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Mechanism of Action for Reduction of Ethanol Intake in Rats by the Tachykinin NK-3 Receptor Agonist Aminosenktide

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CICCOCIOPPO, R., I. PANOCKA, C. POLIDORI, R. FROLDI, S. ANGELETTI AND M. MASSI. *Mechanism of action for reduction of ethanol intake by the tachykinin NK-3 receptor agonist aminosenktide.* PHARMACOL BIOCHEM BEHAV **61**(4) 459–464, 1998.—Intracerebroventricular (ICV) injection of tachykinin (TK) NK-3 receptor agonists inhibits alcohol intake in genetically selected alcohol-preferring rats. The present study investigated the mechanism of action by which the selective TK NK-3 receptor agonist aminosenktide (NH_2-SENK) attenuates ethanol intake in Marchigian Sardinian alcohol-preferring (msP) rats. The effect of NH_2-SENK was studied by ICV injection in the conditioned taste aversion (CTA) and in the conditioned place preference (CPP) paradigms; moreover, the effect of NH_2-SENK on blood alcohol levels (BAL) following intragastric ethanol administration was investigated. The ICV dose of 125 ng/rat of NH_2-SENK, that markedly reduces ethanol intake, did not modify BAL, nor did it increase the CTA induced by intraperitoneal injection of ethanol, 1 g/kg body weight. These findings suggest that the effect of NH_2-SENK on alcohol consumption is not related to modification of the pharmacokinetics of ethanol, nor to increase of the aversive properties of ethanol. On the other hand, the same ICV dose of NH_2-SENK evoked a pronounced and statistically significant CPP. This finding indicates that the TK NK-3 receptor agonist NH_2-SENK possesses rewarding properties in msP rats and suggests that its inhibitory effect on ethanol consumption may be due to substitution of the rewarding properties of ethanol, thus making its consumption redundant. © 1998 Elsevier Science Inc.

Aminosenktide Tachykinin NK-3 receptors Alcohol-preferring rats Alcohol intake Place conditioning

TACHYKININS (TKs) are closely related peptides sharing the common carboxyterminal sequence Phe-X-Gly-Leu-Met.NH_2 (16). Five mammalian TKs have been so far identified in the central nervous system: substance P, neurokinin A, neurokinin B, neuropeptide K, and neuropeptide γ (22). At least three distinct G-protein coupled receptors have been demonstrated for TK peptides (30,36): the NK-1 (which preferentially interacts with substance P), the NK-2 (which prefers neurokinin A) and the NK-3 (which interacts best with neurokinin B). Recently, an orphan receptor resembling the NK-3 receptor, initially claimed to be an atypical opioid receptor, has been shown to bind neurokinin B with high affinity and was proposed as the TK NK-4 receptor (15). Endogenous TKs show low selectivity for different TK receptor subtypes (36,37). NK-1 and NK-3 receptors have a widespread distribution in the central nervous system, while NK-2 receptors appear to be selectively expressed only in specific brain nuclei (13,30,36,38).

Previous studies from our laboratory have shown that the intracerebroventricular (ICV) injection of selective TK NK-3 receptor agonists, such as aminosenktide (NH_2-SENK), senktide, or [MePhe^7]neurokinin B, attenuates alcohol intake in genetically selected alcohol-preferring rats (7–9,33,34). The effect is behaviorally selective, because doses of NK-3 recep-

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tor agonists that reduce alcohol intake do not modify either water intake in water-deprived rats or food intake following food deprivation (8). The effect is centrally evoked; the nucleus basalis magnocellularis (NBM) and the lateral hypothalamus (LH) are the most sensitive sites so far tested (7,31). Moreover, the effect is not related to influence on the gustatory evaluation of the ethanol solution, because doses of NH_2-SENK that reduce ethanol intake do not modify taste reactions to ethanol (35).

The present study was aimed at further evaluating the mechanism of action for the effect on ethanol intake of NH_2-SENK. First, the effect of ICV injections of NH_2-SENK on blood alcohol levels (BAL) following intragastric ethanol administration was investigated to check whether its effect on ethanol intake might be related to influence on the pharmacokinetics of ethanol. Second, the effect of NH_2-SENK was investigated in the conditioned taste aversion (CTA) paradigm (1,42), to evaluate whether its inhibitory effect on ethanol intake might be related to potentiation of the aversive properties of ethanol. Last, the effect of NH_2-SENK was studied in the conditioned place-preference (CPP) paradigm (43), to test the hypothesis that it might reduce ethanol intake by substituting the rewarding properties of ethanol. These studies were stimulated by the work of Huston and co-workers, who showed that the TK substance P and related peptides evoke CPP when injected in discrete brain areas, and that conditioned place aversion follows injection in other brain areas (19,24–27,40).

METHOD

Animals

Male genetically selected alcohol-preferring rats were employed. They were bred for 23 generations in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy), starting from Sardinian alcohol-preferring rats of the 13th generation (17,18). They are referred to as Marchigian Sardinian alcohol-preferring (msP) rats.

Three-month-old msP rats were selected for their preference for 10% ethanol solution (v/v), offering them free choice between water and 10% ethanol 24 h/day for 7 days. The rats employed in the experiments had a 24-h ethanol intake of 6–7 g/kg, with a percent ethanol preference [ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h × 100] higher than 90. Ten days before the experiments ethanol was removed from the cages.

The rats' body weight ranged between 400 and 450 g at the moment in which experiments were carried out. Each experiment was carried out on different groups of animals. Animals were kept in a room with a reverse 12 L:12 D cycle (lights off at 1000 h.), temperature of 20–22°C, and humidity of 45–55%. Rats were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy).

The experimental procedures used in the present study are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Intracranial Surgery

For intracranial surgery, rats were anesthetized by intramuscular injection of 100–150 μl/100 g body weight of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml). A stainless steel guide cannula (outside diameter 0.65 mm) for injections into the lateral ventricle was stereotaxically implanted and cemented to the skull, according to the following coordinates, taken from the Paxinos and Watson atlas (32): AP = 1 mm behind the bregma, L = 2 mm from the sagittal suture, V = 2 mm from the surface of the skull. Drug injections were made by means of a stainless steel injector (outside diameter 0.3 mm), 2.5 mm longer than the guide cannula, so that its tip protruded into the ventricle.

Drugs

The selective TK NK-3 receptor agonist [$Asp^{5,6}$,$MePhe^{8}$] substance P(5–11), also referred to as aminosenktide (NH_2-SENK) (28), was purchased from Peninsula Europe (Merseyside, UK). It was dissolved in 0.9% NaCl and ICV administered in volume of 1 μl/rat.

EXPERIMENTAL PROCEDURE

Experiment 1. BAL Following ICV Injection of NH_2-SENK

Two groups of five rats received ICV injection of 125 ng/rat of NH_2-SENK or isotonic saline (controls) followed by intragastric (IG) administration (by gavage) of 0.7 g/kg of 10% ethanol. This is the usual amount that msP rats voluntarily take in the first 5 min, when they are offered access to 10% ethanol for 2 h/day. Blood samples (50–100 μl) were taken from the tail vein at 15, 30, 60, and 120 min after ethanol administration. BAL were measured by gas chromatography (11).

Experiment 2. Effect of ICV Injections of NH_2-SENK in the CTA Paradigm

Once a day in the 2 days before the experiment, a few drops of sweet solution (containing 0.125% saccharin + 3% glucose) were sprinkled over the rat's mouth, so that it could taste it. This expedient was adopted to familiarize the rat with the new tastant. This sweet solution was chosen because it is highly palatable, has a low caloric content and is consumed in huge amounts by the rat (39,44).

Four groups of water- and food-sated rats were employed. For 5 consecutive days (at 10.00 h), all of them were offered 20-min access to the sweet solution, while food and water were removed from the cage. Immediately after the 20-min access period, two groups of seven rats received ICV injection of either 125 ng/rat of NH_2-SENK or its vehicle followed by intraperitoneal (IP) injections of ethanol, 1 g/kg, as a 10% solution in isotonic saline; the other two groups of animals received either 125 ng/rat of NH_2-SENK ($n = 6$) or its vehicle ($n = 5$) followed by IP administration of isotonic saline. The dose of 125 ng/rat of NH_2-SENK was chosen because in previous studies it evoked a pronounced reduction of ethanol intake (7,8,33). The amount of sweet solution ingested during the 20-min access period was recorded and expressed as ml/kg body weight.

Experiment 3. Effect of ICV Injections of NH_2-SENK in the CPP Paradigm

The study was carried out in a two-compartment box, adopting a classical conditioning procedure that has been successfully used to assess the rewarding properties of drugs of abuse (6). Test sessions were videotaped by means of a Canon VC-20 color videocamera, and analyzed by means of a Panasonic (NV-HD650EG) video cassette recorder. The time spent in each side of the two-compartment box and the num-

ber of crossings from one compartment to the other were measured by an experienced observer who was blind to the treatment conditions. The rat was considered in one side of the box when at least the head and the two forelimbs were inside it.

The conditioning box was divided by a guillotine door in two square-base wooden compartments (h 40 × 30 × 30 cm), one with gray walls with white stripes and a white floor, the other with gray walls with black stripes and white floor with black stripes. The box was placed in a dimly illuminated room with a white noise. Place conditioning sessions were run from 1000 to 1200 h (after the beginning of the dark phase).

Preconditioning. In the first 2 days, rats were allowed to explore the box (guillotine door open) for 15 min, and the time spent in each compartment was recorded. Data of the second day were used as a measure of preconditioning preference. During preconditioning about 70% of the rats preferred the black striped side. The rats employed spent about 70% of the 15 min of the second preconditioning test in the preferred side of the box.

Conditioning. Rats were divided into four groups. Three groups of eight animals received ICV injections of 10, 31, or 125 ng/rat of NH_2-SENK. The fourth group of seven rats received isotonic saline and served as control. Rats were placed in the box immediately after administration, alternating between ICV NH_2-SENK or its vehicle on each consecutive day. During conditioning the guillotine door was closed and rats were confined for 1 h in one compartment of the box. NH_2-SENK was paired to the nonpreferred side. On the first day of training half of the animals received the drug and the other half its vehicle. Four conditioning sessions were run (two isotonic saline and two NH_2-SENK sessions).

Postconditioning test. The day following the last conditioning session, rats were allowed to explore the entire box for 15 min and the time spent in each compartment was measured. Place preference score (referred to as Δ time) for each rat was obtained by subtracting the time spent in the nonpreferred compartment during the second preconditioning day to that spent in the same compartment during the postconditioning test. The Δ time values were submitted to statistical analysis. In addition, the time spent by rats in the nonpreferred compartment in the second preconditioning trial and the time spent in the same compartment in the postconditioning test is reported (Fig. 3B). Last, to provide information on the locomotor activity of the rats, the number of side-to-side crossings measured in these two sessions were reported (Table 1).

Validation of Cannula Placement

After completion of the experiments, rats were ICV injected with 1 μl of Indian ink, and immediately afterwards they were sacrificed and diffusion of ink into the cerebroventricles was evaluated. Only data from animals with valid cannula placement were included in experimental results (the numbers are reported in the legends to figures).

Statistical Analysis

Data are presented as means ± SEM. CTA and BAL data were analyzed by means of split-plot analysis of variance (ANOVA), with between-group comparisons for drug treatment and within-group comparisons for time. Planned pairwise comparisons were carried out by means of *t*-tests. In the CPP study, the one-way ANOVA, followed by Newman–Keuls test, was used. Statistical significance was set at $p < 0.05$.

RESULTS

Experiment 1. BAL Following ICV Injection of NH_2-SENK

As shown in Fig. 1, administration of 0.7 g/kg of ethanol produced pharmacologically relevant BAL. The highest BAL were measured at 30 min, but detectable BAL were also measured 120 min after IG ethanol administration. The ICV treatment with 125 ng/rat of NH_2-SENK did not significantly modify BAL, $F(1, 8) = 0.29, p > 0.05$.

Experiment 2. Effect of ICV Injections of NH_2-SENK in the CTA Paradigm

The overall ANOVA revealed a significant treatment effect, $F(3, 21) = 3.09, p < 0.05$. As shown in Fig. 2, the ICV injection of NH_2-SENK just before IP injection of isotonic saline did not significantly modify the intake of the sweet solution in comparison to that of controls, which received IP

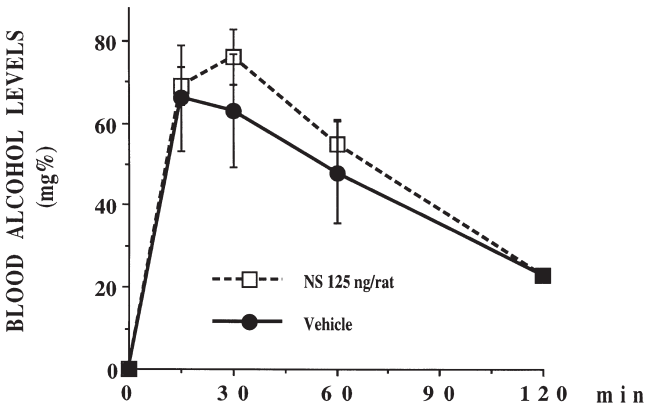

FIG. 1. BAL (mg%) after IG ethanol administration, 0.7 g/kg, in msP rats pretreated with ICV injection of either NH_2-SENK (NS), 125 ng/rat, or isotonic saline (IS). Values represent means ± SEM of FIVE data. Difference from controls was never statistically significant.

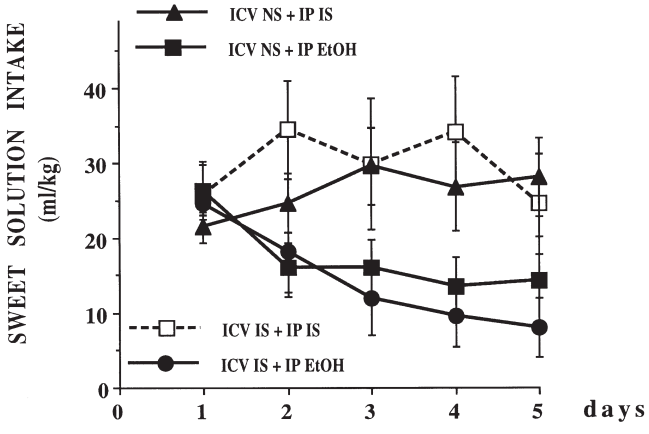

FIG. 2. Effect of ICV injection of either NH_2-SENK (NS), 125 ng/rat, or isotonic saline (IS), followed by IP injection of either ethanol, 1 g/kg, or IS, on the intake of 0.125% saccharin + 3% glucose solution. Values are means ± SEM of five to seven subjects. Statistical difference from controls is reported in the text.

injection of isotonic saline after ICV saline injection. The IP injection of ethanol, 1 g/kg, after ICV injection of isotonic saline resulted in a pronounced and statistically significant reduction in the intake of the sweet solution from the second day of the experiment ($p < 0.05$). The ICV injection of NH_2-SENK, 125 ng/rat, did not significantly modify the CTA induced by IP ethanol administration, although it slightly reduced it in the last 3 days.

Experiment 3. Effect of ICV Injections of NH_2-SENK in the CPP Paradigm

The ANOVA revealed a significant effect of NH_2-SENK treatment on the Δ time measured in the CPP paradigm, $F(3, 27) = 5.47$, $p < 0.01$] (Fig. 3A). Pairwise comparisons revealed a significant effect after ICV injection of 125 ng/rat ($p < 0.01$); in response to this dose rats developed preference for the compartment paired with it (Fig. 3B). The dose of 31 ng/rat increased the Δ time, but the increase was not statistically significant. In response to 10 ng/rat of NH_2-SENK, the place conditioning score was almost identical to that measured in controls.

In regard to the gross behavior of rats during the conditioning procedures, the locomotor activity of the rats was not significantly modified by the NH_2-SENK treatments (Table 1). A slight increase in flat body posture was observed in response to the highest dose of NH_2-SENK, 125 ng/rat. As previously reported (10), the occurrence of wet dog shakes and head shakes after ICV NH_2-SENK injection in our genetically selected rats was very low, far inferior to that observed in genetically heterogeneous rats.

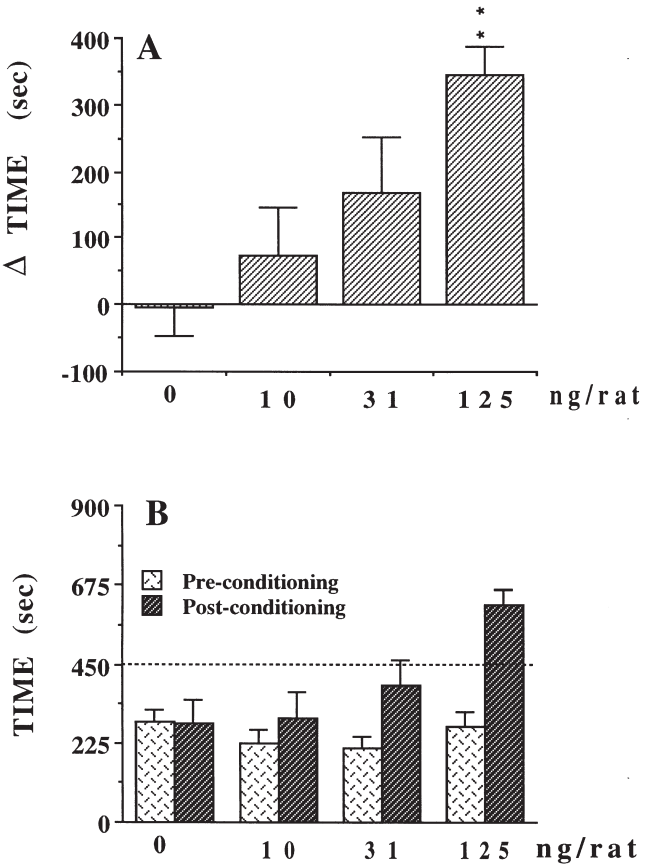

FIG. 3. Place conditioning following ICV administration of 10, 31 and 125 ng/rat of NH_2-SENK or its vehicle. **A** shows the Δ time, obtained by subtracting the time spent in the nonpreferred compartment during the second preconditioning trial to that spent in the same compartment during the postconditioning trial. Values are means ± SEM of seven to eight rats. Difference from controls (0): $^{**}p < 0.01$; where not indicated, difference from controls was not statistically significant. **B** shows the time spent in the nonpreferred side of the box during the pre- and postconditioning trials.

DISCUSSION

Previous studies have shown that ICV injections of 31 or 125, but not 10, ng/rat of the selective TK NK-3 receptor agonist NH_2-SENK reduce ethanol intake in msP rats (7,8,33). The present results reveal that the ICV dose of 125 ng/rat of NH_2-SENK did not modify BAL after IG ethanol administration, suggesting that its effect on alcohol consumption is not related to modification of the pharmacokinetics of ethanol. Moreover, administration of 125 ng/rat of NH_2-SENK did not increase the CTA induced by IP ethanol injection, suggesting that its inhibitory effect on ethanol intake is not dependent upon an increase of the aversive properties of ethanol. On the other hand, in the CPP paradigm the same ICV dose significantly increased the time spent in the compartment paired with it. Indeed, the dose of 31 ng/rat of NH_2-SENK also increased the time spent in the box compartment paired with its administration, but the increase did not reach the level of significance. These findings indicate that NH_2-SENK possesses rewarding properties and suggest that its inhibitory effect on ethanol consumption may be due to substitution of the rewarding properties of ethanol, thus making its consumption redundant.

Studies carried out by Huston and co-workers had already shown that injection of the nonselective TK substance P in the NBM or in the LH evokes CPP in rats (24–26). In their experiments, a strong CPP was also induced by administration of the C-terminal hexapeptide of substance P or the C-terminal heptapeptide analog DiME-C7 (19,20,26), but not by the N-terminal heptapeptide. These findings indicate that the C-terminal sequence of substance P is responsible for the effect (21,26), as required for effects mediated by "classic" TK receptors (36). DiME-C7 shows about 300 times lower affinity than substance P at NK-1 receptors, but approximately three times higher affinity at NK-3 receptors (36). Moreover, C-ter-

TABLE 1

NUMBER OF CROSSINGS FROM ONE SIDE OF THE BOX TO THE OTHER DURING THE SECOND PRECONDITIONING TRIAL AND THE POSTCONDITIONING TEST, FOLLOWING ICV INJECTION OF VEHICLE, 10, 31, OR 125 ng/RAT OF NH_2-SENK

	Number of Crossings	
Treatment	Preconditioning	Postconditioning
Vehicle	12.3 ± 2.5	13.0 ± 4.5
10 ng/rat	14.7 ± 2.3	17.2 ± 2.0
31 ng/rat	17.4 ± 2.6	19.0 ± 3.4
125 ng/rat	12.8 ± 2.9	14.7 ± 1.3

Data are the mean ± SEM of seven to eight rats.

minal sequences of substance P have a higher NK-3/NK-1 receptor affinity ratio, in comparison to substance P (36). Thus, CPP induced by TK peptides in those studies may be mediated by NK-3, rather than NK-1 receptors. The results obtained in the present study with the highly selective TK NK-3 receptor agonist NH_2-SENK support the idea that NK-3 receptors mediate the rewarding properties of TK peptides.

Several findings reported in the literature might provide neurochemical correlates for the rewarding properties of TK NK-3 receptor agonists. Microdialysis studies in freely moving rats have shown that peripheral injection of the nonselective TK substance P or of DiME-C7 results in increased dopamine release in mesolimbic and/or mesostriatal dopaminergic terminals (2–4). Substance P injection in the nucleus basalis magnocellularis evoked increase in the extracellular concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid, in the ipsi- and contralateral nucleus accumbens (5). In addition, it has been shown that central TKergic mechanisms can interact with endogenous opioid mechanisms. In this regard, the substance P-induced CPP has been reported to be blocked by naloxone (20). Moreover, NK-3 receptor agonists have been shown to potentiate morphine-induced analgesia (12), apparently inducing opioid release and/or modulating opioid receptors [see (36) for review]. Interestingly, these effects of TK receptor agonists recall, at least from a qualitative point of view, some of the neurochemical effects of ethanol. Ethanol, in fact, stimulates dopamine (14) and serotonin (29,46) release into the nucleus accumbens. Ethanol stimulates endogenous opioid release (41,45) and ethanol self-administration is blocked by opioid antagonists (23).

In conclusion, the results of the present study indicate that the TK NK-3 receptor agonist NH_2-SENK possesses rewarding properties and suggest that reduction of ethanol consumption induced by NH_2-SENK in msP rats may be due to substitution of the rewarding properties of ethanol, thus making its consumption redundant. A recent mapping study indicates that the brain sites that are most sensitive to the inhibitory effect of NH_2-SENK on ethanol intake are the NBM and the LH, that is, two sites where the injection of the TK substance P induces CPP. These findings are in keeping with the hypothesis that attenuation of ethanol intake by NH_2-SENK may be due to substitution of the rewarding properties of ethanol. Further studies are necessary to clarify the involvement of TKs in the regulation of the brain reward system and to elucidate the role of the different TK receptor subtypes.

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