isoleucine in position 5 of the peptide chain, and so did the other peptides used in this study, which were synthesized by the solid phase method[4,5]. All the peptides were purified by counter-current distribution and characterized by their partition coefficient (K) in n-butanol:acetic acid: water (4:1:5). Their purity was demonstrated by the obtention of a single component on high voltage paper electrophoresis with pyridine acetate buffer, pH 4.9, and by thin layer chromatography on silica gel (TLC) with the following solvent systems: A) n-butanol:acetic acid:water (4:1:1); B) n-butanol:pyridine:water (30:20:6:24); C) propanol:water (2:1); D) ethyl acetate: pyridine:acetic acid:water (3:2:1:1). Amino acid analyses were on a Beckman model 120C amino acid analyzer.

Angiotensin I-yl-leucine had $K = 0.13$ and the following Rf values: A, 0.27; B, 0.56; C, 0.57; D, 0.33. The amino acid molar ratios were: Asp, 1.05; Arg, 0.98; Val, 1.04; Tyr, 0.94; Ile, 0.93; His, 1.96; Pro, 1.01; Phe, 0.97; Leu, 2.07.

Angiotensin I Had $K = 0.21$ and the following Rf values: A, 0.20; C, 0.52; D, 0.24. The amino acid analysis showed the following molar ratio; Asp, 1.09; Arg, 0.99; Val, 1.01; Tyr, 0.93; Ile, 0.91; His, 2.06; Pro, 1.10; Phe, 0.96; Leu, 1.05.

Leu-Val-Tyr-Ser had $K = 0.70$ and the following Rf values: A, 0.52; B, 0.53; C, 0.67; D, 0.69. The amino acid molar ratios were: Leu, 1.02; Val, 1.04; Tyr, 0.95; Ser, 0.97.

Val-Tyr-Ser had $K = 0.31$ and the following Rf values: A, 0.40; B, 0.41; C. 0.55; D, 0.51. The amino acid molar ratios were: Val, 1.04; Tyr, 0.91; Ser, 1.04.

Methods. A solution of renin-substrate in water (1 mg/ml) was adjusted to pH 3.0 or 6.0 by addition of 0.1 N HCl or 0.1 N NaOH, and equilibrated at 37°. The incubation was initiated by addition of 2 µg/ml (at pH 3) or 4 µg/ml (at pH 6) of 3×-crystallized pepsin (Sigma). Aliquots of 0.2 ml were removed at various times, boiled for 3 min, and submitted to TLC with solvent system D and to paper electrophoresis at 1000 volts for 90 min, in 2 M acetic acid (pH 2.4). After 24 h the remaining solution was boiled for 3 min, freeze-dried and submitted to preparative paper electrophoresis in the same conditions as for the previous aliquots. The separated compounds were eluted with water, the eluates were evaporated to dryness, hydrolyzed for 72 h with 6 N HCl at 110° under N_2, and submitted to amino acid analysis.

Results and discussion. The main products of peptic proteolysis of renin-substrate, at either pH studied, were identified as angiotensin I and Leu-Tyr-Ser by TLC and paper electrophoresis. In neither pH was it possible to detect the appearance of angiotensin I-yl-leucine, Val-Tyr-Ser or angiotensin II in the incubation mixtures.

The solution remaining after 24 h of peptic hydrolysis at pH 6.0 was submitted to preparative paper electrophoresis and the two Pauly-positive fractions were eluted, hydrolyzed and analyzed for their amino acid composition. The results, shown in the Table, confirm that the main products were angiotensin I and Leu-Val-Tyr-Ser, although molar ratios indicate in fraction I the presence of other peptides, including some intact substrate. Since all of the other possible fragments of peptic hydrolysis of the tetradecapeptide would have negligible biological activity (with the exception of angiotensin II), it may be concluded that pepsitensin, whether produced at pH 3 or pH 6, is identical with angiotensin I. This agrees with the results obtained with native renin substrate[1], and it seems probable that the same would happen also in the case of the denatured substrate[6].

Amino acid analyses of the 2 fractions from the hydrolysate of renin substrate by pepsin at pH 3.0

Amino acid	Fraction I		Fraction II	
	Expected	Found	Expected	Found
Asp	1	1.28	0	0
Arg	1	1.11	0	0
Val	1	0.81	1	1.00
Tyr	1	0.87	1	1.03
Ile	1	0.70	0	0
His	2	1.87	0	0
Pro	1	1.00	0	0
Phe	1	1.22	0	0
Leu	1	1.46	1	0.95
Ser	0	0.29	1	1.45

Résumé. L'angiotensine I et le Leu-Val-Tyr-Ser sont les principaux produits des hydrolyses peptiques du tétradécapeptide substrat de la rénine, réalisés à pH 3 et à pH 6. Ces résultats indiquent qu'il n'y a qu'une seule pepsitensine laquelle est identique à l'angiotensine I.

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Basal Forebrain Heating and Osmotic Reactivity of the Thirst Mechanism in Dogs

There is controversial data regarding the coupling between the hypothalamic temperature and feeding as well as drinking behavior. Andersson and Larsson[1] found that heating of the preoptic anterior hypothalamus (PO/AH) region in goats increased drinking and suppressed food intake. On the other hand Spector et al.[2] and Hamilton and Ciaccia[3] reported that the same procedure in rats produced increase of food consumption and a tendency to reduce water intake. The present study was performed on conscious dogs in order to check whether local heating of the basal forebrain influences water intake and changes the osmotic reactivity of the thirst mechanism.

Material and methods. Experiments were carried out on 5 male mongrel dogs. Each of them was chronically implanted with 4 thermodes and 2 copper constantan thermocouples. The heater of the thermode consisted of a miniature carbon resistor[4] placed at the end of a 0.8 mm

stainless steel tube. Inside the tube there was a copper wire insulated except for a tip making contact with the resistor. All thermodes were connected to pins of a plug fastened in a Plexiglas socket which was screwed into the parietal bone and fixed with acrylic cement. The experiments were undertaken 2 weeks after the surgery. The influence of the heating of some sites in the basal forebrain on water intake and osmotic reactivity of the thirst mechanism and thermoregulatory functions was examined. The osmotic reactivity was expressed as a threshold value of osmotic stimulus[5] producing cellular dehydration necessary to induce drinking response. The magnitude of the cellular dehydration produced by osmotic stimulus (i.v. infusion of a 5% NaCl solution) was calculated for each dog from osmometric equations[5] using the values of the total body water, extracellular water and extracellular sodium concentration which have been obtained during previous measurements. The osmotic reactivity was examined 3 or 4 times under control conditions in one group and in the course of the brain heating in another group of experiments. The dogs were kept fasting for 18 h before the experiment, having free access to water. The basal forebrain region was heated by connecting the thermode to a battery so that the power delivered was 100 mW. This produced a 0.5 °C increase of the temperature of the brain tissue at a distance of 3 mm. The region examined was heated for 10 min and the dog's behavior was observed. Then a 5% solution of saline was infused at a rate of 7.5 ml/min into the cephalic vein of the dog, whereas the heating was continued. When the dog began to drink the infusion was stopped and cellular dehydration produced by the infusion was calculated. The influence of the heating of the same sites on thermoregulatory functions (respiratory rate, rectal and skin temperatures) was also examined in another set of experiments.

The extracellular water was measured using sodium thiocyanate. Total body water was calculated as the percentage of body weight. The plasma Na concentration was also measured and the total amount of extracellular sodium was calculated. Respiratory rate was determined using a resistance transducer placed around the dog's chest. Skin and rectal temperatures were measured by means of copper – constantan thermocouples. After the termination of the experiments the animals were sacrificed, the brains were fixed in formalin, sectioned and stained after Weil.

Results and discussion. The influence of the heating of 18 sites in the basal forebrain was examined. The tips of the thermodes were localized in the following areas: the region of the tuberculum olfactorium, nucleus accumbens septi, nucleus comissurae anterioris, lateral preoptic area, anterior part of the 3rd ventricle, ventromedial part of the septum, internal capsula, lateral hypothalamus, fasciculus mammillothalamicus and dorsomedial part of the hypothalamus. Heating of the sites mentioned above never produced spontaneous drinking or any interest toward water. On the contrary, more or less evident suppression of the osmotic reactivity of the thirst mechanism was seen when the following sites were heated: the region of the nucleus comissurae anterioris, lateral preoptic area and nucleus accumbens septi. In other words, in the course of the heating the animals started to drink when the degree of the cellular dehydration produced by infusion of the hypertonic solution was higher (from 50 to 300%) than under control conditions (Figure). Heating of the same sites produced also thermoregulatory responses – increase of the respiratory rate as well as in some cases increase of the skin temperature and decrease of the rectal temperature. Thus heating of the region in which highly thermosensitive neurones are described to occur[6], does not stimulate but inhibits the water intake in dogs. As heating to the same regions increases also the plasma ADH level[7], it seems that stimulation of the thermosensitive neurones in the basal forebrain involves dissociation in the activity of the two systems regulating body water balance by activation of the hypothalamo-hypophysial antidiuretic system and inhibition of the thirst mechanism.

Résumé. Les expériences ont été conduites sur des chiens auxquels on a chroniquement implanté des thermodes dans la région du prosencéphale basal. On a constaté que l'échauffement de la région située en avant de la comissure antérieure a abaissé la réactivité osmotique du mécanisme de la soif. L'augmentation de la température de la même région a produit aussi une accélération de la polypnée. Il faudrait alors admettre que chez le chien l'échauffement de la région thermosensitive supprime la sensibilité du mécanisme de la soif aux stimulus osmotiques.

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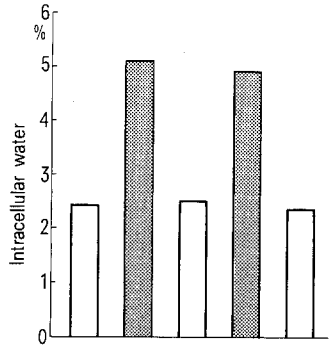

Degree of cellular dehydration expressed as a percent of initial (control) value of the intracellular water at which the thirst mechanism is activated under control conditions (white columns) and during heating of the region of the nucleus accumbens septi (dashed columns).

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