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Sodium and Water Intake of Sheep, Rabbits and Cattle During ICV Infusion of Eledoisin

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TARJAN, E., J. R. BLAIR-WEST, G. DE CARO, D. A. DENTON, M. I. McBURNIE, M. MASSI, J. SZMYDYNGER-CHODOBSKA AND R. S. WEISINGER. *Sodium and water intake of sheep, rabbits and cattle during ICV infusion of eledoisin.* PHARMACOL BIOCHEM BEHAV **35**(4) 823–828, 1990.—The present study reports the effects of ICV administered eledoisin, the most potent anti/dipsogenic member of the tachykinin family, in three species. Sheep with chronic parotid fistula lost daily 200–400 mmol sodium in 3–4 l of saliva. During ICV infusion of eledoisin, 2 to 50 ng/min, a decrease in sodium intake was observed. If water was withheld for 22 hours, sheep normally drank 5.4 l water on presentation. During ICV infusion of eledoisin, 50 ng/min, water intake increased significantly. Wild rabbits lost 5 mmol sodium in 50 ml of urine after injection of furosemide. During ICV infusion of eledoisin, 30 ng/min, a decrease in sodium intake and an increase in water drinking was observed. Cows prepared with parotid fistula had access to sodium solution every other day to replace salivary sodium loss. During ICV infusion of eledoisin, 50 and 150 ng/min, a decrease in sodium intake occurred, and water intake was unaffected. These results confirm that central administration of eledoisin specifically influences ingestive behaviour in mammals and draws attention to some species differences in the observed effects.

Sodium appetite Tachykinins Thirst

INTRACEREBROVENTRICULAR (ICV) administration of tachykinins to rats decreased the dipsogenic effect of ICV angiotensin II and carbachol, and decreased the water drinking following water deprivation or injection of hypertonic saline (6). In contrast, in pigeons, ICV tachykinins induced water drinking (11). Tachykinins also inhibited the sodium appetite resulting from sodium depletion in rats (14, 15, 17, 19). The most potent tachykinin influencing both water and sodium intake was eledoisin, and the doses necessary in rats to influence ingestion of water and sodium were much lower than the doses influencing food intake (16) or locomotor activity (10). Because the effects of the peptides on drinking behaviour were not related to their effects on arterial blood pressure it was suggested that the mechanism of action was directly on neurons rather than indirectly by vascular activity (5).

In the present study the effect of ICV administration of eledoisin on the sodium intake of sodium-depleted sheep, wild rabbits and cows, and on the water drinking of thirsty sheep and wild rabbits was investigated. All three species have been extensively studied in our laboratory. Sheep and cattle correct their sodium and water deficit following deprivation during 30 min (8). Wild rabbits also correct their deficit accurately, but correction takes 8–10 hours for water and 12–24 hours for sodium deficits (9). During ICV infusion of eledoisin the sodium intake of all three species was depressed, similar to the results in rats. In contrast to the effect in rats, eledoisin did not suppress the water intake of the thirsty animals, rather a tendency to increased drinking was observed.

METHOD

Sheep (body weight 35–40 kg), wild rabbits (1.4–1.8 kg) and cows (308–394 kg) were studied. All three species received ICV infusion of eledoisin at various concentrations. Eledoisin (Sigma) was dissolved in small volumes of artificial cerebrospinal fluid (csf) (22), aliquots were kept at −20°C and thawed immediately before the experiment. The aliquots were then diluted in the correct artificial csf to the concentration required for infusion.

Studies in Sheep

Five crossbred Merino ewes were housed in individual metab-

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olism cages. They were fed 0.8–0.9 kg oaten-alfalfa chaff (sodium content 50–150 mmol/kg, potassium content 200–300 mmol/kg) at 1630 hr daily. Salivary loss of phosphate was replaced by the addition of 10 g KH_2PO_4 daily to the chaff. The animals were surgically prepared in two stages, both under general inhalation anaesthesia (Fluothane/O_2 mixture) after induction with intravenous sodium thiopentone. In the first stage, oophorectomy was performed and a permanent parotid fistula was made (7). In the second stage, 16-gauge stainless steel guide cannulae were implanted above the lateral cerebral ventricles. Experiments began 2–4 weeks after the completion of surgery. ICV infusions, at a rate of 0.9 ml/hr, were delivered through an indwelling 20-gauge stainless steel needle introduced through the guide cannula and attached by a length of polythene tubing to a pump (Perfusor, Braun).

Sheep were presented with 0.6 M $NaHCO_3$ for 30 min daily after 23.5-hr loss of sodium from the parotid fistula. Infusion of eledoisin commenced 15 min before the access to $NaHCO_3$, and continued throughout the 30 min of access. At least one week was allowed between experiments. Following the completion of studies on sodium intake, for the studies on water drinking the same five sheep were also deprived of water for 22 hr. ICV infusion of eledoisin was started 15 min before the access to water and continued throughout the 30 min of access.

The effect of ICV infusion of eledoisin 2, 10 and 50 ng/min, given at random order, was compared to the effect of ICV infusion of artificial csf by analysis of variance (repeated measures design) with subsequent Newman-Keuls' tests (3).

Studies in Wild Rabbits

Wild rabbits of both sexes were trapped and brought to the laboratory where they were housed in individual metabolism cages. Dry food pellets (sodium content 15 mmol/kg, potassium content 220 mmol/kg), 0.5 M NaCl solution and tap water were continuously available. Intakes of food, water and NaCl solution, urine volume, and urinary sodium excretion were measured daily between 0900 and 1000 hr. The animals had at least one month to adapt to the laboratory conditions and handling before the beginning of the experiments. Wild rabbits, similar to rats, eat and drink nocturnally. During the day, when experiments were performed, wild rabbits do not normally ingest food, water or sodium solution.

A stainless steel 23-gauge guide tube was implanted above the left lateral cerebral ventricle under general anaesthesia (ketamine 15 mg/kg and xylazine 2 mg/kg, intravenously). The animals were allowed to recover for at least two weeks after surgery. ICV infusions, at a rate of 17 μl/hr, were delivered in the metabolism cages through an indwelling 27-gauge needle attached to a tethered plastic tubing and a pump (Perfusor, Braun). Experiments were performed at 1–2-week intervals when the rabbits were in sodium balance. At the completion of studies the patency of access to the lateral ventricle was checked at postmortem examination after injection of blue dye.

On the day of the experiment after the morning measurements, food and fluids were removed from the cages and furosemide (Flusapex, Apex Laboratories) 20 mg/kg was injected intravenously. ICV infusion of artificial csf or eledoisin, 30 ng/min, given at random order, commenced immediately after the injection and continued for 48 hr. The urine voided during the 2 hr following the injection of furosemide was collected and analyzed for sodium concentration. Two hours after the injection of furosemide water and food were returned and intakes were measured after 1, 2 and 22 hr. Twenty-four hours after the injection of furosemide the 0.5 M NaCl solution was returned and intake was measured after 1, 2 and 24 hr (Fig. 1).

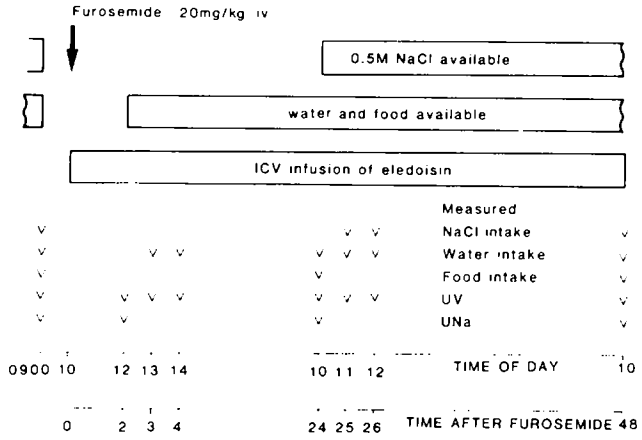

FIG. 1. Experimental protocol for the study of the effect of eledoisin in wild rabbits.

The sodium concentration of urine was measured by Technicon autoanalyzer. Correction of sodium deficit was calculated by dividing the amount of sodium ingested with the 0.5 M NaCl solution and food by the amount of sodium excreted in the urine at each time of measurement. Correction of fluid deficit was calculated by dividing the amount of water consumed with the urine volume at the time of measurement.

Statistical Analysis

Thirteen animals were infused with artificial csf or eledoisin at 30 ng/min. The effect of ICV infusion of eledoisin, 30 ng/min, and csf was compared with paired *t*-test.

Studies in Cattle

The experiments were performed on six 3–5-year-old virgin Aberdeen Angus cows. They were kept in individual pens in a large shed and were fed at 0900 hr and 1700 hr daily 4.5 kg of milled meadow hay. Each animal was surgically prepared with a unilateral parotid fistula (7) and with 16-gauge stainless steel guide cannulae above the lateral cerebral ventricles (21). The animals had free access to water and were provided with unlimited volumes of a solution containing 200 mM $NaHCO_3$ plus 100 mM NaCl for voluntary drinking from 1230 to 1430 hr on Mondays, Wednesdays, Fridays and Saturdays each week, so as to compensate for the loss of sodium from the parotid fistula. The cows were adapted to these conditions for at least a month before the beginning of experiments.

On experimental days a probe (1 mm bore) was inserted through the guide cannula into a lateral ventricle. The probe was connected to a polyethylene cannula and a syringe driven by an infusion pump (Perfusor, Braun). Artificial csf alone or containing eledoisin, 50 and 150 ng/min, was infused from 1130 hr at a rate of 1.9 ml/hr for 3 hr. At 1230 hr the volumes of water drunk since 1130 hr were recorded and the animals were given access to sodium solution. The volumes of sodium solution and water drunk were recorded at 1430 hr and the infusion was stopped. Experiments were performed on Wednesdays and the intakes during ICV infusions were compared with the means of intakes on preceding Wednesdays by analysis of variance (repeated measures design) with subsequent Newman-Keuls' tests (3).

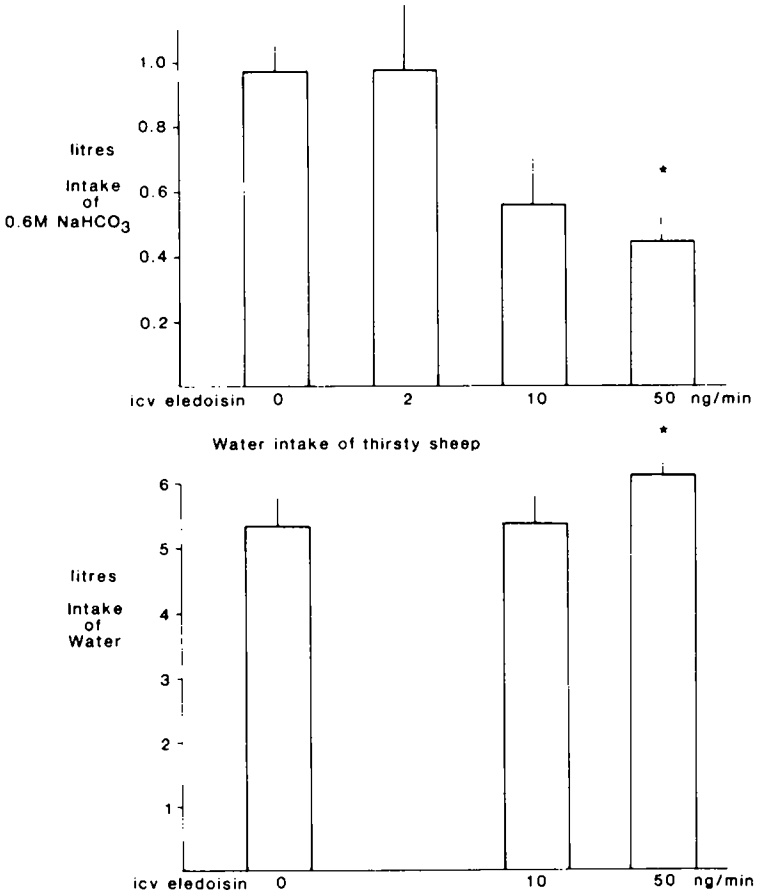

FIG. 2. Sodium intake of sodium-deplete sheep (top graph) and water intake of sheep deprived of water for 22 hr (bottom graph) during ICV infusion of csf (0) and eledoisin, 2, 10 and 50 ng/min. ICV infusion began 15 min prior to and continued until the end of 30-min sodium or water access period. Data are mean values of 5 animals, vertical lines indicate S.E.M. $\star p = 0.05$ compared to csf infusion.

RESULTS

Sheep

Sheep with parotid fistula lost 200–400 mmol sodium in 2.5–4 litres of saliva during the 22 hr of sodium deprivation. When presented with 0.6 M $NaHCO_3$ they drank within 30 min enough sodium to replace their deficit accurately. ICV infusion of eledoisin caused a reduction in the daily sodium intake of the animals relative to consumption measured during ICV infusion of artificial csf, $F(3,12) = 3.999$, $p<0.05$ (Fig. 2). For example, during ICV infusion of eledoisin, 50 ng/min, sodium intake was reduced by 53 percent.

The same sheep, when deprived of water for 22 hr, lost less saliva than during continuous access to water. For example, over two days before fluid deprivation, preceding the ICV infusion of artificial csf, sheep lost a mean 2.98 ± 0.21 litres/day of saliva. During the day when water was removed they lost 2.16 ± 0.32 litres ($p<0.05$, paired t-test). When given access to water 15 min after the commencement of ICV infusion of artificial csf they drank 5.35 ± 0.4 litres during 30 min. ICV infusion of eledoisin, 50 ng/min, increased the thirst-induced water intake significantly (Fig. 2).

At none of the eledoisin doses infused was any specific behavioural change (e.g., exploratory activity, bleating, licking) observed. Daily food intake was not influenced by any of the eledoisin doses.

Rabbits

Furosemide-induced sodium and fluid loss was not different between the experiments in which artificial csf or eledoisin was infused. The total sodium loss in the urine during the 24-hr period following the injection of furosemide was similar with both infusions (during ICV infusion of csf it was 6.14 ± 0.23 mmol, during ICV eledoisin, 30 ng/min, it was 6.02 ± 0.23 mmol, NS). Twenty-four hours after furosemide treatment the rabbits were allowed access to 0.5 M NaCl. The sodium intake of animals during ICV infusion of eledoisin was decreased compared to the intake during ICV infusion of csf at one ($p<0.01$) and two hours ($p<0.01$) after the presentation of 0.5 M NaCl. Correction of sodium deficit, accordingly, was delayed during the first two hours of access to 0.5 M NaCl when the rabbits were infused with eledoisin (at 1 hr correction of deficit was $51.5 \pm 8.5\%$ for csf and $24.9 \pm 2.5\%$ for eledoisin, at 2 hr $64.5 \pm 11.3\%$ for csf and $29.0 \pm 4.6\%$ for eledoisin, $p<0.01$ for both times). The initial reduction in sodium intake was compensated for by an increase in the overnight sodium intake so that over the 24 hr of access to 0.5

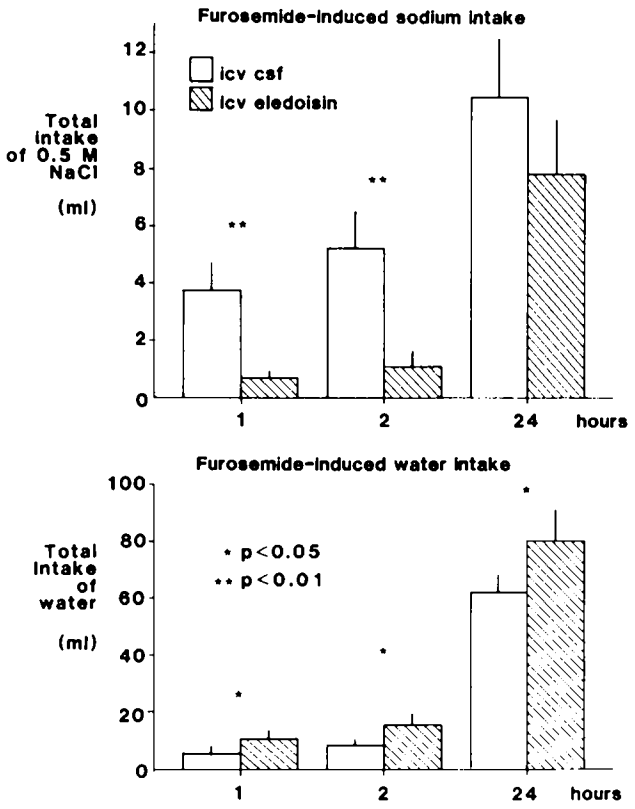

FIG. 3. Sodium intake (top graph) and water intake (bottom graph) of wild rabbits during ICV infusion of csf (empty bars) and eledoisin, 30 ng/min (hatched bars), 1, 2 and 24 hours after the presentation of sodium or water. NaCl solution (0.5 M) was offered 24 hours after IV injection of furosemide and water was offered 2 hr after furosemide. ICV infusion commenced immediately after the injection of furosemide. Data are mean values of 13 animals, vertical lines indicate S.E.M. $*p<0.05$, $**p<0.01$ compared to csf. n = 13.

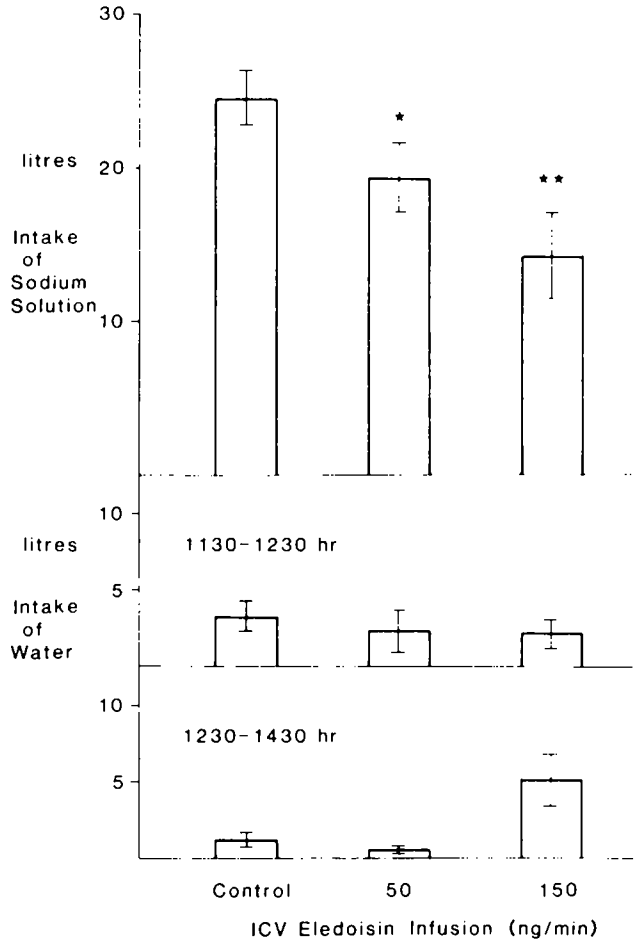

FIG. 4. Sodium intake (top graph) and water intake (middle and bottom graph) of sodium-deplete cows during continuous ICV infusion of csf (control) and eledoisin, 50 and 150 ng/min. Infusion began 1 hr prior to and continued until the end of 2-hr sodium access period. Water was continuously available. Data are mean values of six animals, vertical lines indicate S.E.M. $*p<0.05$, $**p<0.01$ compared to csf.

M NaCl rabbits drank similar amounts of sodium solution with both infusions. By 24 hr during both infusions the rabbits corrected their sodium deficit accurately (correction during ICV infusion of csf was $93.8 \pm 11.9\%$ and during eledoisin $84.7 \pm 17.2\%$, NS).

Some of the total 13 rabbits used were infused ICV with further doses of eledoisin after IV injection of furosemide: 3 ng/min was given to 7 animals, 10 ng/min to 8 and 100 ng/min to 5. The two lower doses tended to decrease sodium intake, but to a lesser degree than did the 30 ng/min dose. Curiously, ICV infusion of eledoisin, 100 ng/min, did not influence sodium intake compared to csf at the times when infusion of 30 ng/min caused a significant reduction (intake of 0.5 M NaCl during ICV eledoisin, 100 ng/min, at 1 hr was 2.58 ± 1.15 ml, at 2 hr 3.90 ± 1.24 ml).

Correction of fluid deficit (urine volume 2 hr after injection of furosemide during ICV csf infusion 46.1 ± 2.0 ml, during ICV eledoisin 44.8 ± 2.6 ml, NS) commenced on presentation of water 2 hours after the injection of furosemide. Rabbits drank more water during ICV infusion of eledoisin than during artificial csf alone at 1, 2 and 24 hr ($p<0.05$ at the three time intervals) (Fig. 3). Correction of water deficit, accordingly, was significantly quicker during ICV infusion of eledoisin than during ICV infusion of csf (during ICV infusion of csf at 1 hr it was $10.1 \pm 3.2\%$, at 2 hr $16.5 \pm 3.9\%$, at 24 hr $82.2 \pm 8.2\%$, while during ICV infusion of eledoisin at 1 hr it was $22.6 \pm 6.3\%$, $p<0.05$, at 2 hr $34.2 \pm 9.1\%$, $p<0.05$, at 24 hr $96.7 \pm 10.0\%$, $p<0.05$).

Water intake during ICV infusion of eledoisin, 3 and 10 ng/min, was somewhat above the intake recorded during ICV infusion of csf, while ICV infusion of eledoisin, 100 ng/min, was accompanied by a mean water intake above that recorded for ICV eledoisin at 30 ng/min.

ICV infusion of eledoisin did not induce any specific change in the behaviour, e.g., grooming, exploratory activity, of wild rabbits. Food intake of rabbits during ICV infusion of eledoisin was not different from that during artificial csf infusion.

Cattle

ICV infusion of eledoisin caused a dose-related reduction in the intake of sodium solution, $F(2,10) = 21.695$, $p<0.001$ (Fig. 4). Intake was reduced to 58 percent of control at 150 ng/min infusion of eledoisin. There was no significant effect on water intake during the first hour of eledoisin infusion, nor during the next two-hour period when the cows had access to sodium solution also. Two cows of the five, however, drank unusually large volumes of water, about 10 litres, during the sodium access period at the highest, 150 ng/min dose.

The cows showed no unusual behaviour, e.g., mooing, licking, scratching, during the ICV infusion of eledoisin.

DISCUSSION

The sodium intake of sodium-depleted sheep, rabbits and cows was reduced by ICV administration of eledoisin, similar to rats (14). Different methods were used to induce sodium depletion in the different species: diuretic-induced loss was the method of choice in rabbits, while sheep and cattle were tested during a chronic sodium depletion/repletion cycle induced by loss from chronic parotid fistula. The common feature of these different experimental models is that these methods were demonstrated to induce reproducible sodium deficits with a defined time course of correction and accurate replacement of deficit at the end point. Eledoisin interfered with the process of correction of deficit, irrespective of the method of induction. However, different amounts of eledoisin were found to reduce the sodium intake in different sodium-depleted species. The doses necessary for halving the sodium intake on a dose/kg body weight basis varied greatly among species. In rats, bolus injection of more than 300 ng/kg halved sodium intake (14). In sheep, a total dose of 20 ng/kg after 15 min of ICV infusion and in cows 25 ng/kg after 60 min of infusion (and a total dose of 75 ng/kg) reduced sodium intake to about half. A total dose of 28 μg/kg decreased the sodium intake of sodium-deplete wild rabbits by more than 80%, but this infusion was considerably longer than the others. Although cross species and cross design comparisons are very difficult, if not impossible due to their very nature, the point that emerges is that the observed reduction in sodium intake was specific in each species, it was not part of a generalised suppression of ingestive behaviour, i.e., water intake was not decreased by eledoisin. It would appear that in the species reported here, endogenously released tachykinins at specific brain areas could influence ingestion of sodium, as demonstrated by ICV administered eledoisin.

The effect of eledoisin on water intake varies between species from inhibition to stimulation. In rats, dose-dependent inhibition of drinking was observed following ICV administration of eledoisin, irrespective of the dipsogenic stimulus (6). In pigeons, ICV administration of eledoisin, and with similar potency, kassinin, was found dipsogenic (18). Of the three other species reported here, increased water drinking was observed following ICV infusion of eledoisin in thirsty wild rabbits, and to a lesser extent in thirsty sheep, but not in satiated cows. Presumably, differences in effects are due to differences in the abundance of diverse types of tachykinin receptors and/or to differences in the neuronal networks into which neurons with a substantial number of specific tachykinin binding sites are connected. Presently, data are not available on the central distribution of tachykinin receptors in all the species employed in our study.

Four mammalian tachykinins have been isolated so far: substance P, neurokinin A, neurokinin B and neuropeptide K (1,13). In addition, eledoisin-like immunoreactivity has been detected in the rat brain (24). Consistent with the occurrence of the multiple tachykinins, three tachykinin receptor subtypes have been proposed: the NK-1, NK-2 and NK-3 subtypes for which substance P, neurokinin A and neurokinin B are, respectively, the preferred endogenous ligands (12,23). All three tachykinin receptors have been demonstrated in the guinea pig central nervous system (23), while in the rat the presence of central NK-2 receptors is still controversial (2). Eledoisin, though rather unselective for the three receptor subtypes, is a potent ligand of the NK-3 receptor (2).

In rats, eledoisin, kassinin, and also selective NK-3 receptor agonists are potent inhibitors of sodium intake (17, 19, 20). ICV infusion of eledoisin suppressed the sodium depletion-induced sodium appetite in pigeons (M. Massi and A. Epstein, unpublished observations). The present study clearly demonstrates that eledoisin is antinatriorexic in sodium-deplete sheep, rabbits and cows and suggests that this effect of tachykinins is widely distributed among the mammalian species.

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