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Brain Tachykinins and the Suppression of Salt Intake

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ABSTRACT

Tachykinins (TKs) are potent inhibitors of salt intake, their inhibition being elicited both in the need-induced and in the need-free intake of sodium. The inhibition is evoked by intracerebroventricular administration, but is particularly evident when TKs are injected into brain areas (the medial nucleus of the amygdala or the dorsolateral tract of the bed nucleus of the stria terminalis) involved in the control of salt intake. The antinatriorexic effect of TKs is selective for this ingestive behaviour and is due to interaction with brain neurokinin-3 (NK-3) receptors. TKs which selectively bind NK-1 or NK-2, but not NK-3, receptors inhibit the intake of water but not that of salt. Experimental data strongly suggest that brain TKs belong to a multi-tachykininergic system which regulates the behavioural control of water and sodium homeostasis, acting as brain inhibitors of salt (NK-3 agonists) and water intake (NK-1 and NK-2 agonists).

INTRODUCTION

The tachykinins (TKs) are neuroactive peptides sharing the common carboxy-terminal sequence: Phe-X-Gly-Leu-Met(NH_2). Some of them are of non-mammalian origin, others are normal components of mammalian tissues, including the brain. The mammalian TKs are substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Kimura et al., 1983; Erspamer, 1984). In mammalian tissues, multiple TKs receptors have been demonstrated: the neurokinin-1 receptor (NK-1) which preferentially binds SP, the NK-2 receptor which prefers NKA and the NK-3 receptor subtype which preferentially interacts with NKB (Quirion and Dam, 1988).

Our studies have strongly suggested that TKs play a role in the neural and behavioural control of body fluid homeostasis. In fact, when administered intracranially to animals of different species, the TKs, taken as a class, inhibit water and salt intake, both effects being selective, for water or salt ingestion, respectively.

In this paper we summarize the data from our laboratory concerning the effects of TKs on salt intake in the rat.

METHODS

Animals

Rats of both sexes of the Wistar (Charles River, Como, Italy) and Sprague–Dawley (Holtzman, Madison, WI, USA) strain were employed. Their weight ranged from 250 to 300 (Wistar) or 300–350 g (Sprague–Dawley) at the beginning of the experiments.

The animals were individually housed in a temperature controlled room on a 12:12 h light:dark cycle and received distilled water and food in pellets (Mucedola, S. Milanese, Italy) at will. When requested by the experimental schedule, they also received 1.5 or 3% NaCl solution or sodium-free food in pellets (Tekland Premier, Medison, WI, USA).

Experimental models of salt appetite

The following models of salt appetite were employed:

Salt intake induced by sodium depletion

Salt appetite was obtained by combining pharmacological natriuresis (2 subcutaneous, s.c., injections of 5 mg per rat of furosemide, separated by 2 h) with removal of ambient sodium. The animals were not deprived of distilled water and received sodium-free food ad libitum. Twenty-two h after the first injection of furosemide, they had free access to 3% NaCl solution and consumption of water and salt was recorded at 15, 30, 60 and 120 min. When requested, salt intake was recorded for longer time, up to 24 h. Testing began with the 3rd depletion, since the first depletions produce a lower intake of salt than the subsequent ones.

Salt intake induced by deoxycorticosterone acetate (DOCA) administration

DOCA, 2 mg per rat in 1 ml of sesame oil, was administered s.c. once a day for 8 days. The animals, which had free access to food in pellets and distilled water, received 3% NaCl 2 h per day, between 11.00 a.m. and 01.00 p.m. Testing began on day 9 of DOCA treatment, when the 2 h intake of salt was reliable and stable.

Salt intake induced by pICV injection of renin

Renin, 200 ng per rat dissolved in 1 µl isotonic saline, was given by pICV injection into the lateral ventricles. Animals were tested at intervals of 7 days. Testing began with the 3rd renin injection, since repeated renin injections evoke a progressively larger intake of salt which reaches a plateau beginning with the third treatment. TKs were injected ICV 1 h after renin administration and one min later the rats had free access to 3% NaCl. Salt intake was recorded 15, 30, 60 and 120 min after salt presentation.

Need-free intake of salt

Female Sprague-Dawley rats were submitted to 3 consecutive sodium depletions, at intervals of 7 days. This produces in rats (but particularly in females) an enhanced spontaneous daily intake of 3% NaCl which lasts long, probably a life time, and is referred to as "need-free" since it occurs in animals which are in positive sodium balance. After the 3rd depletion the rats, which were receiving a 3% NaCl solution, had continuous access to 1.5% NaCl for 2 days. Then, they were offered 1.5% NaCl only 2 h per day, between 11.00 a.m. and 01.00 p.m., and were allowed to adapt to this schedule for 10 days. Testing began at the end of this period. TKs were administered just before salt presentation and salt consumption was recorded at 15, 30, 60 and 120 min.

Substances

The following substances were employed: the non-mammalian TKs eledoisin (ELE) and kassinin (KAS); the mammalian TKs substance P (SP), neurokinin A (NKA) and neurokinin B (NKB); the synthetic NK-1 selective agonist SP methylester (SPOMe); the NK-3 selective agonists [$Asp^{5,6}MePhe^{8}$]SP5-11, (aminosenktide, NH_2S) and [$MePhe^{7}$]NKB; the SP derivatives [Sar^{9}]SP and [$MePhe^{8}Sar^{9}$]SP, endowed with NK-1 and NK-3, but devoid of NK-2, activity. We also employed angiotensin II (Peninsula Labs. Europe, Merseyside, UK), furosemide (Lasix, Hoechst, Frankfurt, Germany), deoxycorticosterone acetate (DOCA, Sigma, Saint Louis, MO, USA) and renin (purified from hog kidney by affinity chromatography), which was a gift of Prof. D. Ganten, German Institute for High Blood Pressure Research.

Surgery

The animals were anaesthetized (ketamine HCl and acepromazine, intramuscularly) and fitted by stereotaxic surgery with stainless steel guide cannulae aimed 1 mm above the lateral ventricle or at selected brain areas (the medial nucleus of the amygdala, MA, or the dorso-lateral tract of the bed nucleus of the stria terminalis, BNST).

Stereotaxic coordinates were those of Paxinos and Watson (1986).

Guide cannulae were permanently attached to the skull by stainless-steel screws and dental acrylic cement. In rats employed in cICV infusion experiments the cannulae were part of swivel apparatuses which were attached to the skull with the same technique as before.

Cannula placement was validated by measuring the animal's response to 50 ng of pICV angiotensin II. Animals were allowed to recover one week before testing began.

Drug administration

The drugs, dissolved in sterile isotonic saline, were given in a volume of 1 (ventricles) or 0.2 µl (MA and BNST) through a stainless-steel injector temporarily inserted into the guide-cannula and protruding into the ventricle or into the MA or BNST. In infusion experiments the infusion rate was 0.1 µl per rat per min.

Injections were given with the aid of a 10 (ventricles) or 1 µl (MA and BNST) Hamilton microsyringe, while infusions were made with the aid of an electronic pump (Precidor Infors, Basel, CH) driving a 1 ml syringe.

Experimental procedure

Experiments were carried out according to a within subject design at intervals of at least 7 days.

Pulse ICV injections took place 1 min before 3% NaCl solution presentation, while cICV infusions began 2 h before and were interrupted 2 h after the animals had free access to salt.

Statistical analysis

Statistical analysis of the data (which are presented as means±SEM) was performed by multifactorial analysis of variance to check the overall significance. Planned pairwise comparisons were carried out by means of *t* tests. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of TKs administration on salt appetite induced by sodium depletion

The nonselective NK-receptors agonist, ELE, at pICV doses of 100 and 250 ng per rat produces a marked inhibition of salt intake which, at the largest dose, is statistically significant even 120 min after the injection (Massi et al., 1986a).

SP, which preferentially binds NK-1 receptors, and the synthetic selective NK-1 agonist, SPOMe, inhibit salt intake, but only at very large doses (2 μg per rat) (Massi et al., 1991b).

KAS, which is a NK-2 agonist with good affinity for NK-3 receptors, is even more effective than ELE itself while NKA, which preferentially binds NK-2 receptors, is practically devoid of any effect on salt appetite (Massi et al., 1986a, 1988a).

The NK-3 agonist, NKB, is poorly soluble in water and, given as a micro suspension, has no effect at doses up to 2 μg per rat. Instead, NH_2-S, which is a synthetic, highly selective, hydrosoluble NK-3 agonist, produces a potent, reliable, dose-dependent inhibition of salt appetite which is statistically significant ($P < 0.05$) even at 31.25 ng per rat (de Caro, 1988; Massi et al., 1988b). At this dose the inhibition lasts only 15 min, but at larger doses (500–2000 ng per rat) it is very pronounced (100% at 15 min) and is significant throughout the 2 h test ($P < 0.01$). Similar effects have the synthetic NK-3 agonist, [MePhe^7]NKB, and NK-1, NK-3 agonist, [Sar^9]SP (Massi et al., 1991).

These data are summarized in Fig. 1.

The antinatriorexic effect of TKs is very potent also when these peptides are administered by cICV infusion. In fact, ELE, KAS and NH_2-S, infused at rates of 10 ng per rat per min or less, produce a potent, easily reproducible and dose dependent inhibition of salt intake (Massi et al., 1986a; de Caro, 1988, 1991).

An interesting phenomenon, which we have called "after inhibition of salt appetite", can be observed in these cICV infusion experiments. In fact, if the rats have a history of depletions of salt and cICV infusions of TKs, an additional depletion not followed by the infusion of TKs arouses a very poor intake of salt. In other words, repeated cICV infusions of TKs in sodium depleted rats produce a sort of "after inhibition" of salt appetite which becomes evident when the rats are submitted to salt depletions not followed by cICV infusions of the peptides.

The after inhibition lasts for 3-4 weeks or longer. Then, the sensitivity of the rats to the antinatriorexic activity of TKs returns to normal (de Caro, 1988, 1991) (Fig. 2).

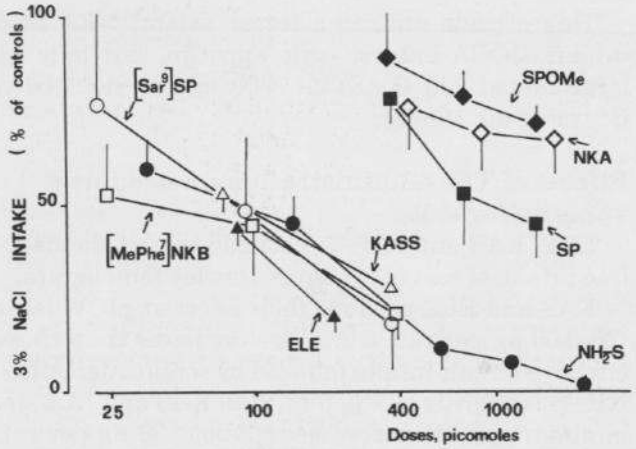

Fig. 1. Percent inhibition of sodium-depletion induced salt intake elicited by pICV injection of several TKs. Each point is the mean of 8–10 subjects. The effect of [MePhe^7]NKB, NH_2-S, ELE and KAS is statistically significant ($P < 0.05$–0.001) throughout the range of the doses employed. The activity of [Sar^9]SP is significant ($P < 0.05$ at doses of 100–400 pmoles, while that of SP, NKA and SPOMe is significant ($P < 0.05$) only at the maximum doses employed.

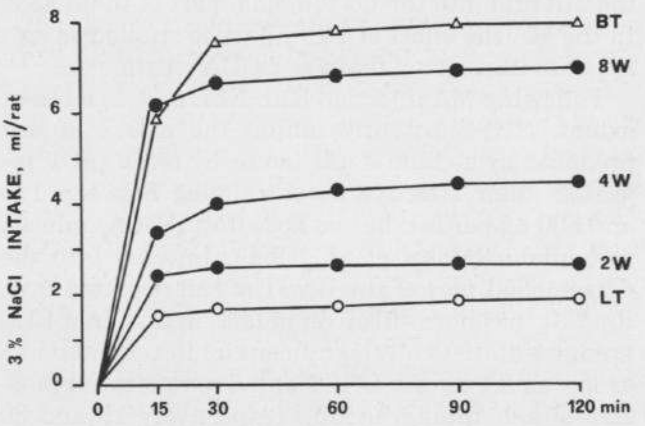

Fig. 2. "After-inhibition" of salt intake following repeated cICV infusions of eledoisin (ELE) in sodium-depleted (SD) rats. BT: 3% NaCl intake in SD rats infused ICV with isotonic saline 1 week before ELE treatment; LT: 3% NaCl intake in SD rats recorded in the last of 4 treatments with cICV infusions of ELE, 10 ng/rat/min, given at intervals of 1 week; 2 W, 4 W and 8 W represent 3% NaCl intake in SD rats infused with isotonic saline 2, 4 and 8 weeks after the last ELE treatment (LT). Both ELE and isotonic saline were infused over a 4 h period (2 h before and 2 h after access to 3% NaCl). The intake of salt of LT, 2 W and 4 W, but not of 8 W, is statistically different ($P < 0.01$–0.001) from that recorded before eledoisin treatment (BT).

Effects of TKs administration on salt intake induced by renin or by DOCA treatment

KAS at doses of 100–500 ng per rat significantly inhibits the intake of salt induced by pICV administration of renin.

This peptide and, to a lesser extent, NKA also inhibit DOCA-induced salt appetite, but only at large doses: 500 (KAS) or 1000 ng per rat (NKA) (Massi et al., 1988a).

Effects of TKs administration on need-free appetite for salt

ELE, KAS and NH_2-S, potently inhibit the need-free intake of salt of Sprague–Dawley female rats.

KAS and ELE produce their effect at pICV doses (20–100 ng per rat) which are far lower than those effective on salt intake induced by sodium depletion. NH_2-S is slightly less potent than KAS and ELE, its minimum effective dose being about 30 ng per rat. NKA produces an evident suppression of salt intake at doses of 125 ng per rat or larger, while SP evokes its effect only at doses as large as 1000 ng per rat (Massi et al., 1991) (Fig. 3).

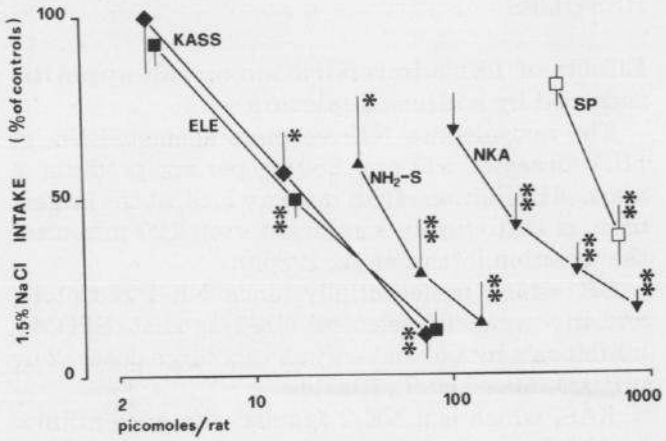

Fig. 3. Percent inhibition of need-free salt intake following pICV injection of several TKs (picomoles/rat). Each point is the mean of 6–7 subjects. Difference from controls: *, $P<0.05$; **, $P<0.01$; when not indicated, $p > 0.05$.

Effects on salt intake of TKs injections into selected brain areas

The effect of TKs on salt intake was studied in sodium depleted rats, following their injection into the MA and into the dorsomedial part of the BNST. In the MA the effect of TKs was also studied on salt appetite induced by renin or DOCA treatment.

Following MA injection both ELE and, to a lesser extent, NH_2-S potently inhibit the intake of salt produced by sodium depletion or by renin pICV injection, their effective doses ranging between 125 and 500 ng per rat, but do not affect DOCA-induced salt intake (Massi et al., 1990). Injected into the dorsomedial part of the BNST of salt depleted rats, the TKs are more effective: in fact, in this area ELE produces statistically significant inhibitions at doses as low as 3.1 ng per BNST and a complete suppression of salt intake, lasting respectively 15 and 30 min, at doses of 50 and 100 ng per BNST (Pompei et al., 1991) (Fig. 4).

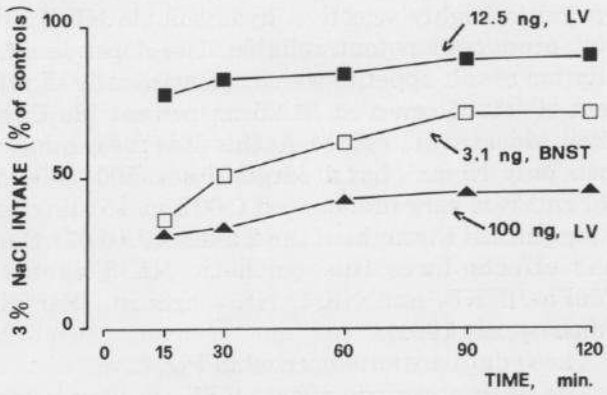

Fig. 4. Percent inhibition of salt intake in sodium depleted rats following pulse injections of different doses of eledoisin (ELE) into the lateral ventricles (LV) or into the dorsomedial part of the bed nucleus of the stria terminalis (BNST).

DISCUSSION

Our data clearly indicate that in the rat TKs are potent inhibitors of salt intake, their inhibition being elicited in the need-induced salt intake of sodium depleted animals, in angiotensin II- and DOCA-induced sodium appetite, and in the need-free intake of sodium.

The antinatriorexic effect of TKs is not due to malaise, neurological artifacts or elicitation of competing behaviours since at antinatriorexic doses they do not suppress the intake of solid or fluid (15% fat milk) food and not even that of 10% sucrose. Indeed, they can elicit, immediately after their administration, grooming and increased motor activity. However, these effects cannot account for the inhibition of sodium intake since they last a short time, far less than the inhibition of salt intake itself, and do not produce, as could be expected, a general impairment of rats' ingestive behaviour (Massi et al., 1986b, 1991; de Caro, 1988).

Salt intake inhibition is not due to alteration of the act of licking or swallowing, since TKs do not inhibit the intake of milk, of 10% sucrose or of solid food. And it is not even related to the antidipsic activity of TKs, since selective NK-3 agonists inhibit salt intake at extremely low doses but do not suppress water intake at all, while selective NK-1 and NK-2 agonists have a potent antidipsic effect but are very poor inhibitors of salt intake (Massi et al., 1991).

The mechanism of the antinatriorexic effect of TKs as well as the site of their action have not yet been completely clarified. However, our data strongly suggest that TKs do not act simply as inhibitors of angiotensin II or of mineralocorticoids. In fact, they inhibit the need-induced intake of salt, which is due to the synergistic action of brain angiotensin II and peripheral mineralocorticoids, but they also inhibit the need-free appetite for sodium, which is independent of these hormones and is only related to a hedonic evaluation of salty taste.

The antinatriorexic effect of TKs seems to be due to interaction with NK-3 brain receptors, while the antidipsic one is very easily due to activation of NK-1 or NK-2 receptors. In fact, selective NK-3 agonists or non-selective agonists having a marked affinity for NK-3 receptors are potent inhibitors of sodium appetite, while selective NK-1 and NK-2 agonists or non-selective agonists endowed with a weak affinity for NK-3 receptors either do not inhibit sodium intake at all or produce only a negligible inhibitory effect. These NK-1 and NK-2 agonists, instead, have a potent inhibitory effect on drinking induced by angiotensin II (NK-1 agonists) or by cellular dehydration (NK-2 agonists), while selective NK-3 agonists, having no affinity for other NK-receptors, do not affect drinking at all (Massi et al., 1991).

The MA is known to be involved in the control of salt appetite in the rat (Denton, 1984; Schulkin et al., 1989) and contains receptors for both angiotensin II (McKinley et al., 1986) and mineralocorticoids (Birmingham et al., 1979), the hormones controlling salt appetite in salt depleted rats. On the other hand, the dorso medial part of the BNST receives inputs from the MA (Weller and Smith, 1982) and contains numerous NK-1 and NK-3 receptors (Saffroy et al., 1988). In these areas, particularly in the BNST, the injection of TKs produces a potent antinatriorexic effect, and this strongly suggests that MA and BNST are sites for TKs' antinatriorexic activity (Pompei et al., 1991).

The effect of TKs on sodium appetite is a phenomenon of general biological interest. In fact, TKs have been tested for their antinatriorexic activity not only in rats but also in other animals of different species: sheep, rabbits, cattle (Tarjan et al., 1990) and pigeons (Massi, 1987). In all these animals at very low doses TKs exert a potent, dose related, easily reproducible inhibition of salt intake and neither produce malaise, neurological alterations or autonomic modification, nor inhibit food intake or elicit any competing behaviour.

Several data strongly suggest that TKs play in the brain a role of relevant importance. This is suggested by the following: (1) TKs and NK-receptors are selectively distributed in the brain, particularly in areas which are known to be involved in the behavioural control of body fluid homeostasis; (2) TKs are antinatriorexic in all the animals tested up to now; (3) their antinatriorexic effect is the expression of a selective interaction with NK-3 receptors; (4) salt intake inhibition is evoked by TKs injections into selected brain areas, MA and BNST, which contain NK-receptors and are involved in the control of salt intake; (5) antinatriorexic TKs are not antidipsic; (6) TKs produce their inhibitory effect at low, and occasionally at extremely low doses, and (7) at antinatriorexic doses do not produce any other behavioural alteration.

CONCLUSIONS

Thus, on the basis of these data and considering that TKs exert a variety of effects on body fluid regulation (inhibition of water intake, vasopressin release due to activation of central NK-3 receptors, antidiuresis in water replete rats) (Cantalamessa et al., 1984; de Caro et al., 1988; Polidori et al., 1989), we can hypothesize that, taken as a class, in the central nervous system TKs play a fundamental role and control sodium and body fluid homeostasis, acting as inhibitors of salt and water intake.

To have demonstrated that TKs are potent, selective antinatriorexic agents and to have hypothesized that they regulate the behavioural control of salt intake might be of practical importance. In fact, the relationship existing between excessive, hedonic intake of salt and hypertension (for review, see Denton, 1984) is well known. In theory, the administration of TKs or of TKs derivatives could turn off the hedonic assumption of salt, thus reducing the consequences of chronic, excessive intake of sodium. To demonstrate this is the goal of our future studies.

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