7. N. F. Podvigin, F. N. Makarov, and Yu. E. Shelepin, Elements of Structural and Functional Organization of the Visual-Oculomotor System [in Russian], Leningrad (1986).
8. A. Ya. Supin, The Neurophysiology of Vision in Mammals [in Russian], Moscow (1981).
9. J. Altman and M. B. Carpenter, J. Comp. Neurol., 119, 77 (1961).
10. G. Amato, G. Gravante, V. La Grutta, et al., Arch. Sci. Biol., (Bologna), 61, 61 (1977).
11. G. Amato, G. Solerno, and F. Sorbera, Boll. Soc. Ital. Biol. Spher., 52, 1183 (1977).
12. A. Molotchnikoff, D. Delaunais, C. Casanova, and P. Lachapelle, Brain Res., 375, 66 (1986).
13. G. Orban, E. Vandenbusshe, and M. Callens, Brain Res., 44, 225 (1972).
14. C. H. Sawyer, J. W. Everett, and J. D. Green, J. Comp. Neurol., 101, 801 (1954).
15. F. Schimielau, Exp. Brain Res., 36, 20 (1979).

CONSUMPTION OF WATER AND SODIUM CHLORIDE SOLUTION BY RATS AFTER CENTRAL INJECTION OF LITORIN AND AFTER IMMUNIZATION WITH LITORIN–BOVINE SERUM ALBUMIN CONJUGATE

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Central and peripheral administration of the regulatory peptides (RP) bombesin and litorin inhibit the feeding and drinking behavior of animals [4-7]. However, the effects observed after administration of exogenous bioregulators are not always identical with their physiological functions. It is important to assess not only the consequences of an increase in their concentration in the body, but also the manifestation of their deficiency. A promising method in this respect is active immunization of animals with peptides conjugated with high-molecular-weight carrier antigens [1, 2].

The aim of this investigation was to study the pattern of consumption of water and sodium chloride solution by rats after a single intracerebral injection of litorin and after long-term immunization of animals with a conjugate of litorin and bovine serum albumin (BSA), in order to elucidate the role of the bombesin-like factor in the "salt appetite" phenomenon in rats.

EXPERIMENTAL METHOD

Before the experiments the physiological activity of litorin was tested on a separate group of rats for the characteristic hypothermic effect of this RP. The animals were kept in a Kogan chamber and were given an intraperitoneal injection of physiological saline or of litorin in a dose of 10 μg/kg body weight. The rectal temperature was measured by means of a TPM-1 electric thermometer. The air temperature was 18°C. The rectal temperature of animals receiving an injection of litorin was significantly reduced: on average by 1°C. Behavioral tests were carried out on 46 noninbred male rats weighing 250-300 g. Those animals which subsequently received an intracerebral injection of litorin or physiological

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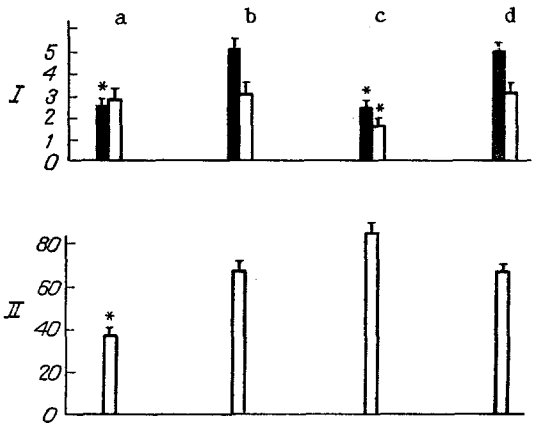

Fig. 1. Consumption of water and sodium chloride solution and CAS after 1 h of testing. Ordinate: I) volumes of fluid drunk (in ml); II) CAS (in percent). Black columns – salt solution, white columns – water; a) litorin, b) physiological saline, c) conjugate, d) albumin. Asterisk indicates significance of differences ($p < 0.05$).

saline were first cannulated in the region of the lateral cerebral ventricles. Rats of the other two groups were immunized as follows: first with a conjugate of litorin and BSA mixed with Freund's complete adjuvant (first and second immunizations) and with Freund's incomplete adjuvant (third and fourth immunizations); the rest were immunized with a solution of BSA treated with 2% glutaraldehyde and with the same adjuvant (control). Immunization was carried out four times with intervals of 7 days and each immunization was done as several subcutaneous injections in a total volume of 0.2 ml per rat. The concentrations of the solutions, expressed as protein, were 600 μg/kg and the ratio of solutions of conjugate (or BSA) to Freund's adjuvant was 1:1. The dose of centrally injected litorin was 500 ng/20 μl per rat, and the volume of conjugate injected was 0.073 ml per rat. The animals were kept one to a cage, where they were provided with pellet food for laboratory rats and water, and also with 1% sodium chloride solution. The volumes of water and salt solution drunk were noted daily for 3-4 days. The animals were then deprived of fluids and food for 16 h (noctural deprivation), after which the bowls containing the same fluids were restored. Central injections were given at the time when the bowls were returned to the rats. During testing, lapping movements of the rats' tongue were recorded during drinking by means of a sensor system based on a K176LA7 microcircuit and KT 315 b transistors. Pulses formed by the device were recorded on a N338-1P high-speed automatic recorder. The duration of grooming (washing, licking the fur, scratching) also was recorded. These tests continued for 1 h. When the results were analyzed, average daily volumes of water and 1% salt solution drunk, volumes drunk per hour of testing, and also a coefficient of attraction for salt (CAS), which we suggested, was determined by the formula:

$$\text{CAS} = \frac{\text{Number of lappings of salt solution after first passage from bowl to bowl}}{\text{Total number of lappings after first passage from bowl to bowl}} \times 100\%$$

The duration of grooming during the first and second halves of an experiment lasting 1 h also was noted (in sec). After the experiments the rats were killed by decapitation and their blood sampled. The blood was centrifuged and the resulting plasma used to isolate antibodies specific for litorin. In the animals with cannulas, the brain was perfused after sacrifice with 10% formalin and the locations of the tips of the cannulas in the region of the lateral ventricles identified by a rapid photographic method.

EXPERIMENTAL RESULTS

Animals immunized with the conjugate of litorin and BSA showed considerable preference for salt solutions over water compared with all the remaining groups of rats on average after one day, and without any increase in total fluid consumption (the ratio of salt solution to water was 23.83 ± 2.36: 11.93 ± 2.21 for immunized animals and 20.54 ± 2.10:16.42 ± 2.40 for rats of the control group). However, after deprivation for 16 h the animals of

TABLE 1. Duration of Grooming (in sec) during First and Second Halves of Experiment

Procedure	Grooming, per hour	
	0–30 min	30–60 min
Injection of litorin into cerebral ventricles	745,74±85,73*	251,50±20,15
Injection of physiological saline into cerebral ventricles	75,32±29,30	197,15±71,15
Immunization subcutaneously with conjugate	146,92±56,86	359,41±52,27**
Immunization subcutaneously with BSA	113,57±34,73	508,49±100,88**

Legend. *$p < 0.005$, **$p < 0.05$ – significance of differences compared with injection of physiological saline.

this group drank less fluid (both water and salt solution) than the control rats. CAS reached its highest value in the immunized animals, but in rats receiving an intracerebral injection of litorin, it reached its lowest values compared with animals of the control groups (Fig. 1). This coefficient, in our opinion, reflects to a greater degree the preference of the rats for salt solution to water than the actual volumes drunk. Rats after deprivation could approach either of the two bowls with about equal probability at first, but the dipsogenic effect of nocturnal deprivation was such that the animals could drink the unpreferred fluid for a long time also. Counting from the time when the animals passed to the second bowl or subsequently returned to the first bowl therefore reflects true preference.

Rats into whose cerebral ventricles litorin was injected showed a general decrease in fluid consumption on average per hour of the experiment. The volume of salt solution drunk by these rats, moreover, did not exceed the volume of water consumed, by contrast with the pattern of prevalence of preference for salt solution to water in animals of all the remaining groups. Injection of litorin into the cerebral ventricles caused excess grooming in the rats, mainly in the form of scratching (Table 1), and led to an increase of total motor activity. This action of litorin was particularly marked during the first 20 min of the experiment. Unexpectedly for us, animals immunized with litorin conjugate, and also rats receiving injections of BSA, exhibited a marked increase in the duration of grooming in the second half of the experiment (Table 1). It can be tentatively suggested that these animals had itching of their skin because of the considerable number of infiltrations as a result of the repeated injections of the substances used.

The titer of antibodies on the 30th day after the first immunization was 1:128-1:2048 in the immunized experimental animals and 1:16-1:32 in the controls. This indicates a strong immune reaction in the experimental rats.

Active immunization to litorin, aimed at creating a deficiency of the endogenous peptide, thus led to intensification of the average consumption of sodium chloride solution per day and to an increase in preference for salt, judging by the CAS, during testing for 1 h. Reduction of the quantity of water and salt solution consumed during the short time interval of 1 h can probably be explained by the weaker dipsogenic action of deprivation for 16 h for the immunized rats, for no decrease in fluid consumption was observed for them on average for the day. A single central injection of litorin depressed the initial preference for salt solution in an experiment lasting 1 h. The intensification of grooming accompanying this reaction may be connected with an increase in dopamine metabolism in the rat brain, just as is observed after central injection of bombesin [10]. Some workers consider that suppression of food and fluid consumption in rats after central injection of certain peptides is due to changes in noradrenalin and GABA metabolism, modulating taste perception in animals [9]. There is evidence that the taste of solutions can influence the degree of the antidipsogenic effect, in particular, of substance P [8]. Bombesin-like peptides are known to resemble in some of their effects substance P, and they are frequently put together into a single group [3]. What the action of centrally injected litorin on

preference by rats for salt solution may be similar to the antidipsogenic effect of substance P and may be effected through modulation of taste perception as a result of a change in monoamine and GABA metabolism. Intensification of the attraction for salt, under the influence of a procedure aimed at lowering the endogenous peptide level in immunized rats, indicates the important role of the bombesin-like factor in the "salt appetite." The fact that immunocorrection is a long-lasting effect is of definite interest. The data described in this paper are thus an example of the possibilities of long-term changes in physiological functions by immunization against biological regulators.

LITERATURE CITED

1. A. V. Kotov, S. M. Tolpygo, K. V. Sudakov, et al., Byull. Éksp. Biol. Med., No. 11, 518 (1987).
2. O. N. Serova, I. P. Ashmarin, M. F. Obukhova, et al., Physiology of Peptides [in Russian], Leningrad (1988), pp. 178-179.
3. J. F. Bishop, F. W. Moody, and T. L. O'Donohue, Peptides, 7, 835 (1987).
4. F. Cantalamessa, G. De Caro, M. Massi, and L. G. Micossi, Pharmacol. Res. Commun., 14, 163 (1982).
5. J. Gibbs, Life Sci., 37, 147 (1985).
6. P. J. Kulkosky, D. J. Fauser, and J. Gibbs, Soc. Neurosci. Abstr., 7, 852 (1981).
7. P. J. Kulkosky, J. Gibbs, and G. R. Smith, Physiol. Behav., 28, 505 (1982).
8. J. E. Morley, A. S. Levine, and S. S. Murray, Brain Res., 226, 334 (1981).
9. J. E. Morley, A. S. Levine, B. A. Gosnell, and C. J. Billington, Fed. Proc., 43, 2903 (1984).
10. W. Widerlöv, R. A. Mueller, G. D. Frye, and C. R. Breese, Peptides, 5, 523 (1984).

PULMONARY MICROCIRCULATION DURING ARTIFICIAL VENTILATION WITH DIFFERENT FREQUENCY AND VOLUME PARAMETERS

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Artificial ventilation of the lungs (AVL) is widely used in clinical practice to treat patients with acute respiratory failure [2, 3, 5]. The effect of AVL on the microcirculation of the lungs has received little study. Some research has been carried out in this direction but under open chest conditions, and on isolated or quickly frozen lungs [1, 9, 12], and their results may therefore differ significantly from changes in the pulmonary circulation during AVL in animals with a closed chest. Research in which the state of the microcirculation during AVL was judged by the hematocrit index in pulmonary capillaries, the vascular resistance in the pulmonary circulation, the respiratory quotient, and other indirect parameters [1, 8, 10], was uninformative.

The aim of this investigation was to study changes in the diameter of arterioles, venules, and wide capillaries and in the length of functioning narrow capillaries, during AVL with different frequency and volume parameters on animals with a closed chest.

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