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Inhibition of Isotonic Sodium Chloride Intake in the Rat by Selective Tachykinin Agonists

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CICCOCIOPPO, R., C. POLIDORI, P. POMPEI, G. DE CARO AND M. MASSI. *Inhibition of isotonic sodium chloride intake in the rat by selective tachykinin agonists.* PHARMACOL BIOCHEM BEHAV 47(3) 609–615, 1994. – The present study investigated the effect of the central injection of selective tachykinin (TK) agonists on the need-free intake of 0.9% NaCl in rats. Isotonic NaCl was offered for 60 min (between 1800 and 1900 h); water was offered for 4 h (between 1800 and 2200 h). The TK agonists were injected into the third ventricle just before access to fluids. The NK_3-selective agonists [Asp^{5,6},MePhe^8]substance P(5–11) and Succ[Asp^6,MePhe^8]substance P(6–11), as well as the NK_1-selective agonist [Sar^9, Met(O$_2$)^{11}]substance P, markedly reduced salt intake, the threshold dose for their effects being 5 ng/rat. The NK_2-selective agonist GR64349 reduced salt intake only at 500 ng/rat. At the dose of 31.2 ng/rat, neither the NK_1 nor the NK_3 agonists inhibited water intake, when water was the only fluid offered (between 1800 and 2200 h), or modified food intake in food-deprived rats. The present study shows that a) TKs inhibit not only the need-induced salt intake, but also the need-free intake of isotonic saline, b) this effect is behaviorally selective, and c) the effect is apparently mediated by NK_1 and NK_3 receptors. The finding that TKs suppress salt intake in a large variety of experimental conditions supports the idea that the antinatriorexic effect of TKs is independent of the physiological and hormonal status of the animal. It is hypothesized that TKs might modify taste sensitivity or the hedonic evaluation of the salty taste.

Tachykinin Tachykinin receptors Salt intake

SALT appetite (or need-induced salt intake) can be evoked by dietary sodium deficiency (3), pharmacological natriuresis and removal of ambient sodium (35), extracellular sodium loss due to dialysis, sweating, diarrea, vomiting, hemorrage (33), or increased metabolic need for salt, as in pregnancy (21).

On the other hand, salt intake can also occur in the absence of a sodium deficit; this need-free salt intake is unhomeostatic and might be related to the hedonic evaluation of the salty taste (6).

Our previous studies have shown that the pulse intracerebroventricular (ICV) injection of tachykinins (TKs) inhibits the need-induced salt intake evoked by sodium depletion, by adrenalectomy, and by administration of components of the renin-angiotensin-aldosterone system (13,14,16,17,23,34), which mediates salt appetite induced by sodium depletion (29). The bed nucleus of the stria terminalis and, to a lesser extent, the medial amygdala have been shown to be highly sensitive to the antinatriorexic effect of TKs (14,23). This finding, the rich TKergic innervation (5,12,27,36) in these areas involved in salt intake control, plus the recent finding that adrenalectomy markedly reduces the mRNA for preprotachykinin A in the bed nucleus of the stria terminalis (22), suggest that at least in these brain areas TKs might play a physiological role in salt intake control.

In a recent paper TKs were proven to also inhibit the need-free salt intake of female rats, whose salt intake had been enhanced by previous sodium depletions (15). Their intake is known to occur without mediation of the renin-angiotensin-aldosterone system (15,28).

The present study evaluated the effect of TKs on the need-free intake of 0.9% NaCl in rats that had not experienced previous sodium depletions or pharmacological treatments, aimed at enhancing salt intake (that might have induced long-term biochemical and/or neurochemical changes). The aim of this study was twofold:

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1. to further challenge the idea that the inhibitory effect of TKs on salt intake occurs independently of the experimental conditions under which it is expressed;
2. to further investigate the TK receptor subtypes involved in the antinatriorexic effect.

Multiple TKs are present in the mammalian brain (2,7, 9,19), and at least three TK receptor subtypes mediate their effects (4,11,24,25): the NK_1, for which substance P (SP) is the preferred ligand; the NK_2, which preferentially binds neurokinin A; and the NK_3, for which neurokinin B is the preferred ligand. Because naturally occurring TKs are rather nonselective for the different receptor subtypes, the present study was carried out employing synthetic TK agonists highly selective for each of the three TK receptor subtypes: the NK_1-selective agonist [Sar^9,$Met(O_2)^{11}$]SP (26), the NK_2-selective agonist GR64349 (8), and the NK_3 selective agonists [$Asp^{5,6}$,-$MePhe^8$]SP(5-11) [also referred to as NH_2-senktide (NH_2-SENK)] and Succ[Asp^6,$MePhe^8$]SP(6-11), also referred to as SENK (10,37).

METHOD

Animals

Male Wistar rats (Charles River, Calco, Como, Italy) weighing 300-325 g at the beginning of the experiments were employed. They were individually housed in a temperature-controlled room on a 12 L : 12 D cycle. They were offered ad lib access to Na-sufficient food pellets (diet # 4RF18, Mucedola, Settimo Milanese, Italy) containing 0.5% NaCl. They had access to water and to 0.9% NaCl according to the modalities reported in the experimental procedure.

Drugs

The following TK peptides were used: [Sar^9,$Met(O_2)^{11}$]SP, GR64349, NH_2-SENK, and SENK. GR64349 was a generous gift of the Glaxo Group Research Ltd, Ware, UK, and the other three peptides were purchased from Peninsula Europe, Merseyside, UK.

Surgery

All the animals used were anesthetized (ketamine hydrochloride and acepromazine, IM) and fitted by stereotaxic surgery with a stainless steel guide cannula (C313G, Plastic Products Co., Roanoke, VA) that opened into the anteroventral third ventricle. The guide cannula was permanently attached to the skull by stainless steel screws and dental acrylic cement. Animals were allowed 1 week to recover from surgery before testing began. Prior to the beginning of the experiments, the cannula placement was validated by measuring the animal's drinking response to an ICV injection of 10 ng of angiotensin II. A minimum criterion of 5 ml of water drunk in 15 min was used. After completion of the experiments, the cannula placement was evaluated by injecting 1 μl of India ink into the ventricle and observing the ventricular diffusion of the ink after the animal was sacrificed.

Drug Administration

The drugs, dissolved in sterile isotonic saline (ISO), were given in a volume of 1 μl through a stainless steel injector temporarily inserted into the guide cannula and protruding into the ventricle. Injections were given with the aid of a 10-μl Hamilton microsyringe.

Sequence of Testing

The experiments were carried out according to a within-subjects design, in which each animal received all the doses of a single drug in counterbalanced order. The intervals between subsequent treatments are given in each experiment.

Experiment 1. Effects of Selective TK Agonists on Simultaneous Intake of 0.9% NaCl and Water

Rats were offered access to fluids at 1800, that is, at the beginning of the dark phase of the light : dark cycle. Sodium chloride solution (0.9%) was available for 60 min (between 1800 and 1900). Tap water was offered simultaneously with NaCl solution, but rats had free access to it for 4 h (between 1800 and 2200). This schedule was adopted: a) to concentrate salt intake in a short period of time (as required to test peptides with a rather short half-life) and b) to provide enough time to rats for drinking and eating during the dark phase, when they are highly active. Animals were adapted to this schedule of fluid presentation for 10 days before the beginning of the experiments. Food pellets were available 24 h a day. Drug injection took place 1 min before access to fluids. Rats were treated at intervals of 3-4 days.

Experiment 2. Effect of Selective TK Agonists on Water Intake, When 0.9% NaCl Was Not Available

Water was the only fluid offered; it was provided for 4 h, between 1800 and 2200. Animals were adapted to this schedule of water presentation for 10 days before the beginning of the experiment. Again, food pellets were available 24 h a day. Peptides were injected, as in Experiment 1, 1 min before access to water. Experiments were carried out at intervals of 3-4 days.

Experiment 3: Effect of Selective TK Agonists on Food Intake Induced by Food Deprivation

To further evaluate the behavioral selectivity of the effect on salt intake of [Sar^9,$Met(O_2)^{11}$]SP, NH_2-SENK, and SENK, the present experiment investigated their effect on food intake induced by food deprivation. Rats were deprived of food, but not of water, for 20 h, from 2200 to 1800 of the following day. One minute before access to food, rats were given an ICV injection of either isotonic saline (controls) or the TK peptide tested. The three peptides were administered at the dose of 31.2 ng/rat, which had proven to evoke a marked suppression of salt intake in Experiment 1. Each animal received both saline and one of the peptides tested at interval of 6 days. Before the beginning of the experiment, animals had already experienced a food deprivation.

Experiment 4: Motor Effects Induced by Selective TK Agonists

Water- and food-replete rats were given an ICV injection of either saline (controls) or the TK tested, and immediately afterwards they were placed in a perspex box (35 × 40 cm, and 40 cm high), where their behavior was recorded by means of a videocamera for subsequent analysis. The animals were made familiar with the box for 7 days before the beginning of the experiment. As in Experiments 2 and 3, [Sar^9,$Met(O_2)^{11}$]SP, NH_2-SENK, and SENK were tested at the ICV dose of 31.2 ng/rat. The categories of behavior evaluated were: feeding, drinking, grooming, resting, locomotion, rearing, sniffing, licking, standing, urination, defecation, stretching,

yawning, treading, head and body shaking, coprophagia, and miscellaneous other behaviors (1). Data were expressed either as number of episodes or as number of seconds spent in a single behavior in the first 15 min following drug administration. This time interval was adopted because afterwards the animals appeared to be just resting in the box.

Statistical Analysis

Data are presented as averages (mean ± SEM). Statistical analysis of data was performed by multifactorial analysis of variance (ANOVA) (repeated measures) to check the overall significance. Post hoc comparisons were carried out by means of the Dunnett's test. Statistical evatuation of the results of Experiment 4 was carried out by means of the Student's *t*-test. Statistical significance was set at $p < 0.05$.

RESULTS

Experiment 1. Effects of Selective TK Agonists on Simultaneous Intake of 0.9% NaCl and Water

Salt intake. The NK_1-selective agonist [Sar^9,$Met(O_2)^{11}$]SP markedly reduced salt intake (Fig. 1A). The ANOVA revealed a highly significant treatment effect, $F(4, 20) = 6.26$, $p < 0.01$. Post hoc comparisons showed that a significant reduction of salt intake was obtained even at the dose of 5 ng/rat. At the dose of 31.2 ng/rat, salt intake was significantly suppressed for the entire 60-min observation period. At the dose of 250 ng/rat, salt intake was almost completely suppressed in the first 15 min. Grooming was evident following administration of the NK_1 agonist in response to 31.2 ng/rat and proved to be very pronounced at 250 ng/rat.

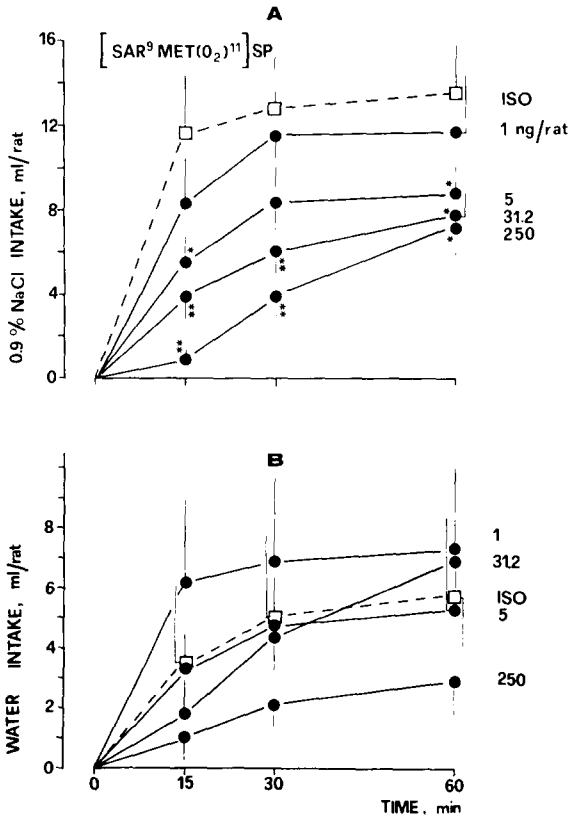

FIG. 1. Cumulative 60-min intake of both 0.9% NaCl (A) and water (B) following the ICV injection of different doses (ng/rat) of [Sar^9,-$Met(O_2)^{11}$]SP. Each point is the mean ± SEM of six data. Statistical difference from controls (ISO): $*p < 0.05$; $**p < 0.01$; where not indicated, difference from controls was not statistically significant.

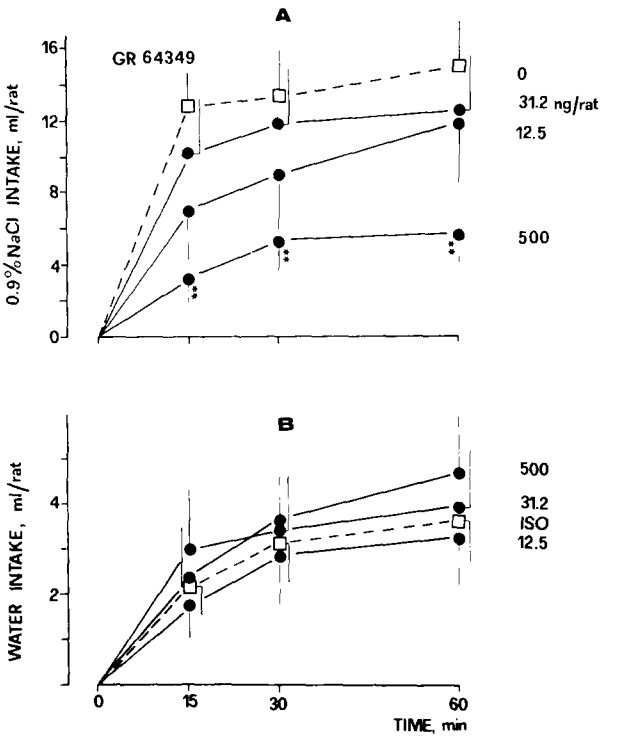

FIG. 2. Cumulative 60-min intake of both 0.9% NaCl (A) and water (B) following the ICV injection of different doses (ng/rat) of GR64349. Each point is the mean ± SEM of seven data. Statistical difference from controls (ISO): $**p < 0.01$; where not indicated, difference from controls was not statistically significant.

The NK_2-selective agonist GR64349 was tested at doses of 31.2–500 ng/rat. It evoked a significant inhibitory effect on salt intake, $F(3, 18) = 4.54$, $p < 0.05$, but pairwise comparisons showed a statistically significant difference from controls only at the dose of 500 ng/rat (Fig. 2A).

The NK_3-selective agonists tested, NH_2-SENK and SENK, potently inhibited 0.9% NaCl intake, $F(4, 24) = 8.83$, $p < 0.001$ and $F(4, 28) = 5.45$, $p < 0.01$, respectively. Pairwise comparisons showed that both peptides produced a statistically significant suppression of salt intake even at 5 ng/rat (Figs. 3A and 4A). Episodes of wet dog shakes and head shakes were commonly observed in response to SENK administration at the dose of 500 ng/rat. Similar behaviors were observed only occasionally in response to NH_2-SENK, even at the highest dose of 500 ng/rat.

Water intake. The salt concentration employed in the present study is preferred to water. Therefore, during the 1-h test, water intake was lower than that of the salty solution. As shown in Figs. 1B, 2B, 3B, and 4B, in the range of doses tested the four peptides only slightly affected water intake, and the ANOVA revealed no significant treatment effect, and no significant treatment × time interaction for any of them.

Consequently, total fluid intake (data not shown) at doses that reduced salt intake was lower than that of controls.

Experiment 2. Effect of Selective TK Agonists on Water Intake, When 0.9% NaCl Was Not Available

One dose of each peptide was employed in this experiment. The NK_1-selective agonist [Sar^9,Met$(O_2)^{11}$]SP and the selective NK_3 agonists NH_2-SENK and SENK were tested at the dose of 31.2 ng/rat. These peptides evoked inhibion of salt intake in the previous experiment with a threshold dose of 5 ng/rat, and showed a marked inhibitory effect at 31.2 ng/rat for the entire 60-min test. The NK_2-selective agonist was tested at the dose of 500 ng/rat, which was only effective on 0.9% salt intake. As shown in Fig. 5, none of the peptides tested significantly modified water intake in these experimental conditions.

Experiment 3: Effect of Selective TK Agonists on Food Intake Induced by Food Deprivation

The ICV administration of 31.2 ng/rat of [Sar^9,Met$(O_2)^{11}$]SP or of NH_2-SENK essentially did not modify food intake. Following ICV administration of 31.2 ng/rat of SENK, food intake was partially reduced (Fig. 6); however, the overall ANOVA revealed neither a drug effect nor a significant drug × time interaction at the dose tested.

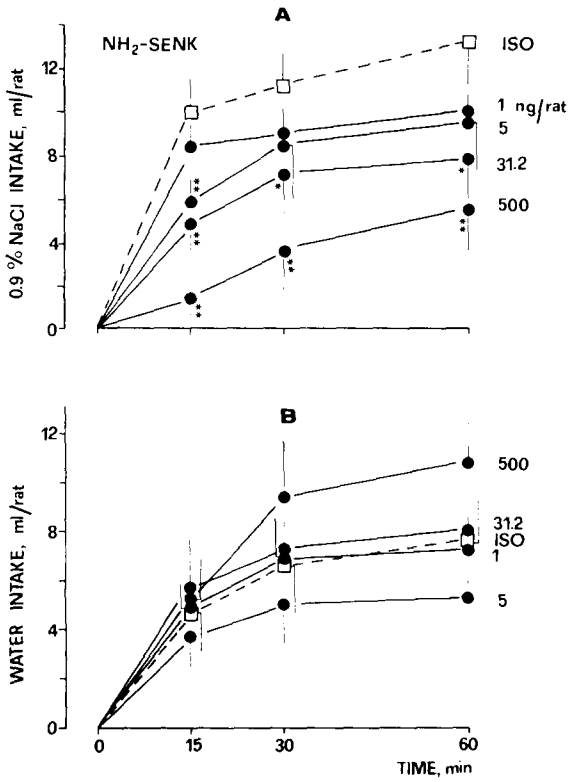

FIG. 3. Cumulative 60-min intake of both 0.9% NaCl (A) and of water (B) following the ICV injection of different doses (ng/rat) of NH_2-SENK. Each point is the mean ± SEM of seven data. Statistical difference from controls (ISO): $*p < 0.05$; $**p < 0.01$; where not indicated, difference from controls was not statistically significant.

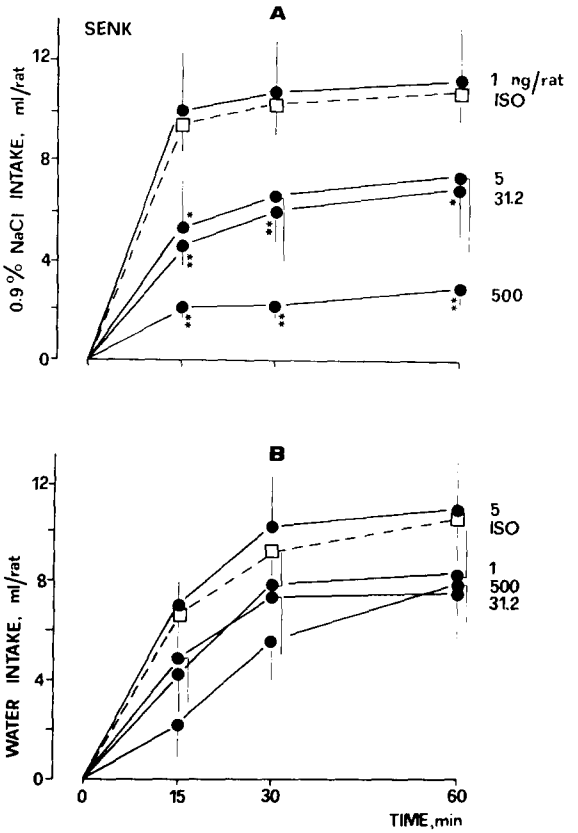

FIG. 4. Cumulative 60-min intake of both 0.9% NaCl (A) and water (B) following the ICV injection of different doses (ng/rat) of SENK. Each point is the mean ± SEM of eight data. Statistical difference from controls (ISO): $*p < 0.05$; $**p < 0.01$; where not indicated, difference from controls was not statistically significant.

Experiment 4: Motor Effects Induced by Selective TK Agonists

In addition to resting and locomotion, the categories mainly observed were grooming, head shakes, wet dog shakes, and flat body posture, their occurrence varying in relation to the peptide administered.

As shown in Fig. 7A, control rats spent 119 ± 19 s grooming. [Sar^9,Met$(O_2)^{11}$]SP produced a significant increase in grooming behavior; the mean time spent in this behavior was 252 ± 49 s. On the other hand, the selective NK_3 agonists SENK and NH_2-SENK did not stimulate grooming. Indeed, the time spent grooming was, respectively, 69 ± 16 s and 40 ± 19 s, the latter being significantly lower that that of controls.

Controls showed 3.0 ± 1.0 episodes of head shakes and 5.6 ± 0.8 episodes of wet dog shakes in the 15-min observation period. The ICV administration of the three TKs tested slightly increased the number of these episodes; however, the difference from controls was never statistically significant (Fig. 6B, C).

Controls maintained flat body posture only for 3.0 ± 2.9 s during the 15-min observation period. [Sar^9,Met$(O_2)^{11}$]SP and NH_2-SENK left essentially unmodified the time spent in flat body posture. Following SENK, one of the treated animals remained in flat body posture for 494 s in the 15-min

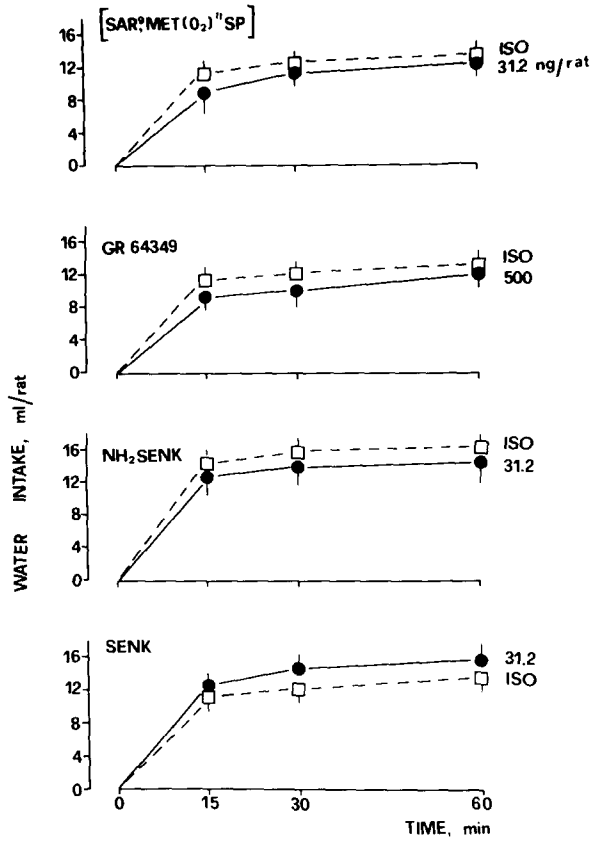

FIG. 5. Cumulative 60-min intake of water, when water was the only fluid offered, in rats treated with an ICV injection of a selective TK agonist (ng/rat). Each point is the mean ± SEM of nine data for SENK and [Sar⁹,Met(O₂)¹¹]SP, seven data for NH_2-SENK, and eight data for GR64349. Difference from controls was never statistically significant.

observation period, while all the other animals spent just a few second in this behavior. The statistical analysis revealed the absence of a significant effect (Fig. 7D).

The remaining part of the observation period was essentially spent by the rats in locomotion or resting behavior. At the dose tested, none of the three TKs significantly modified the time spent in these two behaviors (data not shown).

Other behaviors were only occasionally and sporadically observed, and the paucity of observations did not justify a statistical evaluation.

DISCUSSION

The experimental conditions adopted in the present study were designed to concentrate fluid ingestion in a limited period of time, thus allowing the study of the effect of rather short-lasting peptides given by injection. The animals had a sodium-sufficient diet available 24 h a day, they were not given natriuretic treatments, and their cages were not washed, thus leaving high amounts of sodium in the environment, and in addition they used to take between 12 and 15 ml/rat/day of 0.9% NaCl in the 60 min of access to it. Moreover, the animals employed had not experienced previous pharmacological treatments or sodium depletions that might have produced long-term biochemical and/or neurological consequences, as in the case of the enhanced need-free salt intake of multidepleted female rats (15). Therefore their intake of salt can be considered just the expression of a need-free preference for isotonic saline.

In addition, rats were offered 4-h access to fluids during the dark period when they are highly active and express most of their ingestive behavior. The mean total fluid intake (0.9% NaCl + water) in the 4-h test ranged between 30 and 35 ml/rat, thus providing good hydration of the experimental animals.

In these experimental conditions, the selective NK_3 agonists NH_2-SENK and SENK, as well as the selective NK_1 agonist [Sar⁹,Met(O₂)¹¹]SP potently inhibited the intake of 0.9% NaCl, the threshold dose for their effect being 5 ng/rat. On the other hand, the selective NK_2 agonist GR64349 evoked a significant suppression of salt drinking only in the high dose of 500 ng/rat. These findings indicate that TK agonists exert a marked antinatriorexic effect in this model of need-free salt intake and suggest that both NK_1 and NK_3 receptors might mediate the effect. Probably the effect observed at the high dose of the NK_2 agonist GR64349 might be due to interaction with a TK receptor subtype different from the NK_2.

A previous study had already suggested the involvement of NK_3 receptors in the mediation of the antinatriorexic effect of TKs in sodium-depleted rats (18). The present study, while confirming a role for NK_3 receptors, suggests, on the basis of the results obtained with the potent and selective NK_1 agonist [Sar⁹,Met(O₂)¹¹]SP, that NK_1 receptors might also be involved in the antinatriorexic action of TKs.

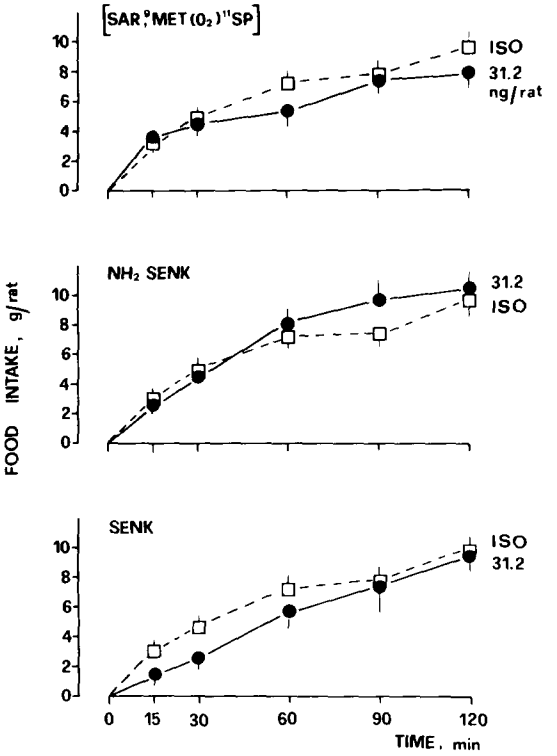

FIG. 6. Cumulative 2-h food intake in food-deprived rats treated with an ICV injection of a selective TK agonist (ng/rat). Each point is the mean ± SEM of seven data. Difference from controls was never statistically significant.

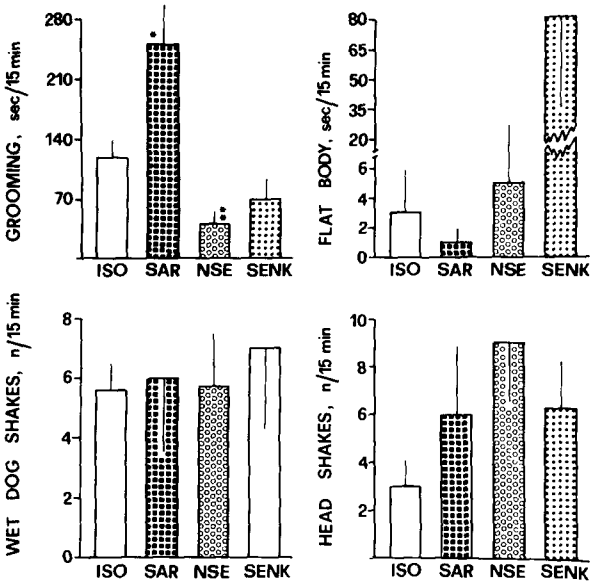

FIG. 7. Motor behaviors following ICV injection of isotonic saline (ISO), or of 31.2 ng/rat of [Sar^9,Met(O$_2$)^{11}]SP (SAR), of NH$_2$-SENK (NSE), or of SENK. Each point is the mean ± SEM of six data. Difference from controls: $*p < 0.05$; $**p < 0.01$; where not indicated, difference from controls was not statistically significant.

It is interesting to notice that the doses required to suppress salt intake in the present experimental model were very low. The threshold dose for NH$_2$-SENK in suppressing depletion-induced salt appetite has been shown to be 31.2 ng/rat (17), and in the present study it was only 5 ng/rat.

In Experiment 1, the NK$_1$-selective agonist and the two NK$_3$-selective agonists suppressed 0.9% NaCl intake, leaving water intake essentially unmodified; consequently, total fluid intake following their administration was significantly reduced. This finding raises the question whether the reduction in total fluid intake might be due to behavioral impairment, or alternatively, to the fact that saline intake was not needed for adequate hydration, so that its suppression does not necessarily imply a compensation. In this context, Experiment 2 clearly shows that the animals took essentially the same amount of water as controls when they were offered only water to drink. These findings suggest that the reduction of the total fluid intake observed in Experiment 1 is not expression of behavioral incompetence. Probably the saline intake was not necessary to the animals owing to the sodium-rich diet offered and to the environmental sodium available, and it might have been just related to the hedonic evaluation of its taste.

Also, the results of Experiment 3 support the idea that the antinatriorexic effect of TKs is a behaviorally selective effect. In particular, [Sar^9,Met(O$_2$)^{11}]SP and NH$_2$-SENK essentially did not modify food intake at a dose (31.2 ng/rat) that markedly suppressed salt intake. Further evidence in favor of the behavioral selectivity of the effect of NH$_2$-SENK comes also from Experiment 4, which showed that the peptide did not produce competing behaviors at the dose tested. As far as [Sar^9,Met(O$_2$)^{11}]SP is concerned, Experiment 4 showed that at the dose of 31.2 ng/rat it produced intense grooming, in accordance with the notion that NK$_1$ receptors mediate grooming induced by naturally occurring TKs (32). However, the results of Experiments 2 and 3 indicate that the grooming induced by [Sar^9,Met(O$_2$)^{11}]SP is not compulsive, as it allows the expression of a normal ingestive behavior.

As far as SENK is concerned, we know from the literature that this TK has a particular pharmacological profile. At appropriate doses the drug elicits serotonin-mediated behaviors, apparently linked to release of serotonin (30,31). Moreover, the drug can also reduce food intake, another effect probably mediated by serotonergic mechanisms (20). In our experimental conditions, the dose of 31.2 ng/rat of SENK was sufficiently low to produce no significant behavioral alteration in Experiments 3 and 4, even though there was a trend to the reduction of food intake and to the induction of flat body posture. These findings indicate that at doses up to 31.2 ng/rat the antinatriorexic effect of the drug is behaviorally selective.

In conclusion, the present results show that central TKergic mechanisms suppress the need-free salt intake of isotonic saline in the rat, which was not enhanced by previous pharmacological treatments. The large variety of experimental conditions under which the antinatriorexic effect of TKs has been observed apparently suggests that their effect is independent of the physiological and hormonal status of the animal and raises the question whether TKs might interfere with gustatory mechanisms for salt or with the hedonic evaluation of the salty taste.

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