ROLE OF ANGIOTENSIN-II IN MECHANISMS OF REGULATION OF ETHANOL CONSUMPTION IN RATS

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The writers showed previously that the formation of alcohol motivation in rats is accompanied by significant changes in the structure of the neurochemcial mechanisms of thirst regulation [2, 3]. More recently work has been published which shows a direct involvement of vasoactive neuropeptides (angiotensin-II, bradykinin) in the central mechanisms of regulation of water consumption [5-9]. The dipsogenic action of angiotensin-II under these circumstances has been linked by some workers with activation of the hypothalamic-hypophyseal neurosecretory system [4, 10].

The object of this investigation was to study the state of the hypothalamic-hypophyseal neurosecretory system and the renin-angiotensin system in animals under normal conditions, during prolonged alcohol administration, and during the formation of an artificial alcohol motivation.

EXPERIMENTAL METHOD

Experiments were carried out on 92 male Wistar, August, and noninbred rats weighing initially 200-250 g. An artificial alcohol motivation was formed in the rats by the method described by the writers previously [1-3]. Depending on the experimental conditions all the animals were divided into three principal groups: Group 1 consisted of 10 rats with marked alcohol motivation (alcohol deprivation for 2-3 days), group 2 consisted of 40 rats with chronic alcohol administration (enforced or voluntary consumption of 20% ethanol solution for 30-40 days), and group 3 consisted of 25 intact rats.

All animals except the controls (17 rats), kept under unrestrained conditions, were given single intraventricular (100 mg/μl, 3 μl) and intrahypothalamic (50 ng/μl, 3 μl) microinjections of angiotensin-II (from Serva, West Germany) by means of a specially designed microinjector. The quantity of fluid (alcohol or water) consumed by the rats was recorded for 1 h of the postinjection period, and also daily during the subsequent 1-2 weeks of observation. At the end of the experiments the location of the tips of the cannulas was confirmed histologically either by a rapid photographic method or by staining sections by Nissl's method. The state of the hypothalamic-hypophyseal neurosecretory and renin-angiotensin systems was studied in some animals (six rats of group 1, five of group 2, and five of group 3) on the 5th-6th days after single microinjections of angiotensin-II. The state of the above-mentioned systems was judged from changes in the RNA content in the nucleoli of neurons of the supraoptic nucleus (SON) of the hypothalamus (cytophotometrically), and by the change in the number of granules of neurosecretion in axovasal synapses of the neurohypophysis and the number of renin granules in the cells of the juxtamedullary apparatus of the kidneys (electron-microscopically). For comparative analysis, similar tests were carried out on animals of the control group, which did not receive angiotensin-II: seven intact rats, five rats receiving alcohol, and five rats with marked alcohol motivation. Material for cytophotometry was fixed in Carnoy's fluid. Serial paraffin sections 7 μ thick were stained with gallocyanin

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Table 1. Effect of Intraventricular (I) and Intrahypothalamic (II) Injections of Microdoses of Angiotensin II on Consumption of 20% Alcohol Solution and Water by Rats during First Hour after Injection (M ± m)

Group of rats	Mode of administration	Number of rats	Mean volume of fluid consumed, ml		Behavioral reactions unconnected with drinking water and 20% alcohol solution
			20% alcohol solution	water	
1	I	10	2,4±0,7	—	Orienting-investigative reactions, washing, grooming
	II	6	2,7±0,5	—	
2	I	30	—	—	Orienting-investigative, feeding, sex behavioral reactions, washing, grooming
	II	10	—	—	
3	I	15	—	5.1±2.9	—
	II	6	—	4,7±1,8	

TABLE 2. Duration of Effect of Single Intraventricular (I) and Intrahypothalamic (II) Microinjections of Angiotensin II into Rats of Different Groups (M ± m)

Group of rats	Mode of administration	Volume of liquid consumed, ml		Volume of fluid consumed after injection of angiotensin II				Duration of effect, days
				ml		%relative to background		
		20% alcohol solution	water	20% alcohol solution	water	20% alcohol solution	water	
1	I	15,1±0,4	0,6±0,3	8,2±0,7	1,7±0,7	45	183	2—13 (mean 6)
2	II	12,3±0,9	0,8±0,3	6,5±1,1	1,6±0,4	47	100	
3	I	—	4,2±2,3	—	9,6±2,8	—	129	1—10 (mean 5)
	II	—	3,9±2,0	—	9,4±2,5	—	141	

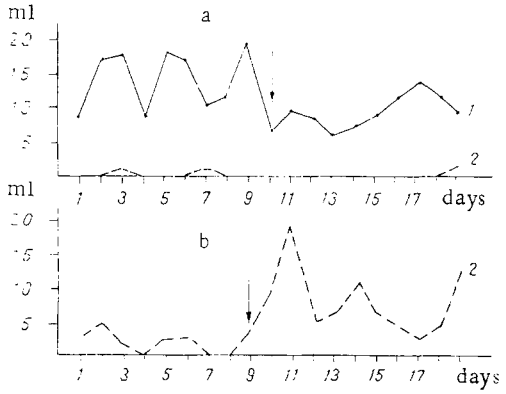

Fig. 1. Time course of consumption of 20% alcohol solution (1) and water (2) by rats after single microinjections of angiotensin II: a) rat with chronic ethanol consumption, b) intact rat. Vertical arrow indicated injection of angiotensin II.

and chrome alum by Einarson's method. The RNA concentration in SON neurons of the hypothalamus was judged from the optical density of the nucleolus, measured on a microphotometer at a wavelength of 570 nm. Material for electron microscopy was fixed in 1% OsO_4 solution and embedded in Araldite. The numerical results were subjected to statistical analysis.

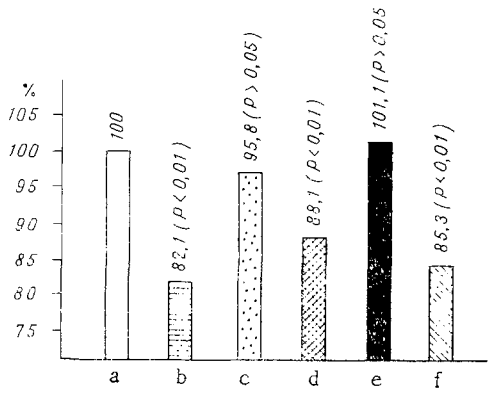

Fig. 2. Changes in RNA content in nucleoli of SON neurons of hypothalamus of control and experimental rats belonging to different groups (in % of value obtained in intact animals). Control rats: a) intact; b) with chronic alcohol administration; c) with alcohol deprivation for 2-3 days. Experimental animals on 5th-6th days after single intraventricular injection of angiotensin-II; d) alcohol deprivation for 2-3 days; e) chronic alcohol administration; f) intact.

EXPERIMENTAL RESULTS

Single microinjections of angiotensin-II into the "thirst center" of the perifornical zones of the hypothalamus in all intact rats led to the appearance of behavioral reactions of seeking and actively drinking water. In the animals of group 2, exposed beforehand to chronic alcohol administration, in response to similar chemical stimulation of the same hypothalamic zones no activation of drinking behavior was observed (consumption of alcohol or water). Microinjections of angiotensin-II into these animals caused the appearance of intensive grooming, feeding, sex, and orienting-investigative behavioral reactions. Intrahypothalamic chemical stimulation with angiotensin-II in rats with marked alcohol motivation (group 1) also was found to reduce the quantity of alcohol consumed on average by 20-30%.

Microinjections of angiotensin-II into the lateral ventricles induced effects similar to those observed after intrahypothalamic injections in the experimental animals of all three groups.

The quantitative results showing water and 20% alcohol solution consumption during the first hour after intrahypothalamic and intraventricular microinjections of angiotensin II are given in Table 1.

Recording the volumes of water and alcohol consumed voluntarily by the experimental animals over a period of time showed the presence not only of the short-term effects described above, but also of a marked long-term inhibitory action of angiotensin II on alcohol drinking by the rats of groups 1 and 2. This inhibitory effect of angiotensin II on alcohol consumption by the rats persisted until a few weeks after the beginning of observation (1-2 weeks). In the intact animals (group 3), on the other hand, an increase was observed in the daily water consumption during 4-5 days of the postinjection period. Data showing the time course of water and alcohol consumption by the rats are given in Table 2 and Fig. 1.

Cytophotometric studies of the RNA concentration in nucleoli of SON neurons of the hypothalamus in control rats showed a marked increase compared with intact rats in this parameter in the animals with chronic alcohol administration (on average by 82.1%; $P < 0.01$), which was more marked in the rats with a developed addiction for alcohol. In the rats with deprivation of alcohol for 2 days a tendency was noted for the state of the neurons to return to normal. The study of the RNA concentration 5-6 days after injection of angiotensin-II showed a decrease in SON nucleoli of the hypothalamus in the experimental animals of groups 1 and 3, whereas in the rats of group 2, on the other hand, the RNA level was completely back to normal, and in some cases it was actually increased a little.

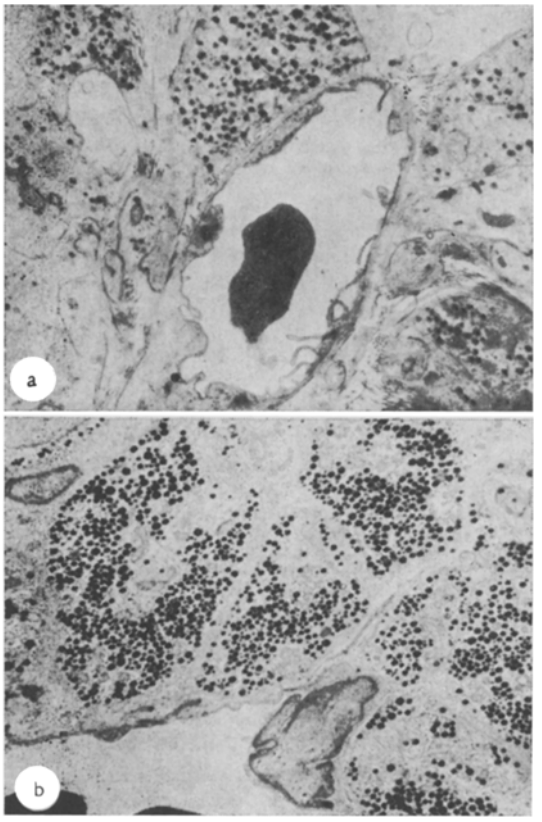

Fig. 3. Ultrastructural changes in cells of neurohypophysis in rats after chronic alcohol consumption: a) decrease in number of neurosecretory granules in control animals consuming alcohol for a long time; b) normalization of number of neurosecretory granules in rats with chronic alcohol consumption after injection of angiotensin-II. 8000 ×.

The mean statistical data on RNA concentration in SON nucleoli of the hypothalamus of the control and experimental animals of the different groups are illustrated in Fig. 2.

Electron-microscopic investigation of the state of the neurosecretory granules in axovasal synapses (herring's bodies) showed a decrease in the number of neurosecretory granules in the neurohypophysis of control rats with chronic alcohol administration, and also accumulation of granules along the course of the neurosecretory cells and in certain areas of the axovasal synapses (Fig. 3a). In the experimental animals of group 2, consuming alcohol for a long time, the picture observed after injection of angiotensin II differed only a little from normal (Fig. 3b).

An electron-microscopic study of the state of the juxtamedullary apparatus of the kidneys showed an increase in the number of granules, including immature ones, in the epithelioid cells of all rats studied except intact animals of the control group. The number of lipid inclusions showed no significant change in the interstitial cells of the medulla, which are known to produce prostaglandins. The changes described above in the kidneys of the rats receiving alcohol and injections of angiotensin II can be interpreted as signs of activation of the peripheral zones of the renin-angiotensin system.

The results of experiments to study the long-term inhibitory effect of angiotensin-II on alcohol intake in rats and the data showing a decrease in activity of the vasopressin-synthesizing neurosecretory cells of SON of the hypothalamus and of the Herring's bodies with compensatory activation of the peripheral zones of the renin-angiotensin system are thus evidence of specific changes in the properties of the hypothalamic-hypophyseal neurosecretory and renin-angiotensin systems during the formation and realization of alcohol motivation in rats. Incorporation of ethanol metabolites in the normal metabolic processes of the brain evidently modifies considerably the role of angiotensin-II in the acitvity of the two systems.

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ABILITY OF DIPHOSPHONATES TO BIND CALCIUM AND THEIR EFFECT ON OSMOTIC PERMEABILITY OF THE FROG BLADDER WALL

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Calcium plays an exceptionally important role in the regulation of various physiological processes, stabilization of membranes, and phenomena of cell adhesion [13]. Diphosphonates have begun to be used in recent years in the treatment of disturbances of calcium metabolism [1, 9, 11]. Together with other effects, the possibility of binding of calcium with these substances in biological systems must probably be taken into account in the mechanism of their action. To study the degree of extraction of calcium from biological structures by diphosphonates, changes in permeability of the wall of the isolated frog urinary bladder for water were determined. This object has been widely used in recent years in experimental physiology in order to study membrane transport [6].

The object of the present investigation was to compare the effect of diphosphonates on the osmotic permeability of the frog's urinary bladder, depending on their ability to bind calcium ions.

EXPERIMENTAL METHOD

The action of a series of organophosphorus complexones with different stability constants with calcium ions and being analogs of methylenediphosphonic acid (Table 1, compounds Nos. 2-6), and also of compound No. 7, similar in its structure to ethylenediaminetetraacetic acid (compound No. 1) was studied. The experiments were conducted as follows. The isolated urinary bladders of frogs (*Rana temporaria*) were filled with Ringer's solution diluted with water (1:10) and were placed in aerated Ringer's solution. After various time intervals the bladders with their contents were weighed, and the decrease in weight was used to calculate the volume of water which had passed through the bladder wall along the osmotic gradient [7]. Complexones were added to the Ringer's solution on the side of the serous membrane.

EXPERIMENTAL RESULTS

Under ordinary conditions the bladder wall possesses very low osmotic permeability and water transport along the osmotic gradient amounts to 0.03-0.05 $\mu l/cm^2 \cdot min$. At the beginning of the experiment inside the bladder there was 700-800 µl of water and the loss of water dur-

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