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BRIEF COMMUNICATION

Inhibitory Effect of Intracranial Injections of Tachykinins on Angiotensin-Induced Drinking in the Cat

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BAROCELLI, E., M. CHIAVARINI, M. IMPICCIATORE, M. MASSI AND G. DE CARO. *Inhibitory effect of intracranial injections of tachykinins on angiotensin-induced drinking in the cat.* PHARMACOL BIOCHEM BEHAV 31(2) 493–497, 1988.—The tachykinins eledoisin, substance P and kassinin were administered by pulse intracerebroventricular (ICV) injections to cats made thirsty by ICV angiotensin II, 100 ng per cat. Eledoisin, 100 ng per cat, produced an inhibition of drinking which was larger (56.0 vs. 45.2%) and lasted longer than that evoked by 400 ng per cat of substance P. Kassinin, 100 ng per cat, did not evoke any effect at all. The treatment with these peptides neither produced signs of discomfort nor induced any other behavioural alteration. The results of present experiments suggest that the antidipsogenic effect of tachykinins is a phenomenon of general interest among mammals.

Eledoisin Substance P Kassinin Cat Brain tachykinins Angiotensin II-induced drinking

THE tachykinins are a family of deca-, endeca- and dodecapeptide amides of natural origin sharing the common C-terminal sequence Phe-X-Gly-Leu-Met(NH_2).

They have a wide distribution and have been detected in different tissues of invertebrates, amphibians and mammals, including man. As far as mammals are concerned, these peptides have been detected also in the brain, and numerous data suggest that at least two of them play physiological roles acting as neurotransmitters (8,10).

The injection of tachykinins into the brain ventricles produces inhibition of water intake in rats (2) and rabbits (M. Perfumi, unpublished observation).

On the basis of several experimental data we hypothesized that, at least in rats, brain tachykinins play a physiological role and participate in the control of body fluid homeostasis (1). Thus, to evaluate whether the effect of tachykinins on water and electrolyte balance is a phenomenon of general interest among mammals, and in an attempt to obtain information on the role of brain tachykinins in the control of body fluid homeostasis, we considered it interesting to check whether these peptides affect drinking behaviour also in animals belonging to zoological orders different from those considered in our previous experiments. Since in those experiments the mammals that we had employed were herbivorous (rabbits) or omnivorous (rats), we decided to carry out present experiments on a carnivore and we choose the cat, on which we have studied the effect on drinking of pulse ICV injections of substance P, eledoisin and kassinin.

Substance P was chosen because it is the best known tachykinin of mammalian origin and is a normal component of mammalian brain. Moreover, very likely it acts in the brain as a neurotransmitter in sensory structures carrying nociceptive and visceral information as well, the latter

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originating from baroreceptors and chemoreceptors (6,8).

Eledoisin and kassinin are not of mammalian origin (5), however both have their counterpart in mammalian brain. In fact, eledoisin-like immunoreactive substances have been detected in rats' brain (11) and three kassinin-like peptides (neurokinin A, neurokinin B and neuropeptide K) have been isolated in the brain of rats and pigs (7, 9, 13). Eledoisin possesses the widest spectrum of antidipsogenic activity and elicits the most reliable effects (1), while kassinin has a more limited spectrum of effects on drinking behaviour and almost exclusively inhibits cell-dehydration drinking (3). Moreover, both peptides are more resistant to enzymatic hydrolysis (12), and influence drinking behaviour more effectively than the tachykinins of mammalian brain (4). Thus, eledoisin and kassinin may be considered important tools in the study of the effects and of the role of endogenous tachykinins of mammalian brain. For these reasons in the present study eledoisin and kassinin have been employed in addition to substance P.

METHOD

Animals

Eight cats of both sexes (5 males and 3 females) weighing 2.5 to 3.0 kg were employed. The animals, which had been supplied by Al Serio, Bergamo, were housed at room temperature in individual cages in which water was freely available and received food (minced meat, 66%, and cow milk, 33%, 300 g per cat) once a day at 10:00 a.m.

Implantation of ICV Cannulae

Under ketamine anesthesia (50 mg/kg, intraperitoneally) a stainless-steel indwelling cannula (o.d. 600 μm) was stereotaxically implanted into the brain and was secured to the skull by means of stainless-steel screws and dental cement. The following coordinates, experimentally determined in a pilot study, were employed: A=13 mm from the interaural line, L=3 mm from the sagittal suture and V=10 mm from the surface of the bone. In these experimental conditions the tip of the indwelling cannula protruded into the lateral brain ventricle. To prevent leakage of cerebrospinal fluid the cannula was occluded by an obturator.

Immediately after surgery each animal received an intramuscular injection of ampicillin, 35 mg per kg. The antibiotic treatment was repeated at intervals of 12 hr for seven consecutive days. The cats were allowed at least 10 days to recover before receiving the first treatment.

Intracranial Injection

The peptides were dissolved in sterile 0.9% NaCl solution and were injected into the brain ventricles through a stainless-steel injector temporarily inserted into the indwelling cannula and protruding 2 mm beyond the cannula tip.

The cats received into the ventricles a constant volume of 10 μl of either simple NaCl isotonic solution or of saline containing different concentrations of the peptides tested.

Food and Water Intake Stimulation

Food intake was induced by depriving the cats of food for 24 hr. Drinking was induced by means of pulse ICV injection of angiotensin II, 100 ng per cat.

Experimental Procedure

The experiments were carried out on water-replete cats which were always tested in the morning, at 11:00 o'clock.

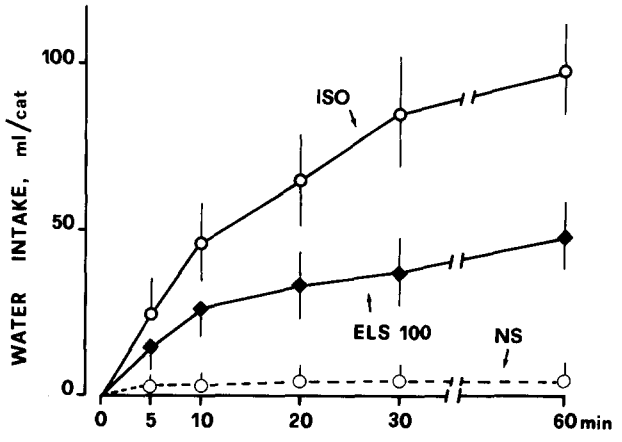

FIG. 1. Water intake (ml per cat) of angiotensin II-treated cats following ICV administration of 10 μl of simple isotonic NaCl solution (ISO) or of isotonic saline containing 100 ng of eledoisin (ELS100). Not stimulated (NS) cats received into the lateral brain ventricles 10 μl of isotonic NaCl solution but were not treated with angiotensin II. Vertical bars are standard errors.

At the beginning of the experiment the cats received an ICV pulse injection of tachykinins alone, of angiotensin II alone or of tachykinins followed 1 min later by an additional ICV injection of angiotensin II.

Immediately after the ICV administration of the peptides the cats had free access to water and food. The intake of water and of food was determined by weighing to the nearest g the containers of water and of food respectively 5, 10, 20, 30 and 60 min and 30 and 60 min after the treatment.

Each cat was tested in several experiments at intervals of at least 7 days. Each animal acted as its own control and the effect of the single tachykinins was evaluated on the basis of the drinking response obtained in the last control experiment (injection of angiotensin II alone or of simple isotonic NaCl solution) before the treatment with the peptides.

Substances

Angiotensin II, substance P and kassinin were supplied by Peninsula Laboratories Europe, Ltd., St. Helens, UK. Eledoisin was a generous gift of Farmitalia Research Laboratories, Milan, Italy, Ketamin (ketalar) was supplied by Parke-Davis Labs., Milan, Italy.

Statistical Analysis

All data are presented as means±SEM. Statistical analysis was performed by multifactorial analysis of variance (repeated measures). Planned pairwise comparisons were made by means of *t*-tests. Statistical significance was set at $p<0.05$.

RESULTS

The ICV administration of 10 μl of isotonic NaCl solution to seven water-replete cats elicited negligible drinking (2.75±2.1 ml of water per cat over a 60 min period of observation). In the same experimental conditions 5 hungry cats took 252.6±27.3 g of food in a period of 30 min following the

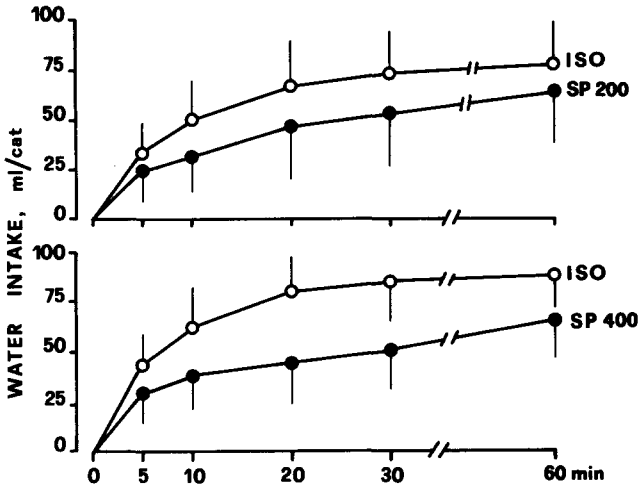

FIG. 2. Water intake (ml per cat) of angiotensin II-treated cats following ICV injection of 10 μl of simple isotonic NaCl solution (ISO) or of isotonic saline containing 200 (SP200) or 400 (SP400) ng of substance P. Vertical bars are standard errors.

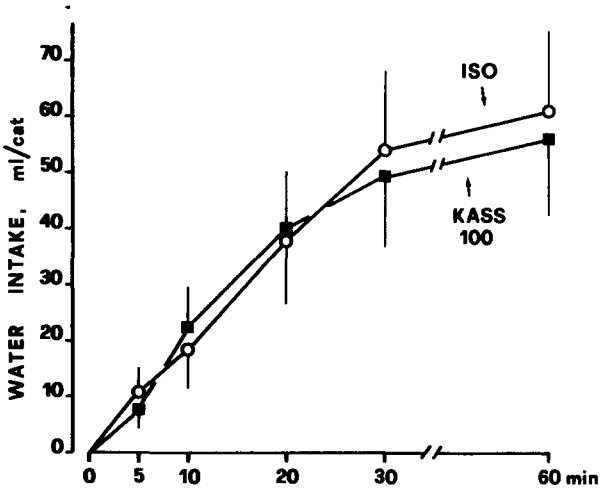

FIG. 3. Water intake (ml per cat) of angiotensin II-treated cats following ICV administration of 10 μl of simple isotonic NaCl solution or of this solution containing 100 ng of kassinin. Vertical bars are standard errors.

ICV injection; however, if food replete, they did not take any food at all.

The same volume of saline containing 100 ng of angiotensin II induced profuse drinking. The effect became statistically significant at 10 min and was generally complete in 30 min. However, in 2 of the 7 cats tested, some drinking was recorded up to 60 min after the treatment (Fig. 1).

The intake of water produced by angiotensin II was copious: in about 30 min the cats took much more water than they usually do over an entire day (85.00±17.2 versus 19.4±5.2 ml per cat).

The ICV injection of the single tachykinins, alone or followed by the ICV administration of angiotensin II, yielded the following results:

Eledoisin

ICV eledoisin, 100 ng per cat, did not produce any drinking at all in eight water replete animals. At a larger dose (1000 ng) the ICV injection of the peptide was followed by a negligible intake of water (1.0±0.76 ml per cat, over a 60 min period of observation), drinking having been observed only in 1 out of the 4 animals tested at this level of dose. In this cat latency to drinking was 9 min.

At the same dose of 100 ng per cat, eledoisin significantly inhibited the intake of water elicited by 100 ng of ICV angiotensin II (Fig. 1). Five min after the treatment the inhibition was about 30%. The inhibition increased afterwards, being 45 and 51% respectively at 10 and 20 min, reached the maximum (56%) 30 min after the injection of angiotensin II and decreased (51%) thereafter (Fig. 4). The inhibition became statistically significant 30 min after the treatment. We tested three cats with larger doses of eledoisin (200 and 400 ng per cat) and we observed that in two animals the effect increased with the dose.

While treated with eledoisin alone or eledoisin plus angiotensin II the cats appeared to be absolutely normal: they were neither excited nor depressed, vocalization or pilo-erection were not observed and the animals never appeared to be engaged in any other behaviour. Moreover, eledoisin did not suppress food intake of food-deprived cats. In fact, in five hungry rats, the intake of food recorded 30 min after the ICV injection of 100 ng of eledoisin was 243±19.9 g per cat. This intake was not statistically different from that observed in hungry cats which received into the brain ventricles simple isotonic saline solution (252.6±27.3 g per cat).

Substance P

The ICV injection to seven cats of 100 ng per cat of substance P did not produce any inhibition at all of drinking evoked by angiotensin II. Two hundred and 400 ng of the peptide inhibited drinking, but only the inhibition elicited by the largest dose of substance P was highly significant ($p<0.01$) (Fig. 2). At this level of dose, 5 and 10 min after the injection of angiotensin II the inhibition was about 33% and 40% respectively. The inhibition reached a maximum 20 min after the treatment (45.2%) and decreased thereafter (40.6 and 26.9% respectively 30 and 60 min after the injection of the dipsogen) (Fig. 4). The effect of 200 ng of substance P was statistically significant ($p<0.05$) only 10 and 20 min after the treatment, while that of 400 ng became significant 10 min after the treatment ($p<0.01$) and was still statistically significant ($p<0.05$) at the end of the experiment.

The ICV injection of substance P did not produce any disturbance: the animals appeared to be normal and never were engaged in any other behaviour, even at the maximum dose employed.

Kassinin

The intake of water recorded in 5 cats after ICV injection of angiotensin II plus kassinin, 100 ng per cat, was essentially identical to that observed after ICV angiotensin II alone (Fig. 3). Larger doses of kassinin (200, 400 and 800 ng) were tested in two cats, but none of them produced any inhibition of angiotensin-induced drinking.

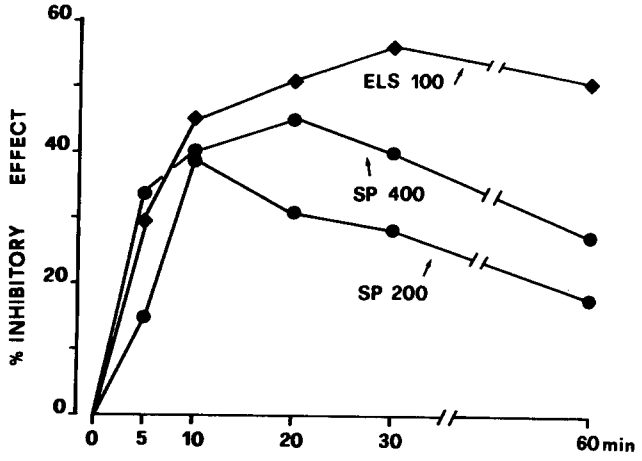

FIG. 4. Comparison of the percent antidipsogenic effect evoked by ICV injection of eledoisin 100 ng per cat (ELS100) or of substance P, 200 or 400 ng per cat (SP200 and SP400), in cats made thirsty by ICV administration of angiotensin II, 100 ng per cat.

DISCUSSION

The ICV administration of eledoisin and substance P, but not that of kassinin, inhibits the intake of water elicited in the cat by injection of angiotensin II into the lateral brain ventricles.

We have demonstrated that in rats made thirsty by ICV angiotensin II eledoisin elicits a potent antidipsogenic effect, the inhibition being practically complete after an ICV dose of 50 ng per rat (4). In present experiments the peptide evoked a clear-cut inhibition, but it seemed to be less effective than in rats. In fact, a dose which was two times as large as that which in rats produced an almost complete inhibition of drinking, in cats reduced only by 30–50%, at different times after the treatment, the intake of water. However, in evaluating the sensitivity of cats and rats to eledoisin, we have also to take into consideration the different size of their brains and the related problems of pharmacokinetics.

Due to the limited number of animals available, in present experiments it has not been possible to study the relationship between the dose and the antidipsogenic effect of eledoisin. However, in at least two of the three cats treated with doses of eledoisin larger than 100 ng the antidipsogenic response apparently increased with the dose.

Experiments carried out in rats demonstrated that in this animal species eledoisin is more effective than substance P in inhibiting the intake of water elicited by ICV angiotensin II. This is confirmed in present experiments, which demonstrated that in the cat eledoisin is an antidipsogen more effective than substance P, since 100 ng of the former peptide yielded a 56% inhibition versus a 45% inhibition elicited by 400 ng of substance P.

Present experiments have also shown that in the cat the antidipsogenic effect of eledoisin lasts longer than that of substance P. In fact, 10 and 20 min after the treatment, 100 ng of eledoisin and 400 ng of substance P produce practically the same inhibitions. However, in the following times the effect of substance P progressively decreases, while that of eledoisin increases, reaching a maximum 30 min after the treatment and remaining practically the same thereafter.

It has been reported that substance P is a substrate for angiotensin converting enzyme, while eledoisin is not (12,14). Thus, the different time courses of eldoisin and substance P inhibitions might be accounted for by the different sensitivity of the two peptides to the hydrolyzing effect of the brain angiotensin converting enzyme.

In the rat, kassinin has a rather selective antidipsogenic effect, since it potently inhibits cell-dehydration thirst but practically does not affect at all that evoked by angiotensin II (3). In present experiments 100 ng of kassinin did not influence at all the intake of water induced by ICV angiotensin II. We cannot state whether doses larger than 100 ng have any inhibitory effect, since these doses have been tested only on a negligible number of cats. However, the few data in our hands suggest that in these animals not even at doses as large as 800 ng does kassinin have any effect on angiotensin II-induced drinking. Thus, there is the possibility that not only in rats, but also in cats kassinin elicits a selective antidipsogenic effect.

In previous papers we stated that the inhibitory effect elicited by eledoisin and substance P on water intake is specific for this ingestive behaviour and it neither is the consequence of malaise nor is due or is related to other specific or aspecific behavioural alterations (4). In present experiments we observed that under eledoisin or substance P treatment the cats seem to be perfectly normal and are not engaged in other behaviours. Moreover, we observed that 100 ng of eledoisin, which produces an evident inhibition of water intake, does not inhibit at all the intake of food in hungry cats. The intake of food has been recorded only after eledoisin treatment, however, taken all together, these data seem to suggest that also in the cat the antidipsogenic effect of tachykinins is specific.

We hypothesized that, at least in the rat, brain tachykinins play a physiological role in the control of body fluid homeostasis, acting as thirst inhibitors (1,4). The results of present experiments clearly demonstrate that the antidipsogenic effect of tachykinins is a phenomenon of general interest, since it involves not only rats and rabbits, but also cats, that is, animals belonging to completely different zoological orders.

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