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The Tachykinin NH_2-Senktide Inhibits Alcohol Intake in Alcohol-Preferring Rats

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PERFUMI, M., C. POLIDORI, P. L. POMPEI, G. DE CARO AND M. MASSI. *The tachykinin NH_2-senktide inhibits alcohol intake in alcohol-preferring rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 881–887, 1991.—The present study evaluated the effect of the intracerebroventricular injection of the tachykinins, substance P, neurokinin A and [Asp^{5,6},MePhe^8]substance P(5–11) (also referred to as NH_2-senktide), on the alcohol intake of genetically selected, alcohol-preferring rats. Animals were offered both water and 8% ethanol 2 h/day; tachykinins were administered just before access to fluids. Neurokinin A and substance P did not modify alcohol intake at doses up to 1000 and 2000 ng/rat, respectively. On the other hand, NH_2-senktide potently suppressed alcohol intake at doses of 31.2–500 ng/rat. At the same doses, however, it did not significantly affect water intake. This finding suggests that its effect on alcohol intake might be rather selective and not due to general impairment of the behavior. Activation of tachykinin NK-3 receptors, for which NH_2-senktide is a highly selective agonist, produces angiotensin II release in the brain; however, the effect of NH_2-senktide on alcohol intake is probably not mediated by angiotensin II, as suggested by the fact that it is not modified by captopril pretreatment.

Tachykinins NH_2-senktide Alcohol intake

TACHYKININS are biologically active peptides sharing the common carboxyterminal sequence PHE-X-GLY-LEU-MET.NH_2 (8). Several tachykinins have been isolated from the mammalian nervous system: substance P (SP), neurokinin B (NKB), neurokinin A (NKA), NKA (3–10), neuropeptide K (NPK) and neuropeptide (NPγ). All of these tachykinins have been identified in the central nervous system and show regional differences in their distribution (1, 16, 27, 40). Three distinct tachykinin receptor subtypes have been proposed for these peptides: the NK-1 (which preferentially interacts with SP), the NK-2 (which prefers NKA, NPK and NPγ) and the NK-3 (which interacts best with NKB) (2, 3, 18, 20, 32, 34). A large body of evidence indicates the presence of NK-1 and NK-3 receptors in the central nervous system, while the presence of NK-2 receptors is still debated (18).

Previous studies indicate that tachykinins exert potent effects on the rat ingestive behavior. Work done in our laboratory has shown that tachykinins potently affect the behavioral regulation of body fluid in the rat. Their intracerebroventricular (ICV) injection inhibits water intake induced by several dipsogenic treatments (4–6, 23, 29), as well as salt intake elicited by several natriorexic determinants (22, 24, 25). Their effects on water and salt intake appear to be mediated by different receptor subtypes, which might account for the different spectrum of antidipsogenic and antinatriorexic action of different tachykinins. A recent paper from another laboratory reported that NPK exerts an anorexic effect in the rat, following both peripheral and central administration (38).

The present study was aimed at investigating the possible effects of tachykinins on another aspect of the ingestive behavior, the ingestion of ethanol solutions. Our interest in this study was stimulated by three main reasons:

a. tachykinins exert many effects on the rat ingestive behavior, probably in relation to their rich hypothalamic localization (2, 21, 37),
b. in several experimental models ethanol intake has been shown to be related to salt appetite, which is potently modified by tachykinin administration (11–13),
c. tachykinins have been shown to interact with neurochemical systems (such as the central angiotensin and serotonin systems) which are known to affect ethanol intake (14, 15, 28, 35, 36, 41).

Our study began to evaluate the effect of three tachykinins, SP, NKA and [Asp^{5,6},MePhe^8]SP(5–11), also referred to as NH_2-senktide (17). SP and NKA were employed as potent agonists, respectively, at NK-1 and NK-2 receptors (7). Since NKB is essentially insoluble in water, NH_2-senktide was chosen as a selective and potent NK-3 agonist. In addition to its high selectivity of action (17), it has the advantage of being freely soluble in water.

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METHOD

Animals

Male Wistar rats, weighing between 250 and 300 g at the beginning of the experiments, were employed. They were alcohol-preferring rats selected for 14 generations, according to the method of Lumeng et al. (19). Breeding of animals in our laboratory started from Sardiman alcohol-preferring rats of the 13th generation, kindly provided by the Department of Neurosciences of the University of Cagliari, Italy (9). Animals were individually housed in a temperature-controlled room on a 12:12-h light-dark cycle.

Drugs

The following substances were employed: 1) angiotensin I (Ang I), 2) angiotensin II (Ang II), 3) SP and 4) NKA, which were purchased from Peninsula Laboratories Inc., Merseyside, UK, 5) NH_2-senktide, which was a generous gift of Menarini Farmaceutici, Florence, Italy, and 6) captopril, which was a gift of Squibb Italia, Rome, Italy.

Surgery

All the rats employed were anaesthetized (Equithesin, 3 ml/kg, b.wt.; intraperitoneally) and fitted by stereotaxic surgery with a stainless-steel guide cannula aimed at 1 mm above the lateral ventricle. The cannula was permanently attached to the skull by stainless-steel screws and dental acrylic cement. Animals were allowed one week to recover from surgery before being tested. Prior to the beginning of the experiments, the cannula placement was validated by measuring the animal's drinking response to a pulse ICV injection of 100 ng of Ang II. A minimum criterion of 10 ml of water drunk in 15 min was used.

Moreover, validation of the ICV injection was also made post-mortem. Following completion of the experiments, animals were given an ICV injection of 1 μl India ink and then sacrificed. Brains were removed and diffusion of the ink in the cerebroventricles was evaluated.

Intracranial Injections

The drugs tested were dissolved in sterile isotonic saline and were given by pulse ICV injection in a volume of 1 μl through a stainless-steel injector temporarily inserted into the guide cannula. The injector was 2 mm longer than the guide cannula, so that its tip was aimed at the cavity of the ventricle. Injections were given with the aid of a 10 μl Hamilton microsyringe.

Sequence of Testing

All the experiments were carried out according to a within-subject design, in which each animal received different treatments in counterbalanced order. The intervals between subsequent tests are given in the experimental procedure.

Food and Fluids

Food in pellets (Mill, Morini, Reggio Emilia, Italy) was freely available throughout the experiments. Tap water and 8% (volume/volume) ethanol solution were offered in graduate drinking tubes, allowing measurements of fluid taken to the nearest 0.1 ml. Eight percent ethanol concentration was preferred to 10%, used by Lumeng et al. (19), since at this higher concentration alcohol intake of our rats was clearly lower. Immediately after surgery animals were allowed continuous access to both water and ethanol solution for 15 days. Following this period, access to water and ethanol solution was restricted to 2 h a day, between 10.00 and 12.00 a.m. A short schedule of access was adopted in relation to the short half-life of the peptides tested. Access was restricted not only to alcohol, but also to water, because we were interested in a simultaneous analysis of the effect of tachykinins on the intake of both fluids, in relation to the inhibitory effect on some tachykinins on water intake induced by several dipsogenic determinants (4–6).

The experiments started after 15 days of 2-h access to fluids, when the intake of water and ethanol appeared to be stable enough.

Experimental Procedure

Experiment 1. Effect of pulse ICV injections of NH_2-senktide, SP and NKA on ethanol and water intake. One min before access to fluids, rats were given a pulse ICV injection of isotonic saline (controls) or of one of the tachykinins tested. One group of 8 animals was used for NH_2-senktide experiment; they received 5 ICV treatments. A second group of 8 animals was employed to test SP and NKA; also the animals of this group received 5 ICV treatments. Each animal received different treatments at intervals of 2–3 days.

Experiment 2. Effect of captopril pretreatment on the inhibitory effect of NH_2-senktide on ethanol intake. Six rats were employed in this experiment. All of them received the 6 treatments reported in Fig. 3. Captopril was given in 2 ICV injections, of 1 μg/each, 60 and 10 min before access to water and ethanol (controls received two ICV injections of isotonic saline with the same time schedule). Two captopril injections, separated by 50 min, were employed to block the angiotensin converting enzyme well before the test, thus minimizing brain levels of Ang II generated before captopril treatment. The effectiveness of our captopril treatment was behaviourally evaluated by checking the dipsogenic response to the ICV administration of Ang I, 10 ng/rat. This dose of Ang I produced a cumulative water intake in a 2-h test slightly lower (about 26%) than the 24-h cumulative intake of our deprived rats. Therefore, the results of this test were considered predictive of the degree of blockade of the central renin-angiotensin system also in our experimental conditions. One min before access to fluids, animals received a further ICV injection of either isotonic saline or of NH_2-senktide.

Statistical Analysis

Data are presented as means ± S.E.M. Statistical analysis of data was performed by multifactorial analysis of variance (repeated measurements) to check the overall significance. A priori pairwise comparisons were carried out by means of Student's *t*-test. Statistical significance was set at $p<0.05$.

RESULTS

Experiment 1. Effect of Pulse ICV Injections of NH_2-Senktide, SP and NKA on Ethanol and Water Intake

NH_2-senktide.

Ethanol intake. The ICV injection of NH_2-senktide, in the range of doses between 31.2 and 500 ng/rat, produced a marked, dose-dependent and easily reproducible suppression of 8% ethanol intake. The analysis of variance revealed the presence of a potent drug effect, $F(4,28)=17.404$, $p<0.0001$. It revealed also significant time effect, $F(4,28)=19.604$, $p<0.0001$, and drug-time interaction, $F(16,112)=3.036$, $p<0.0005$. The drug-time interaction was probably related to the fact that in controls most of drinking

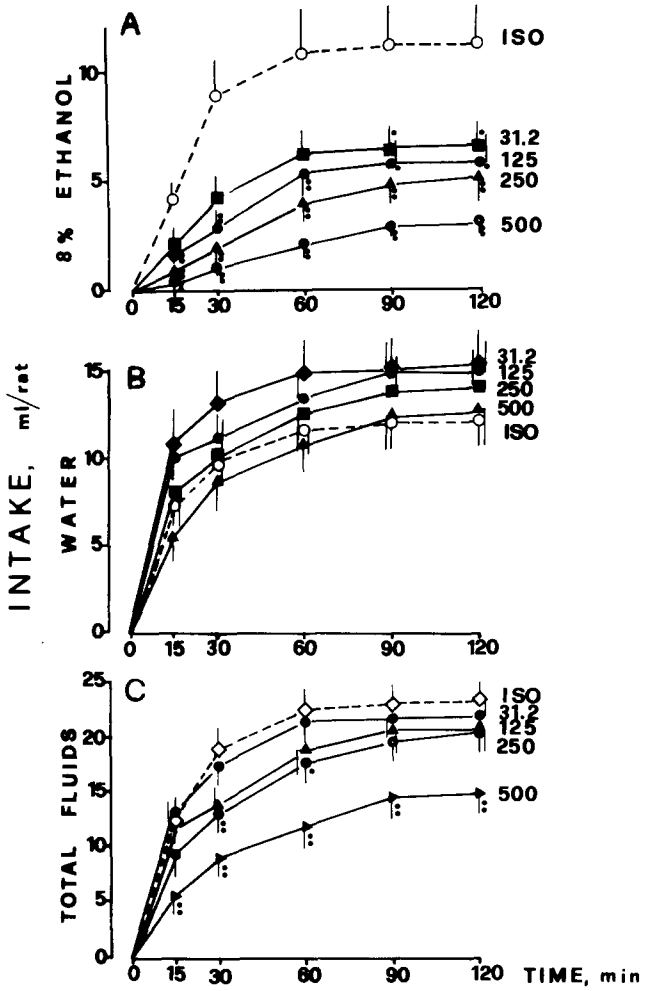

FIG. 1. Effect of the ICV injection of different doses of NH_2-senktide (ng/rat) or of isotonic saline (ISO) on (A) 8% ethanol intake, (B) water intake and (C) total fluid intake. Values are means ± S.E.M. of 8 subjects. Difference from controls: :$p<0.01$; ·$p<0.05$; where not indicated, difference from controls was not statistically significant.

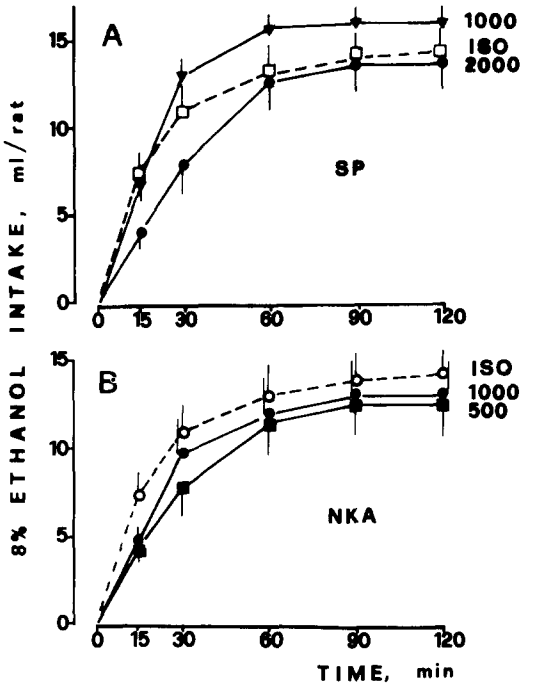

FIG. 2. Effect of ICV injection of isotonic saline (ISO) or of different doses (ng/rat) of (A) SP and (B) NKA on 8% ethanol intake. Values are means ± S.E.M. of 8 subjects. Difference from controls was never statistically significant.

occurred in the first 30 min, while the intake was very low in treated animals.

As shown in Fig. 1A, control animals took 4.2 ± 0.7 ml of ethanol solution in the first 15 min after access to it; at the end of the 2-h period of observation their cumulative intake was 11.1 ± 1.9 ml of ethanol solution (i.e., about 2.7 g/kg b.wt.).

A significant suppression of ethanol intake was observed in response to the dose of 31.2 ng/rat. Treated rats took 2.1 ± 0.7 at 15 min (percent inhibitory effect: 48.9%) and 6.5 ± 1.0 ml at 2 h after access to it (percent inhibitory effect: 41.0%). The cumulative intake of treated rats was significantly lower than that of control rats for the entire period of observation ($p<0.05$).

Higher doses of NH_2-senktide progressively increased the inhibitory effect on ethanol intake. In response to the highest dose tested, 500 ng/rat, the intake of ethanol solution was almost completely suppressed in the first 15 min of access to it (percent inhibition: 91.5%), and remained strongly inhibited even at the end of the 2-h period of observation (percent inhibitory effect: 73.1%).

Water intake. While the intake of ethanol was markedly depressed by the ICV injection of NH_2-senktide, the concomitant intake of water was not significantly modified (Fig. 1B). The overall analysis of variance, in fact, showed a nonsignificant drug effect, $F(4,28)=1.193$, $p>0.05$, as well as of a nonsignificant drug-time interaction, $F(16,112)=1.025$, $p>0.05$. There was, however, a significant time effect, $F(4,28)=19.566$, $p<0.0001$.

A small reduction in water intake was detected only at the highest dose of 500 ng/rat and only in the first 30 min of observation. However, the reduction was modest, variable among subjects and never statistically significant.

Total fluid intake. As shown in Fig. 1C, the total fluid intake (ethanol solution + water) was significantly inhibited by NH_2-senktide, $F(4,28)=10.188$, $p<0.0001$. The analysis showed also a significant time effect, $F(4,28)=57.538$, $p<0.0001$, and a nonsignificant drug-time interaction, $F(16,112)=1.254$, $p>0.05$. Pairwise comparisons showed that the total fluid intake of NH_2-senktide-treated rats was lower than that of controls only in response to the doses of 500 and 250 ng/rat.

SP.

Ethanol intake. Substance P was tested at doses of 1000 and 2000 ng/rat. Our previous experiments have shown that SP 1000 ng/rat potently inhibits angiotensin II- or carbachol-induced water intake, while a modest and short-lasting inhibition of water deprivation-induced water intake had been observed at 2500 ng/rat (4).

The ICV injection of SP at doses of 1000 and 2000 ng/rat produced only small modifications of ethanol intake (Fig. 2A). The analysis of variance revealed the absence of a drug effect, $F(2,14)=1.770$, $p>0.05$, while showing significant time effect, $F(4,28)=109.230$, $p<0.0001$, and drug-time interaction, $F(8,56)=5.090$, $p<0.001$. Planned pairwise comparisons revealed the absence of significant difference between rats treated with both doses of SP and controls at the different times of observation. Higher

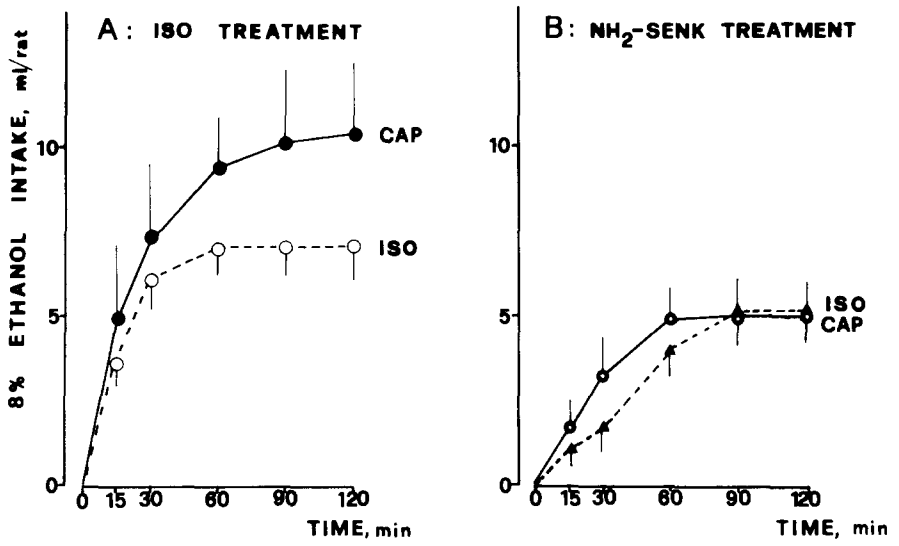

FIG. 3. (A) Eight percent ethanol intake after ICV pretreatment with 2 injections of captopril (CAP; 1 μg/rat) or of isotonic saline (ISO), followed by ICV ISO treatment. Values are means ± S.E.M. of 6 subjects. (B) Effect of captopril or of ISO pretreatment on the inhibitory action of NH_2-SENK, 500 ng/rat, on ethanol intake. Values are means ± S.E.M. of 7 subjects. Difference from controls was never statistically significant.

doses were not used in our experiments since they produce excessive grooming, which might cause a nonselective inhibition of the ingestive behavior.

Water intake. The same doses of SP did not significantly modify water intake in these experimental conditions.

NKA.

Ethanol intake. NKA was tested at doses of 500 and 1000 ng/rat, which have been shown to exert marked inhibitory effects on the ingestive behavior (29). In the present experiment, however, NKA did not produce any significant alteration of 8% ethanol intake (Fig. 2B). The analysis of variance showed the absence both of a drug effect, $F(2,14)=0.765$, $p>0.05$, and of a drug-time interaction, $F(8,56)=1.645$, $p>0.05$. On the other hand, there was a significant time effect, $F(4,28)=43.425$, $p<0.0001$.

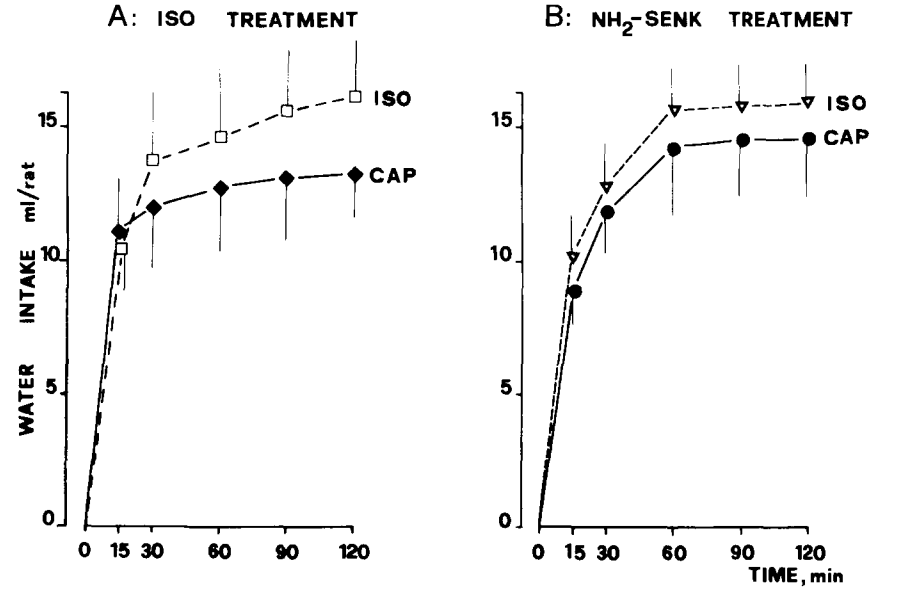

FIG. 4. (A) Water intake after ICV pretreatment with 2 injections of captopril (CAP; 1 μg/rat) or of isotonic saline (ISO), followed by ICV treatment with ISO. Values are means ± S.E.M. of 6 subjects. (B) Effect of captopril or of ISO pretreatment on water intake following ICV NH_2-SENK, 500 ng/rat. Values are means ± S.E.M. of 7 subjects. Difference from controls was never statistically significant.

Water intake. At the same doses of NKA, no significant modification of water intake was observed.

Experiment 2. Effect of Captopril Pretreatment on the Inhibitory Effect of NH_2-Senktide on Ethanol Intake

Ethanol intake. As shown in Fig. 3A, the intake of 8% ethanol solution of control rats was slightly, but nonsignificantly increased by captopril ICV administration (2 injections of 1 μg/each, 60 and 10 min before access to ethanol). The analysis of variance revealed a nonsignificant captopril effect, $F(1,5)=0.874$, $p>0.05$, and a nonsignificant drug-time interaction, $F(4,20)=2.307$, $p>0.05$. However, a significant time effect was obtained, $F(4,20)=30.564$, $p<0.0001$.

In rats treated with NH_2-senktide, 500 ng/rat, captopril pretreatment did not significantly affect the inhibitory effect of NH_2-senktide on ethanol intake (Fig. 3B). In fact, the analysis of variance revealed no significant effect of captopril pretreatment, $F(1,6)=0.658$, $p>0.05$, as well as lack of drug-time interaction, $F(4,24)=0.882$, $p>0.05$. Only a significant time effect was observed, $F(4,24)=21.592$, $p<0.0001$.

Water intake. The results obtained are reported in Fig. 4A. The analysis of variance showed a nonsignificant drug effect, $F(1,5)=2.501$, $p>0.05$, but significant time effect, $F(4,20)=13.393$, $p<0.0001$, and drug-time interaction, $F(4,20)=7.498$, $p<0.05$.

In rats treated with NH_2-senktide (Fig. 4B), captopril pretreatment did not modify water intake, $F(1,6)=1.424$, $p>0.05$. The analysis revealed a nonsignificant drug-time interaction, $F(4,24)=0.069$, $p>0.05$. Again, there was a significant time effect, $F(4,24)=9.373$, $p<0.0005$.

To get evidence that the captopril treatment employed had been effective in blocking the brain angiotensin-converting enzyme, the dipsogenic effect of Ang I was evaluated following captopril pretreatment. In the absence of captopril pretreatment, Ang I, 10 ng/rat, produced a prompt and copious dipsogenic response. In 15 min after Ang I injection, rats drank 9.1 ± 0.8 ml of water per rat; at the end of the 2-h observation period their intake was 17.2 ± 2.1 ml. Rats treated with captopril had a markedly lower dipsogenic response: they drank 1.8 ± 0.4 in 15 min, and 6.2 ± 1.3 ml in 2 h.

DISCUSSION

The results of the present study show that the central administration of NH_2-senktide, but not of the other tachykinins tested, SP and NKA, produces a potent suppression of 8% ethanol intake.

In the absence of potent and selective antagonists for the 3 tachykinin receptor subtypes, the rank order of potency of agonists has been used for receptor classification in both functional and binding assays. In this context, the development of synthetic tachykinin analogues selective for one particular receptor subtype has provided important tools for defining the participation of a given receptor subtype to specific responses. NH_2-senktide has been reported to be a highly selective agent for NK-3 receptors, as shown by its pattern of agonist potency in in vitro and in binding tests (7,17). Therefore, present findings suggest the involvement of NK-3 receptors in the inhibitory effect of the drug on ethanol intake. The idea of the involvement of NK-3 receptors in the effect is also supported by the fact that the endogenous agonists for NK-1 and NK-2 receptors, SP and NKA, are essentially inactive in these experimental conditions.

The second finding that emerges from the present study is that the inhibitory effect of NH_2-senktide on ethanol intake appears to be a rather selective effect, apparently not due to a general behavioral impairment. This is clearly evident at 31.2 and 125 ng/rat of NH_2-senktide, which inhibit ethanol intake, but do not suppress water intake, which at these doses is slightly higher than in control rats. Following these doses the total fluid intake of treated rats is not significantly different from that of controls. In response to larger doses, 250 and 500 ng/rat, ethanol intake was dramatically suppressed, but again water intake was not significantly affected. Indeed, at these higher doses, the total fluid consumption proved to be reduced, as the lowered ethanol intake was not compensated by a higher water intake. This finding should probably be interpreted as the consequence of other central effects of NH_2-senktide, which become relevant with increasing the dose. For instance, a previous study has shown that the ICV injection of NH_2-senktide, 500 ng/rat, produces a significant reduction of water intake induced by central injection of angiotensin II (25). Therefore, high doses of the peptide might have prevented the compensatory increase in water intake, following ethanol intake inhibition, by inhibiting the angiotensinergic component of the central mechanisms controlling deprivation-induced water intake (33).

Further evidence in favor of a selective behavioral effect of NH_2-senktide comes also from our previous study (25), which has shown that this compound does not inhibit water intake induced by osmotic load even at doses of 1000 or 2000 ng/rat injected (as in the present study) into the lateral cerebroventricle. On the other hand, in the same range of doses tested on ethanol intake, NH_2-senktide proved to be a very potent inhibitor of salt appetite (25).

All these findings clearly support the idea that administration of NH_2-senktide into the lateral brain ventricle, at the doses employed in the present study, leaves the animal behaviorally competent for the intake of fluids. Therefore, the inhibitory effects exerted by the compound, that is the inhibition of ethanol intake observed in the present study and the inhibition of salt intake previously described, should be considered as expression of selective actions of the drug on the central mechanisms controlling ingestive behavior.

Previous studies have shown that NK-3 agonists can interfere with both the brain renin-angiotensin and the serotoninergic systems. It has been reported that central administration of NH_2-senktide produces serotonin release in the central nervous system with consequent elicitation of serotonin mediated behaviors (39). Moreover, studies from our laboratory have shown that the central administration of NK-3 agonists is able to induce vasopressin release through the release of Ang II and activation of Ang II receptors in the central nervous system, mainly into the paraventricular nucleus (26, 30, 31). Since these two systems are known to affect ethanol intake, the question rises whether the action of NH_2-senktide on ethanol intake might be related to possible release either of Ang II or of serotonin.

The first point was addressed in the present study by captopril administration. Since fluid deprivation is known to activate the renin-angiotensin system, attention was paid to verify the efficacy of the captopril treatment employed in the experiment. This was done behaviorally, by checking the inhibition of Ang I-induced drinking evoked by the captopril treatment. The dose of 10 ng/rat of Ang I was used since it gave a 2-h water intake of 17.2 ± 2.1 ml/rat, which was only slightly lower than that of the fluid-deprived rats employed (23.3 ± 1.9 ml). Taking into account that water intake following water deprivation is only in part dependent on Ang II [while to a large extent it is due to cell dehydration, and therefore Ang II independent; (11,34)], our test was considered severe enough to provide evidence for a pronounced blockade of Ang II generation, even in these conditions of fluid deprivation. However, the results of Experiment 2 showed that captopril treatment did not modify the effect of NH_2-senktide, thus suggesting that Ang II should not be involved in its inhibi-

tory action on ethanol intake.

The second point, that is the possible involvement of serotonin in the effect of NH_2-senktide, has not been sufficiently investigated up to now. In a preliminary experiment, we have observed that the ICV administration of the serotonin antagonist metergoline (2 and 4 μg/rat), which is rather nonselective for different serotonin receptor subtypes, does not affect the inhibitory action of NH_2-senktide on alcohol intake. However, the variety of serotonin receptor subtypes, so far reported, will require the use of different selective antagonists and of different neurochemical approaches.

In conclusion, the findings of the present study show that NH_2-senktide suppresses ethanol intake in fluid-deprived rats, without significantly affecting their water intake. According to the receptor agonist profile of NH_2-senktide, these findings suggest that NK-3 receptors might be responsible for the observed inhibition of alcohol intake.

Further studies are required to investigate the mechanisms of action for the inhibitory effect of NH_2-senktide. It would be interesting to elucidate whether activation of NK-3 receptors might provide a satiety signal for stopping ethanol intake or, alternatively, it might act by diminishing the positively reinforcing properties of ethanol.

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