±0,03	0,146±0,04	0,226±0,03	0,350±0,1	0,388±0,1	0,296±0,08
SON	0,650±0,02	0,560±0,11	0,240±0,09	0,290±0,08	0,350±0,09	0,084±0,03	0,420±0,18	0,620±0,15	0,360±0,14
SFO	0,198±0,04	0,160±0,05	0,160±0,05	0,082±0,03	0,093±0,02	0,093±0,02	0,440±0,19	0,440±0,19	0,312±0,13
ADN	0,270±0,05	0,316±0,07	0,210±0,04	0,140±0,02	0,102±0,03	0,142±0,02	0,406±0,18	0,324±0,09	0,406±0,18

Legend. Values given in relative units.

$$E = \log (\Phi_0/\Phi),$$

where E denotes the optical density of the medium, Φ_0 the photic flux transmitted through the preparation close to the section; Φ the photic flux transmitted through a section of the preparation [2].

In each animal 30 neurons were investigated in each of the following structures: paraventricular and supraoptic hypothalamic nuclei, subfornical organ, and anterior dorsal thalamic nucleus. In each neuron five measurements of the density of the photic flux transmitted through the cytoplasm and one measurement in the nucleolus were made. The results were subjected to statistical analysis by Student's t test. The morphometric measurements were made with an ocular grid with magnification of 70 times. The numerical data were processed with the aid of R. B. Strelkov's table.

EXPERIMENTAL RESULTS

Of the periventricular nuclear formations responsible for drinking behavior known at the present time, the following were chosen for morphological and histochemical analysis: the subfornical organ (SFO) as a receptor of the blood and CSF A-II, possessing direct axonal connections with many central structures of the food reflex [6, 7]; the supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei, which are similar with respect to the presence of A-II receptors, and for connections with central structures of drinking behavior, and with the median eminence. At the same time these nuclei differ sharply in their connections with extrahypothalamic brain formations. Additionally, to assess the specificity of the cytological changes in the above-mentioned structures, we studied the anterior dorsal thalamic nucleus (ADN). There is no information in the known literature on the role of this nucleus in drinking behavior.

The results given in Table 1 and Fig. 1 show that central administration of A-II leads to significant metabolic changes in neurons in the brain nuclei chosen for study, and to a change in the NA concentration which, in turn, determines changes in synthesis of specific proteins. It was also shown that the three structures of drinking behavior taken for analysis, judging by the morphological and neurochemical parameters, respond to injection of A-II into intact (group 1) and previously stressed (group 3) animals differently.

Neurons of PVN increased their functional activity considerably after injection of A-II: the NA concentration rose in the nucleolus and cytoplasm. By contrast with PVN and SON, the NA concentration in the nucleolus fell, but in the cytoplasm it rose; however, this rise took place against the background of a decrease in area of the neurons, indicating redistribution of NA in the cell rather than its enhanced synthesis.

The picture of the PVN neurons after injection of A-II preceded by stress indicates exhaustion of the neurons, to a more marked degree than after immobilization alone, not followed by injection of A-II: central chromatolysis, hyperchromatosis, and changes in the shape of the nucleus, with ectopia. Morphological and histochemical changes in neurons of SON after injection of A-II, preceded by stress, were less marked than in PVN: perinuclear chromatolysis, a decrease in area of the nucleus, but the shape and color of the nucleus and its position were identical with those in the control animals, and the NA concentration in the cytoplasm was increased. The NA concentration in nucleoli of ADN cells changed after immobilization in the same way as in other structures, possibly due to a general reaction of the neurons to stress. Comparison of the changes in NA in the cytoplasm of ADN (Fig. 1; Table 1) demonstrates the specificity of response of the remaining structures tested to injection of A-II preceded by immobilization stress.

Thus neurons of PVN, after central injection of A-II, exhibit much greater functional activity than neurons of SON, which evidently reflects differences between these nuclei in the organization of drinking behavior [3, 5, 12].

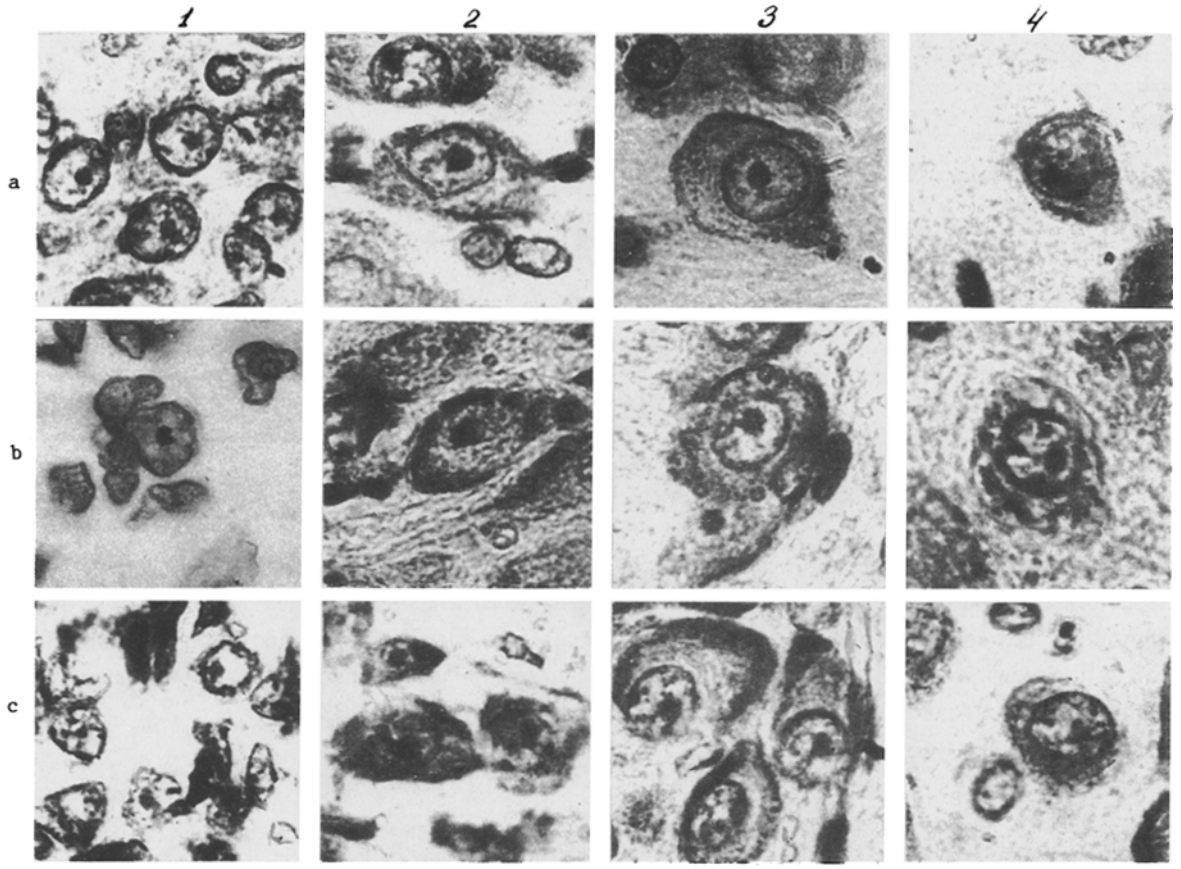

Fig. 1. Morphological changes in brain nuclei in response to injection of angiotensin II preceded by stress. a) Control, b) A-II, c) A-II preceded by stress. 1) SFO, 2) PVN, 3) SON, 4) ADN.

Immobilization stress leads to more marked structural and neurochemical changes in PVN than in SON. PVN is known to have extensive intracentral connections both with structures of the forebrain and with brain-stem and spinal centers, and it is distinguished by complex neurochemical features, which determine the multifaceted role of this nucleus in the realization of stress [1, 4, 8, 10].

Particularly sharp changes were found in the PVN of animals receiving A-II after previous exposure to stress. The result is evidence that disturbances of drinking behavior detected by physiological experiments after immobilization may be largely due to dysfunction of the neurons of PVN.

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IMPROVED METHOD OF ISOLATING THE SUPRAEPITHELIAL LAYER OF MUCUS FROM THE SMALL INTESTINE OF EXPERIMENTAL ANIMALS

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Key Words: small intestine; intestinal mucus; method of obtaining; digestive enzymes

Many recent investigations have shown that the supraepithelial layer of mucus of the small intestine not only performs a protective function but also has a very important role in the final stage of digestion, by participating in the degradation of products of intraluminal hydrolysis and their subsequent transport [3, 6, 13, 14]. The mucous layer has been shown to contain active pancreatic and intestinal enzymes proper, but the role of contact (taking place in the mucous layer) digestion has been interpreted differently [6, 10, 11]. A solution to this problem is possible only by the use of a standard method of obtaining mucous deposits, so that the juxtamural layer can be separated sufficiently completely without damage to the epithelial cells.

The method suggested previously [3], namely removing mucus with a spatula under a binocular microscope is applicable only to the intestine of large animals and cannot be used for quantitative assessment of the role of the enzymes of this layer, because in this case it is possible to obtain only the intraluminal part of the mucus, and there is great probability of injuring the epithelium.

Some studies of the radial distribution of enzymes of the final stages of hydrolysis of food substances in the small intestine have been undertaken by Pitran's method of isolating mucous deposits [6, 11]. In this method the supraepithelial layer of mucus is obtained from the everted intestine, fitted on to a glass rod into a test tube by manual forward and rotary shaking. It will be evident that the inadequate standardization of this procedure and the possibility of damaging the preparation on the wall of the tube, i.e., the fact that the efficacy of separation of the mucus depends on the experimenter's skill, has led to the obtaining of contradictory results.

The aim of this investigation was to develop a standard method of isolation of the supraepithelial layer of mucus with the aid of a special apparatus, eliminating the above deficiencies, and allowing the supraepithelial mucus to be separated more completely, while preventing the possibility of damaged villi and their fragments from the traumatized ends of the intestinal preparation from contaminating the fraction of mucous deposits.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 400-450 g. The rats were deprived of food for 12 h before sacrifice. The rats were killed by decapitation. In all experiments segments of small intestine 6 cm long, taken 10 cm distally to the stomach, were used. A ligature was applied to one end of the intestinal segment, after which the segment was everted and fitted on the working rod of the apparatus for shaking the juxtamural layer of mucus, which consisted of a system of a rigidly fixed rod, in contact with a toothed disk, mounted on the axle of an electric motor (Fig. 1). When the disk rotates, it induces vi-

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