COMPARISON OF THE EFFECTS OF MICROINJECTIONS OF ANGIOTENSIN II AND BRADYKININ INTO THE LATERAL VENTRICLES AND THEIR INSTILLATION INTO THE CONJUNCTIVA ON FEEDING BEHAVIOR OF RATS

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The physiological functions of peptides in the CNS are not always the same as their functions at the periphery. For instance, it has been found that the vasoactive oligopeptides angiotensin-II and bradykinin, if injected systemically, cause changes in vascular tone [4, 10], whereas if given by microinjections into the ventricles and certain other brain structures they have a marked effect on drinking behavior [12, 15] and on alcohol consumption [12] of animals. This specificity of the central intracerebral effect of such peptides has been shown to be largely dependent on their ability to pass through the blood-brain barrier only in small quantities, which have virtually no physiological action [14]. Meanwhile, an essential role in the initiation and realization of goal-directed behavioral acts [8] and in the production of psychopathological states of whatever kind [6, 13], is played by changes in the endogenous peptide levels in the CNS. Accordingly, data on the ability of certain substances including peptides (angiotensin II, vasopressin, and luteinizing hormone releasing hormone), when instilled into the conjunctival sac or into the nasal cavity, to induce the appearance of the same behavioral reactions as after intracerebral microinjections, are of fundamental importance [1, 3, 7, 9, 11].

In view of the potential advantages of the peripheral method of peptide administration for acting upon goal-directed animal behavior under particular experimental conditions (the study of animal behavior in the group, population, etc.) and in the case of the possible use of these preparations in clinical practice, it was decided to compare the effects of angiotensin II and bradykinin on drinking behavior when given by intraventricular injections and by instillation into the conjunctival sac.

METHODS

Experiments were carried out on 104 male Wistar and noninbred rats weighing 200-250 g. Addiction to alcohol was formed beforehand in 76 of these rats on a model of water deprivation, with long-term compulsory consumption of 20% ethanol solution by the animals as the sole source of fluid. In the experiments of series I the dipsogenic effects of angiotensin II (from Ciba, Switzerland) in intact rats were compared when given by intraventricular microinjections (300 ng in 3 µl of physiological saline) and by instillation of various doses of the peptide (1, 10, and 100 µg in 0.05 ml of physiological saline) into the conjunctival sac. The intensity of the animals' drinking behavior was estimated from the number of visits to the drinking bowl, the latent period of onset of drinking behavior, and the volume of water consumed during 1 h of the postinjection period. In the experiments of series II the effect of angiotensin II and of bradykinin (from Serva, West Germany) on consumption of water and of 20% ethanol solution was studied in rats under free choice conditions. Microinjections of peptides into the lateral ventricles (30 ng of angiotensin II and 15 ng of bradykinin, in a volume of 3 µl of physiological saline and distilled water respectively) were given by means of a special microinjector, into unrestrained animals. Instillation of the oligopeptides into the conjunctival sac also was carried out on waking rats. To provide the most atraumatic conditions for these experiments, physiological saline was used as the solvent and peptides

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TABLE 1. Comparison of Dipsogenic Effects of Angiotensin II in Intact Rats after Intraventricular Microinjections and Instillations into the Conjunctival Sac.

Experimental conditions	Dose	Number of animals	Latent period of onset of feeding behavior, min	Total number of visits to drinking bowl	Volume of water drunk, ml
Microinjection of angiotensin II into lateral ventricles	300 ng	10	3,2±1,9	14+3	6,9±1,5
Instillation of angiotensin II into conjunctival sac	1 µg	6	0	0	0
	10 µg	6	7,8±2,1	7+3	2,2+1,7*
	100 µg	6	7,5+2,2	9+3	2,5+1,8*
Instillation of physiological saline into conjunctival sac	50 µl	6	0	0	0

Legend. *P < 0.05.

were instilled by means of a tuberculin syringe with a specially adapted soft catheter (diameter of the tip 0.2-0.5 mm), drawn from polyvinyl chloride tubing. The following doses of peptides were used: 200 ng and 1 and 10 µg of angiotensin II, 10 ng and 3 µg of bradykinin. The quantity of water and of 20% ethanol solution drunk daily was recorded for all the rats during 20-30 days of observation. The locations of the tips of the cannulas were determined by a rapid photographic method. The numerical results were subjected to statistical analysis by standard methods of determination of the arithmetic mean and by Student's test.

RESULTS

The mean data obtained in the experiments of series I are given in Table 1.

It will be clear from Table 1 that instillations of angiotensin II into the conjunctival sac in a dose of 1 µg did not initiate drinking behavior in any of the animals studied. Only intensive grooming, orienting-investigative responses, washing, etc., were observed in rats under these conditions. In control experiments with instillation of physiological saline, six rats only developed mild anxiety accompanied by washing movements, evidently due to the actual procedure of instillation of liquid into the eyes and mechanical stimulation of the conjunctiva. Incidentally, compared with angiotensin II, physiological saline caused only a very brief and transient response.

Instillations of 10 and 100 µg angiotensin II evoked drinking behavior as well as grooming and washing. However, the latent period of the dipsogenic effect was significantly longer in these rats, and the intensity of water consumption was less than after intraventricular microinjections of angiotensin II.

The results of the experiments of series I are evidence that angiotensin II, in certain doses, can induce a central dipsogenic effect specific for this peptide. Since intracerebral microinjections of angiotensin II and of the other vasoactive peptide, bradykinin, as the writers showed previously [2], have an inhibitory action on alcohol consumption, in the next series of experiments the effect of instillation of these peptides into the eyes on alcohol motivation in rats was studied.

Instillation of angiotensin II into the conjunctival sac, like intraventricular microinjections of the peptide, induced consumption neither of water nor of alcohol in rats with established addiction to alcohol. Moreover, a single microinjection into the lateral ventricles or instillation into the eyes led to a decrease in the volume of alcohol consumed during the

TABLE 2. Delayed Effect of Peptides on Consumption of Water and Alcohol after Intraventricular Microinjection (A) and Instillations into the Conjunctival Sac (B)

Peptide	Mode of administration	Dose	Number of rats	Water, ml		20% Ethanol solution, ml	
				before administration of peptide	after administration of peptide	before administration of peptide	after administration of peptide
Angiotensin II	A	300 ng	25	0,6±0,3	1,2±0,6	12,1±0,4	8,2±0,7*
	B	200 ng	6	2,3±0,8	3,1±1,6	5,3±0,6	3,8±0,7*
		1 µg	6	5,6±2,7	4,5±0,6	5,1±1,2	2,2±0,4*
		10 µg	12	9,4±2,8	7,6±1,7	8,6±1,8	3,4±0,8*
Bradykinin	A	15 ng	15	2,3±0,9	3,6±0,9	11,4±1,8	8,5±0,8*
	Б	10 ng	6	3,5±1,2	7,9±1,3*	5,9±0,8	4,1±0,7*
		3 ng	6	0,3±0,1	2,6±1,4*	5,7±1,2	2,7±1,1*

Legend. *P < 0.05.

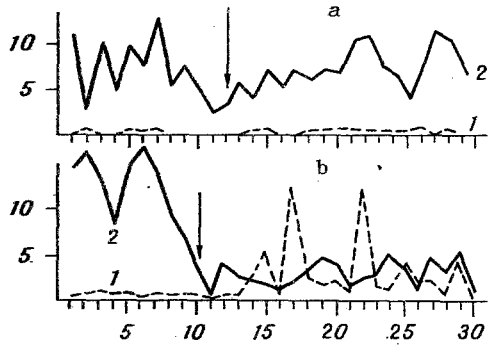

Fig. 1. Effect of angiotensin II on consumption of water (1) and alcohol (2) in rats after a single instillation (arrow) into conjunctival sac. Abscissa, duration of observation (in days); ordinate, volume of fluid consumed (in ml); a, b) 20 ng and 10 µg respectively of angiotensin II.

next 3-10 days or more (Table 2, Fig. 1). The inhibitory effect of angiotensin II on alcohol consumption first appeared, incidentally, with instillation of only 200 ng of the peptide, and it increased with an increase in the dose; in some cases alcohol consumption was almost completely suppressed for 16-23 days. In many animals the character of preference of fluids in a situation of free choice between water and alcohol was changed, as shown by the appearance of preference for taking water.

After instillations of bradykinin into the conjunctival sac its specific inhibitory effect on alcohol consumption was observed in response to a dose of 10 ng of the peptide, and the effect increased with an increase in the dose to 3 µg. The reduction in the daily volume of ethanol consumed after single instillations of bradykinin continued during 15-20 days of observation and was accompanied by an increase in the consumption of water after the 6th-10th day after instillation. In virtually all cases of instillations and microinjection of the peptide the character of preference for fluids was changed, with the appearance of alternate consumption of water and 20% ethanol solution (Table 2, Fig. 2).

Instillation of vasoactive peptides into the conjunctival sac thus has the same effect on consumption of water and alcohol as intraventricular microinjections of these substances. However, in rats with established addiction to alcohol, unlike in intact animals, the spe-

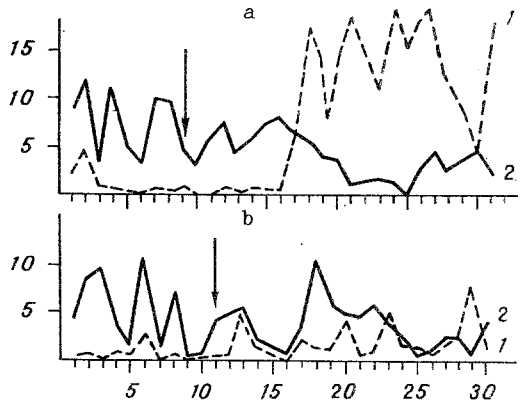

Fig. 2. Time course of consumption of water (1) and alcohol (2) in rats after instillation (arrow) of bradykinin into conjunctival sac. a, b) 10 ng and 3 μg bradykinin respectively. Remainder of legend as to Fig. 1.

cific behavioral responses to administration of the peptides were observed even when the doses used were equal to those given by intraventricular microinjection; the reason for this is evidently increased permeability of the blood–brain or the ciliary barrier in rats during development of alcohol motivation [5].

LITERATURE CITED

1. V. I. Badikov, E. A. Lomakina, and K. V. Sudakov, Byull. Éksp. Biol. Med., No. 10, 442 (1981).
2. K. V. Sudakov, A. V. Kotov, and L. F. Kelesheva, Dokl. Akad. Nauk SSSR, 246, No. 1, 243 (1979).
3. C. Bergquist, S. J. Nillius, and L. Wide, Clin. Endocrinol., 17, 91 (1982).
4. J. T. Fitzsimons, Physiol. Rev., 52, 468 (1972).
5. K. Heilmann, Therapeutic Systems Pattern — Specific Drug Delivery, Concept and Development, Stuttgart (1978).
6. G. Knoll, Neurosci. Lett., 13, Suppl. 3, 19 (1979).
7. F. Laczi, J. M. van Ree, A. Wagner, et al., Acta Endocrinol (Copenhagen), 102, 205 (1983).
8. S. F. Leibowitz and L. Hor, Soc. Neurosci. Abst., 6, 318 (1980).
9. J. C. Merrit, J. Nat. Med. Ass., 74, 715 (1983).
10. M. J. Peach, Physiol. Rev., 57, 313 (1977).
11. V. Popovic, Bull. Mus. Comp. Zool., Harvard, 124, 105 (1960).
12. L. W. Swanson and L. G. Sharpe, Amer. J. Physiol., 225, 566 (1973).
13. H. M. Van Praag and W. M. A. Verhoeven, Comp. Psychiat., 2, 135 (1981).
14. L. Volicer and C. G. Loew, Neuropharmacology, 10, 631 (1971).
15. C. R. Willis, J. H. Ludens, J. B. Hook, and H. E. Williamson, Amer. J. Physiol., 217, 1 (1968).