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Dipsogenic Effect of Angiotensin II, Bombesin and Tachykinins in the Duck

G. DE CARO,[1] M. MARIOTTI, M. MASSI AND L. G. MICOSSI

Institute of Pharmacology, Faculty of Pharmacy, University of Camerino—62032 Camerino, Italy

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DE CARO, G., M. MARIOTTI, M. MASSI AND L. G. MICOSSI. *Dipsogenic effect of angiotensin II, bombesin and tachykinins in the duck*. PHARMAC. BIOCHEM. BEHAV. 13(2) 229–233, 1980.—The effect on drinking behaviour of intracerebroventricular injections of angiotensin II, bombesin, eledoisin and substance P was studied in the duck. While substance P was almost completely ineffective, angiotensin II, bombesin and eledoisin elicited a clear dipsogenic response which was dose-dependent and apparently specific. Angiotensin II was about 10 times more potent than bombesin and far more potent than eledoisin. These results confirm once more the wide phylogenetic distribution of the dipsogenic response to angiotensin II. Furthermore, they show that bombesin and eledoisin, which potently inhibit water intake in the rat, exert in the duck a dipsogenic effect strictly parallel to that elicited in the pigeon. On the basis of the animal species so far tested it is possible to hypothesize that bombesin and tachykinins stimulate water intake in birds, while inhibiting drinking in mammals.

Angiotensin II Bombesin Eledoisin Substance P Water intake Duck

AFTER the first pioneering studies by Fitzsimons *et al.* [10,11], a large body of evidence has been collected suggesting an important role for angiotensin II in the regulation of water intake. This peptide, in fact, proves to be a very powerful dipsogen in all the animal species so far tested including mammals, birds and reptiles [12].

Recently, also bombesin (De Caro, Massi, Micossi, to be published) and the tachykinins substance P, eledoisin and physalaemin [3, 4, 5, 6, 9] have been reported to affect drinking behaviour in rats and pigeons. But while angiotensin II proves to be dipsogenic in these animal species, as well as in all the others studied up to now, the effect of tachykinins and bombesin on drinking behaviour is different in that they stimulate drinking in the pigeon, but inhibit water intake in the rat.

As a working hypothesis, it seems possible to think that the response to these peptides may be different in mammals (rat) and in birds (pigeon) because of the neuroanatomical and neurochemical differences in their central nervous system.

Since more animal species, both mammals and birds, should be tested before checking the validity of this hypothesis, we investigated the effect of these peptides also in the duck. The results of these experiments are reported in the present paper.

METHOD

Thirty ducks (*Anas plathyrinchos plathyrinchos*, Peking strain) of both sexes weighing between 2000 and 2400 g were obtained from domestic suppliers. The animals were housed in groups of 3 or 4 animals and kept in a well illuminated room in which temperature ranged between 20 and 22°C. They were maintained on lettuce, bird-seeds (PAPP, Martini, Forlì) and poultry feed (Cristalli, Castelraimondo, MC). None of the birds was used for experiments until they were consistently accustomed to being handled.

Implantation of ICV Cannulae

The head of the animal was placed in a David Kopf stereotaxic apparatus for rats and the anterior part of the beak was gently fixed to the base of the instrument. The skull was exposed and the bone trephined to expose the sagittal sinus and the confluence between the transverse and the sagittal sinuses.

Stainless-steel cannulae (o.d. 0.6 mm, length 9 mm) were stereotaxically implanted into the brain of the animals under equithesin anaesthesia (3 ml/kg b.w.) and fixed to the skull according to the technique described by Epstein *et al.* for the rat [7].

The following coordinates, histologically determined in a previous study, were employed: A=10.5 mm from the confluence between the sagittal and the transverse venous sinuses, L=0 mm from the sagittal sinus and V=5 mm from the dura. The tip of the cannula remained 1 mm above the third ventricle. Immediately after the operation each animal received an intramuscular injection of penicillin G, 200,000 units.

The ducks were allowed at least ten days to recover from surgery before being tested.

[1]Send reprint requests to Professor Giuseppe de Caro, Institute of Pharmacology, University of Camerino, Via Scalzino 5, 62032 Camerino, Italy.

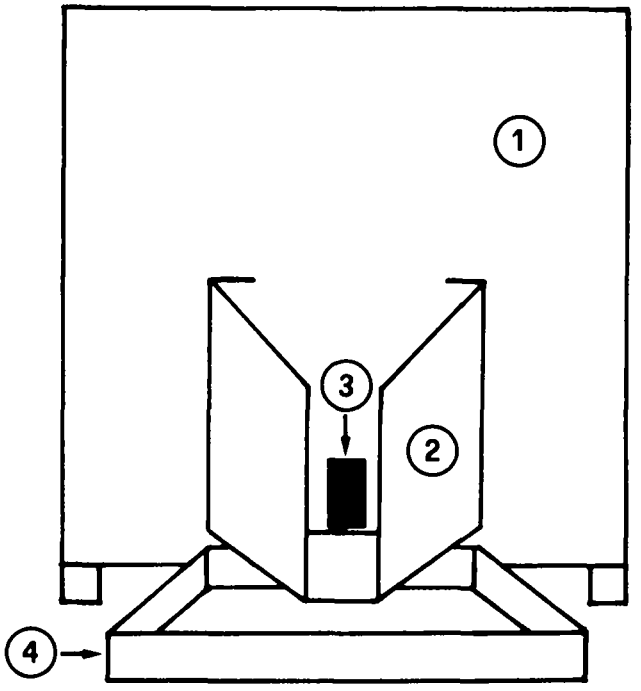

FIG. 1. The drinking apparatus: (1) cage, (2) funnel-like device, (3) window, (4) water container.

Intracranial Injections

The drugs were dissolved in sterile 0.9% NaCl solution and injected into the third ventricle through a stainless-steel injector temporarily inserted into the guide cannula and projecting 2 mm beyond the cannula tip.

The drugs were administered in a constant volume of 1 μl. A larger volume (5 μl) was employed to inject the highest doses of the drugs tested (3000–4000 picomoles). Accordingly, controls received 1 or 5 μl of sterile 0.9% NaCl solution. Thirty minutes before each injection the animal to be tested was left alone in its home cage by removing the other ducks of the group.

The animals received only one drug per experiment and they were not tested more than twice per week. The order of testing was random. At the end of the experiments the animals were sacrificed and their brains sectioned to verify the position of the guide cannula.

The Drinking Apparatus

Ducks drink in a chicken-like fashion by immersing their beak in the water for a few seconds and then raising the head to allow water to pass down into the oesophagus. When the animals raise their head from the water container, usually some water leaks out of their beak and falls onto the floor.

In order to get an accurate determination of the amount of water ingested, it is necessary to collect the water lost during drinking. Therefore, the animals were accustomed to taking water from a suitable drinking apparatus (Fig. 1). Water was provided in a plastic container placed approximately 15 cm outside the box. The ducks had access to water through a small window (6×10 cm) open in a lateral wall of the box about 12 cm from the floor of the cage. A funnel-like device, joining the window to the water container, was adopted to collect the water lost by the ducks in the drinking bouts and to drive it back into the container.

In order to reach water the animals were required to bend their necks forward through the window open in the cage wall. Owing to the low and narrow window and to the remote position of water, treated birds, which showed a strongly motivated drinking behaviour, completed their drinking bouts in close succession outside the box, that is in a position in which the funnel-like device was able to collect all the water lost. In this way only negligible amounts of water fell out of the drinking apparatus. However, it must be pointed out that the determination of water intake of controls animals could not be sufficiently accurate, since they used to interrupt drinking bouts very frequently and to move inside the box, losing amounts of water out of the drinking apparatus.

Water Intake Determination

Immediately after the injection, the ducks were returned to their home boxes where they had free access to food and to water. Water intake was determined at 15 min intervals for a 30 min period by weighing the water container to the nearest 1 g, after removing it from the drinking apparatus. The results were expressed as cumulative water intake in 30 min after drug administration.

Substances

The following substances were employed: synthetic eledoisin and bombesin (prepared at the Farmitalia Laboratories, Milan), bovine substance P (Beckmann Instruments) and angiotensin II (synthetic 5-isoleucin angiotensin II, Calbiochem). Their structure is reported in Table 1.

RESULTS

Since water intake of controls which received 1 μl of sterile saline was not significantly different from that of controls receiving 5 μl of the same solution, data obtained in the two groups were pooled. The mean water intake of controls in thirty minutes after the injection of saline was 0.48 ± 0.1 ml per 100 g b.w.

Angiotensin II, injected by intracerebroventricular route, elicited a rapid and potent dipsogenic response, which was

TABLE 1

AMINO ACID SEQUENCES OF THE PEPTIDES TESTED

Peptide	Structure
Bombesin	PYR-GLN-ARG-LEU-GLY-ASN-GLN-TRP-ALA-VAL-GLY-HIS-LEU-MET NH_2
Eledoisin	PYR-PRO-SER-LYS-ASP-ALA-PHE-ILE-GLY-LEU-MET NH_2
Substance P	ARG-PRO-LYS-PRO-GLN-GLN-PHE-PHE-GLY-LEU-MET NH_2
Angiotensin II	ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE OH

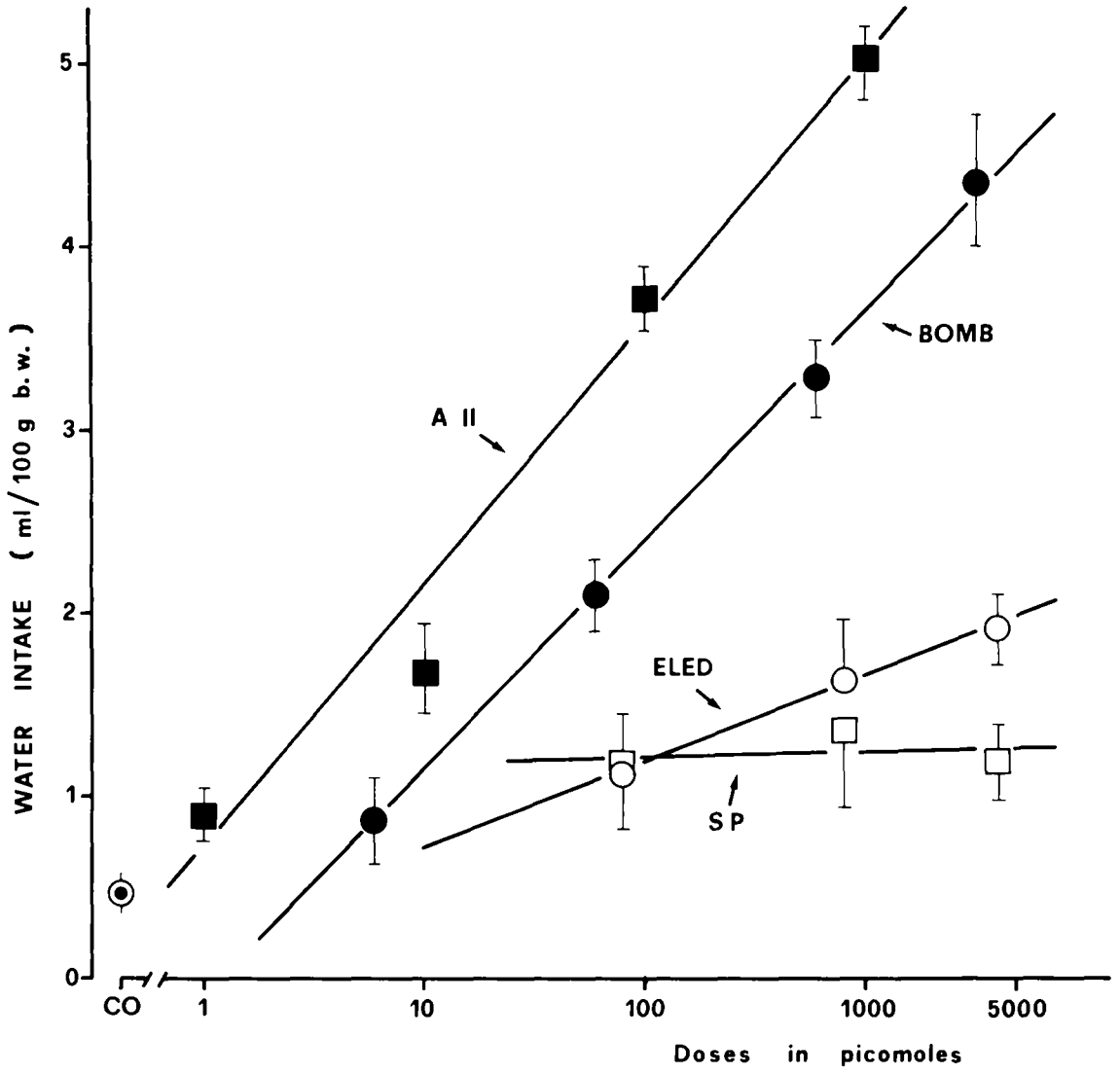

FIG. 2. Dose-drinking relationships following ICV administration of angiotensin II (-■-), bombesin (-●-), eledoisin (-○-) and substance P (-□-) in the duck. Water intake was determined 30 min after drug administration. Each point is the mean of 7-14 data. Vertical lines are SE of the mean.

dose-dependent for the range of doses tested (1-1000 picomoles per bird). Latencies to the onset of drinking were very short, ranging between 30 and 300 sec, the mean latency in 14 ducks in response to 100 picomoles of angiotensin II being 136.8 ± 22.2 sec. Most drinking usually occurred only during the first 15 min after drug administration, however a few drinking bouts were observed up to 30 min following ICV injection. The amount of water taken by animals in response to 1, 10, 100 and 1000 picomoles of angiotensin II was 0.9 ± 0.15, 1.7 ± 0.25, 3.74 ± 0.18 and 5.0 ± 0.20 ml of water per 100 g b.w., in 30 min after injection.

Water intake of angiotensin treated animals was significantly different from that of controls even at the dose of 1 picomole ($p<0.05$).

The response was repeatable from one experiment to another. Drinking was absolutely normal: after injection, as soon as the animals were returned to their home cages, they rapidly moved towards the water container and drank vigorously without showing any sign of excitation or of discomfort. During the experiments the animals never ate nor engaged in any other behaviour.

Water intake after 100 and 1000 picomoles of angiotensin II was very large, since we observed that during an entire day untreated ducks usually drank about 17 ml of water per 100 g b.w. Moreover, it must be pointed out that this datum is really too large since, as stated above, the determination of water intake in unstimulated ducks could not be sufficiently accurate.

Bombesin, as well as angiotensin II, proved to be a clear dipsogen in the duck at ICV doses ranging between 6 and 3000 picomoles per bird. In this dose interval the dipsogenic response was dose-dependent. The amount of water taken by treated animals in response to 6, 60, 600 and 3000 picomoles of bombesin per duck was 0.88 ± 0.24, 2.1 ± 0.20, 3.29 ± 0.22 and 4.36 ± 0.39 ml per 100 g b.w. in 30 min after drug administration. The dose of 6 picomoles induced a water intake which was not statistically different from that of control animals; at the dose of 60 picomoles

drinking became highly significant ($p<0.01$). The time course (latency and duration) of the effect of the two substances was almost identical. Furthermore the same drinking behaviour described after angiotensin II was observed also in response to centrally administered bombesin.

Even the tachykinins tested, eledoisin and substance P, proved to stimulate drinking. Eledoisin at doses of 80, 800 and 4000 picomoles per duck elicited water intakes of 1.13 ± 0.32, 1.63 ± 0.34 and 1.92 ± 0.3 ml per 100 g b.w. respectively. Water intake was statistically significant only at 800 and 4000 picomoles ($p<0.01$). At the same doses substance P elicited water intakes of 1.16 ± 0.32, 1.36 ± 0.45 and 1.18 ± 0.30 ml per 100 g b.w. Drinking induced by substance P was poorly related to the dose (Fig. 2), but water taken by treated ducks was always statistically different from that taken by controls ($p<0.05$). As observed for the other peptides, the dipsogenic response induced by eledoisin and substance P was rapid, the latencies to the onset of drinking ranging between 30 and 120 sec after 800 picomoles of the drugs. Water intake usually lasted up to 15 min following drug administration. No other behavioural alteration nor signs of disturbance were ever observed after the injection of eledoisin or substance P.

In order to compare the dipsogenic potencies of bombesin, eledoisin and substance P to that of angiotensin II, the regressions of the amount of water drunk versus the amount of peptide injected were calculated. The regression lines are reported in Fig. 2; they demonstrate that angiotensin II was the most powerful dipsogenic peptide in the duck, bombesin being approximately 10 times and eledoisin far less effective than angiotensin itself. Substance P was, at least in the range of the doses employed, almost devoid of any activity.

The regression line relative to bombesin proved to be parallel to that of angiotensin II: in fact the deviation from parallelism $F(1,71)=1.132$ was statistically non significant. On the contrary the regression lines relative to the dipsogenic effect of eledoisin and substance P were clearly non parallel to that of angiotensin II.

DISCUSSION

The results of our experiments confirmed, as expected, that angiotensin II potently stimulates water intake even in the duck. Moreover, they demonstrated that bombesin, eledoisin and, to a lesser extent, substance P elicit in ducks a dipsogenic effect similar to that observed in the pigeon.

The effect of these peptides is apparently specific since no other behavioural alteration was ever observed after their administration. The animals in fact were not depressed nor excited by the peptides injected, they never showed panting or vocalization, they did not flap their wings nor show any other behavioural alteration. The specificity of the effect is also supported by the fact that water intake, but not food intake, was stimulated.

As stated above, the regression lines of angiotensin II and bombesin were parallel. This observation, taken together with the fact that the dipsogenic effect of the two substances showed a similar time-course, apparently suggests the possibility that angiotensin II and bombesin may act with similar mechanisms. On the other hand the lack of parallelism between the regression lines of angiotensin II and those of eledoisin and substance P apparently suggests that different mechanisms are involved in their dipsogenic effect.

According to Nicolaïdis and co-workers [15,16] the dipsogenic effect of angiotensin II might be due to the ischaemic action the peptide elicits on the vascular periventricular organs. It seems unlikely that bombesin and the tachykinins affect drinking behaviour in the duck by producing vasoconstriction in the brain vessels, since the former is a poor vascular drug [1,8] and the latter produce vasodilation in all the animal species and in all the vascular beds studied up to now [1, 2, 14]. However we have no data on the effects of these peptides on the cerebral vessels of the duck: thus, we cannot suggest any interpretation of the mechanism(s) of their dipsogenic effect.

For other potent dipsogens it has been suggested that they may act by inducing profuse urine elimination and thus osmolar modifications [13]. This is not the case with our substances, since after their injection latency to drinking was too short and episodes of aqueous feces elimination were apparently not more numerous than in controls.

The results obtained in the duck, to some extent, parallel those previously obtained in the pigeon ([9]; De Caro, Massi, Micossi, to be published), in that all the peptides tested proved to be dipsogenic in the former, as well as in the latter. Moreover, the sensitivity of both animal species to the dipsogenic action of angiotensin II and bombesin was similar, and the ratio of dipsogenic potency between these substances was in the duck almost identical to that observed in the pigeon. The only relevant difference was the response to tachykinins, the duck being less sensitive than the pigeon to them.

In conclusion, our data, while confirming the extremely large phylogenetic distribution of the dipsogenic response to angiotensin II, seem to confirm that bombesin and tachykinins may act as drinking inducers in birds, while acting as drinking inhibitors in mammals. However several other animal species should be tested to have definitive evidence in favour of this hypothesis.

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