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Dipsogenic Action of Brain Natriuretic Peptide and Endothelin-1 in the Japanese Quail, *Coturnix coturnix japonica*

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ABSTRACT—Porcine brain natriuretic peptide (pBNP) elicited a significant increase in water intake, when administered intraperitoneally (5 and 10 μg/bird) or intracerebroventricularly (0.3 μg/bird), in the water-replete Japanese quail, *Coturnix coturnix japonica*. Intraperitoneal injection of endothelin-1 (ET-1; 0.3, 1 and 3 μg/bird) did not affect water intake, but intracerebroventricular administration of ET-1 (10 ng/bird) slightly enhanced water intake in the water-replete Japanese quail. The possible involvement of these peptides in thirst mechanisms is discussed.

INTRODUCTION

Porcine brain natriuretic peptide (pBNP) is composed of 26 amino acid residues [1, 2], and exhibits high sequence homology to α-human atrial natriuretic peptide (α-hANP). Injections of pBNP have natriuretic, diuretic and hypotensive actions similar to those induced by α-hANP in rats [1]. Furthermore, intracerebroventricular (i.c.v.) injections of pBNP [3] or rat BNP (rBNP) [4, 5] suppress the water intake that is stimulated by angiotensin II (ANG II) or dehydration in rats, as seen also with i.c.v. injections of α-rANP (5–28) [6, 7], α-rANP [7], and α-hANP [7–9]. However, pBNP [3] and rBNP [5] appear not to suppress water intake in water-replete rats. In the water-replete Japanese quail, by contrast, both intraperitoneal (i.p.) and i.c.v. injections of α-hANP stimulated water intake [10]. One of the purposes of this study was to examine whether or not pBNP is antidipsogenic or dipsogenic in the Japanese quail.

Endothelin (ET) is a potent pressor/vasoconstrictor peptide, and there are at least three isoforms of this peptide, ET-1, ET-2 and ET-3, in mammals [11, 12]. Samson *et al.* [13] reported that ET-3 administered into the third ventricle inhibited drinking that was stimulated by dehydration, hyperosmotic challenge or ANG II in the rat. A second purpose of this study was to test whether or not ET is involved in thirst mechanisms in the water-replete Japanese quail.

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MATERIALS AND METHODS

Male Japanese quail, *Coturnix coturnix japonica* (8 weeks old) were purchased from a commercial source. They were housed in a room maintained at 21–25°C under a 12L photoperiod (07:00–19:00 h). Birds were kept individually in bird cages [30 (D)×15.5 (W)×22 (H) cm^3] and screened one from another by pieces of cardboard inserted between the cages. Water and food were available *ad libitum* before and during the experimental period. Food consisted of crushed corn, kaoliang, wheat and crushed dry fish meat (Showasangyo Co., Ibaragi). The average body weight was 100.4 g, ranging from 84 to 121 g, at 20 weeks after hatching.

Synthetic porcine BNP, ANG II (Val^5-ANG II) and ET-1 were obtained from the Peptide Institute, Inc., Osaka. Each was dissolved in saline solution, and injections were given between 12:00 and 13:30 h. During the observation period (10:

00–17:00 h), the drinking rate was usually almost constant in the Japanese quail [14].

Measurement of water intake

Birds were trained to drink water from a small hole at the end of a glass tube attached to an up-ended 20-ml cylinder for about 2 weeks before the first injection. The amount of water ingested was estimated by reading the scale on the cylinder to the nearest 0.1 ml. Measurements of water intake started from 2 hr before injections, and were recorded every 30 min.

I.c.v. injection

Birds were anesthetized with Nembutal, and a stainless-steel guide cannula (o.d., 0.7 mm; length, 13 mm) was implanted stereotaxically into the brain with the aid of X-rays, with the tip being guided until it was located in the third ventricle. The cannula was fixed securely to the skull with dental resin and two anchoring screws. The details of the cannulation technique have been described in an earlier paper [15]. The implanted cannula was closed off with nylon thread when it was not in use to prevent blood coagulation in the cannula. Birds were allowed to recover from the operation for at least one week before the i.c.v. injections. Injections were performed with a stainless-steel injector (o.d., 0.4 mm), which was 1 mm longer than the guide cannula. The injector was connected to a 10-μl microsyringe with a 10-cm piece of polyethylene tubing (o.d., 0.4 mm). To verify that the tip of the guide cannula had been successfully located in the third ventricle, ANG II (30 or 50 ng/bird) was injected into the brain through the cannula, and the dipsogenic response of the birds was verified. Only those birds that responded to ANG II by drinking, as shown in a previous paper [10], were used for subsequent experiments.

Experiment I: i.p. injection of pBNP and water intake

Thirty-seven birds (10–19 weeks old) received a single i.p. injection of pBNP at a dose of 0 (saline, n=11), 1 (n=9), 5 (n=8), 10 (n=6) or 25 μg/bird (n=3). The injection volume was 0.1 ml/bird. Water intake after the injection was measured at intervals of 30 min for 2 hr. Dose-response curve was depicted from the data obtained 30 min after the injection.

Experiment II: i.c.v. injection of pBNP and water intake

Seven birds (24–27 weeks old) were given a single i.c.v. injection of pBNP at a dose of 0 (saline), 0.03, 0.1 and 0.3 μg/bird. Each bird received each dose once on different days in random order. Injection volume was 1 μl/bird. Water intake after the injection was measured at intervals of 30 min for 2 hr.

Experiment III: i.p. injection of ET-1 and water intake

Thirty-four birds (10–19 weeks old) received a single i.p. injection of ET-1 at a dose of 0 (saline, n=9), 0.3 (n=7), 1 (n=8), 3 (n=7) or 10 μg/bird (n=3). The injection volume was 0.1 ml/bird. Water intake after the injection was measured at intervals of 30 min for 4 hr.

Experiment IV: i.c.v. injection of ET-1 and water intake

Seven birds (25–28 weeks old) were given a single i.c.v. injection of ET-1 at a dose of 0 (saline), 10 and 30 ng/bird. Birds were injected in random order, each bird receiving each dose once. Injection volume was 1 μl/bird. Water intake after the injection was measured at intervals of 30 min for 3 hr.

Statistical analysis

Data from Experiments I and III were analyzed by the Kruskal-Wallis test. When differences were significant, Mann-Whitney's *U*-test was also employed. Data from Experiment II were analyzed by Friedman's test. When differences were significant, Wilcoxon's test was also employed. Wilcoxon's test was also employed for data from Experiment IV.

RESULTS

Experiment I: i.p. injection of pBNP and water intake

Water intake for 30 min after an injection of

pBNP (1 to 10 μg/bird) increased in a dose-dependent manner ($Y=0.220X+0.147$, $P<0.001$; Fig. 1). Copious water intake was induced by a single i.p. injection of pBNP (5 and 10 μg/bird), starting at 12.4±3.3 min (5 μg/bird) and 15.8±2.7 min (10 μg/bird) after the injection. The effect of pBNP on water intake persisted for 60 min (5 μg/bird) and 90 min (10 μg/bird) (Fig. 2). No behavioral changes were observed after the injection of pBNP at any dosage used, except when the dose was 25 μg/bird (n=3). Since behavioral depression was observed at this dosage, the data of water intake of these birds were excluded.

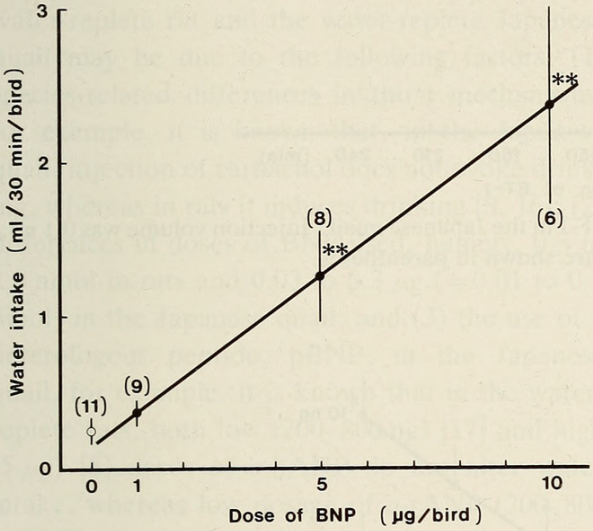

FIG. 1. Dose-response curve for the effects of single i.p. injections of porcine brain natriuretic peptide (pBNP) on water intake over a 30-min period in the Japanese quail. Each point shows the mean with SE. Numbers of birds are shown in parentheses. ○, Saline control; ●, experimental. ** $P<0.01$ compared with saline control.

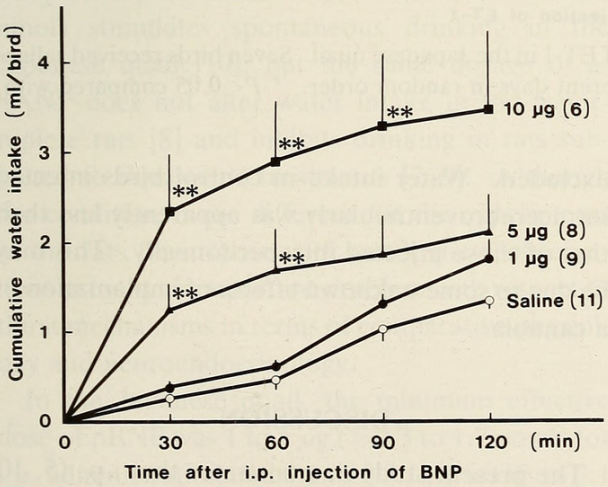

FIG. 2. Cumulative water intake after i.p. injection of pBNP in the Japanese quail. Injection volume was 0.1 ml. Each point shows the mean with SE. Numbers of birds are shown in parentheses. ** $P<0.01$ compared with saline control.

Experiment II: i.c.v. injection of pBNP and water intake

I.c.v. administration of pBNP (0.3 μg/bird) stimulated water intake (Fig. 3), and the latency was 9.7±2.2 min (n=7). Significant increases in cumulative water intake were observed 60 ($P<0.05$) and 90 ($P<0.05$) min after injection. I.c.v. injections of 0.03 and 0.1 μg pBNP had no effect on drinking for 120 min (Fig. 3). No overt changes were observed in the behavior of birds injected at any dosage. Water intake in control birds injected intracerebroventricularly appeared to be less than that of those injected intraperitoneally. This may be due to some unknown effects of implantation of a cannula.

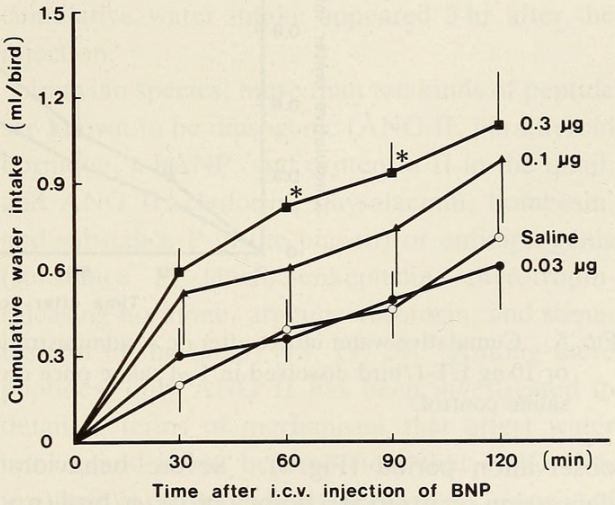

FIG. 3. Cumulative water intake after i.c.v. injection of pBNP in the Japanese quail. For each dosage, seven birds were injected with the peptide dissolved in 1 μl of saline once on different days in random order. * $P<0.05$ compared with saline control.

Experiment III: i.p. injection of ET-1 and water intake

I.p. administration of ET-1 (0.3, 1, 3 μg/bird) did not significantly affect water intake, as compared with saline controls, throughout the

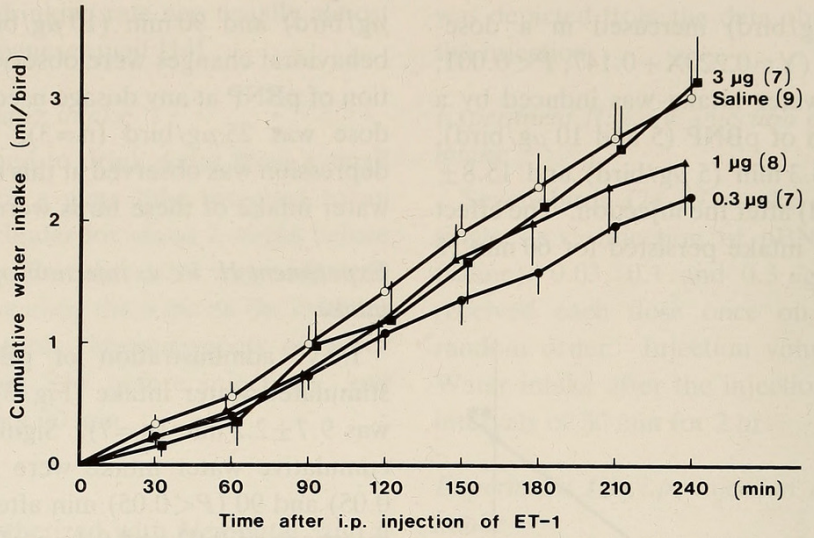

FIG. 4. Cumulative water intake after i.p. administration of ET-1 in the Japanese quail. Injection volume was 0.1 ml. Each point shows the mean with SE. Numbers of birds are shown in parentheses.

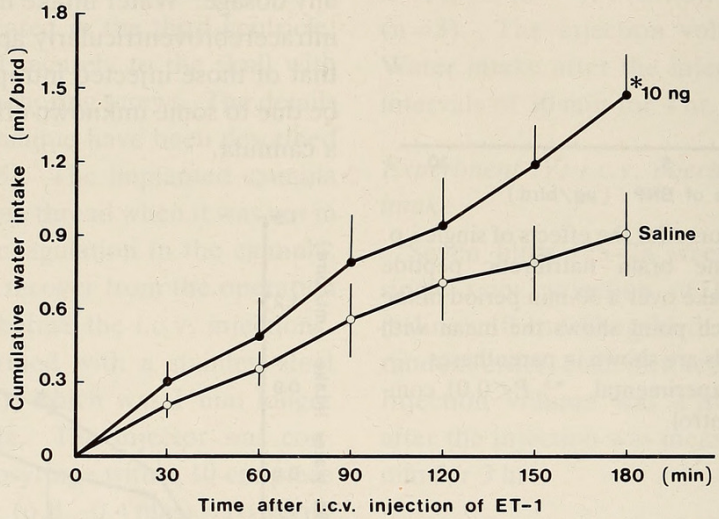

FIG. 5. Cumulative water intake after i.c.v. administration of ET-1 in the Japanese quail. Seven birds received saline or 10 ng ET-1/bird dissolved in 1 μl saline once on different days in random order. * $P<0.05$ compared with saline control.

observation period (Fig. 4). Severe behavioral depression occurred at a dosage of 10 μg/bird (n=3). Therefore the data of water intake of these birds were excluded.

Experiment IV: i.c.v. injection of ET-1 and water intake

A significant increase in water intake was observed 3 hr after a single i.c.v. injection of ET-1 (10 ng/bird, $P<0.05$) (Fig. 5). No behavioral changes were observed at this dosage. At a dosage of 30 ng (n=3), however, depressed behavior was observed and hence the data of water intake were excluded. Water intake in control birds injected intracerebroventricularly was apparently less than that of those injected intraperitoneally. This may be due to some unknown effects of implantation of a cannula.

DISCUSSION

The present study demonstrates that i.p. (5, 10 μg/bird) and i.c.v. (0.3 μg/bird) injections of pBNP significantly increase water intake in the water-replete Japanese quail. These results suggest that i.p. pBNP may reach the receptive site for

BNP in the brain and that pBNP or pBNP-like peptide may act as a dipsogen in the Japanese quail. In rats, by contrast, i.c.v. administration of pBNP [3] or rBNP [5] had no effect on water intake in water-replete rats, although these peptides prevented any increase in water intake as a result of dipsogenic treatments, such as injecion of ANG II [3, 4] or water deprivation [4, 5]. The difference in the response to BNP between the water-replete rat and the water-replete Japanese quail may be due to the following factors: (1) species-related differences in thirst mechanisms, for example, it is known that, in the Japanese quail, injection of carbachol does not evoke drinking, whereas in rats it induces drinking [8, 16]; (2) differences in doses of BNP used, namely, 0.3 to 2.0 nmol in rats and 0.03 to 0.3 μg ($\fallingdotseq$0.01 to 0.1 nmol) in the Japanese quail; and (3) the use of a heterologous peptide, pBNP, in the Japanese quail, for example, it is known that in the water-replete rats, both low (200–800 ng) [17] and high (5 μg) [8] doses of α-hANP do not alter water intake, whereas low dosage of α-rANP (200–800 ng) [17] stimulates drinking in water-replete rats.

Discrepancies between the effects of some neuropeptides on water intake in birds and mammals have also been reported: physalaemine, eledoisin and bombesin stimulate spontaneous drinking in the pigeon and the duck, but they inhibit drinking induced by dipsogenic treatments in the rat [18, 19]. Furthermore, α-hANP (about 1.5 nmol) stimulates spontaneous drinking in the Japanese quail [10], but the same dosage of α-hANP does not alter water intake in the water-replete rats [8] and inhibits drinking in rats subjected to dipsogenic treatments [7–9]. Although the conditions were different for the experiments in the rats from those with quail, these observations present an important problem with respect to thirst mechanisms in terms of comparative physiology and neuroendocrinology.

In the Japanese quail, the minimum effective dose of pBNP was 1 to 5 μg ($\fallingdotseq$0.3 to 1.7 nmol) for i.p. administration and 0.1 to 0.3 μg ($\fallingdotseq$0.03 to 0.1 nmol) for i.c.v. administration. Okawara *et al.* [10] reported that α-hANP, with a structure similar to BNP, stimulated water intake at the dose of 1 nmol i.p. and at the dose of 0.03 nmol i.c.v.. These results indicate that the dipsogenic effect of pBNP is almost similar to that of an equimolar dose of α-hANP.

Samson *et al.* [13] reported that i.c.v. administration of ET-3 (30 to 60 ng) inhibited water intake in rats subjected to dipsogenic treatments, such as injection of ANG II, hyperosmotic challenge and dehydration. These effects appeared within 15 min after the injection. There were no behavioral changes after the injection (30 to 60 ng ET-3). In the present study, however, i.c.v. injection of 10 ng ET-1 had a dipsogenic effect 3 hr later in water-replete Japanese quail. These differences might be explained by the differences in species and in the peptides used, namely, ET-1 in the quail and ET-3 in the rat. Furthermore, i.p. administration of ET-1, even at 3 μg, had no effect on water intake, while i.c.v. injection had a stimulatory effect at 10 ng of ET-1. These observations suggest that, in the Japanese quail, ET may be degraded rapidly in the blood and, furthermore, that ET injected into the brain may secondarily stimulate water intake, since an increase in cumulative water intake appeared 3 hr after the injection.

In avian species, more than ten kinds of peptide are known to be dipsogenic (ANG II, parathyroid hormone, α-hANP, and urotensin II in the quail, and ANG II, eledoisin, physalaemin, bombesin, and substance P in the pigeon) or antidipsogenic (substance P, leucine-enkephalin, thyrotropin-releasing hormone, arginine vasotocin, and somatostatin in the quail) [18, 19, 20]. Among these peptides, only ANG II has been investigated in detail in terms of mechanisms that affect water intake, and it has become clear that ANG II is involved in physiological mechanisms associated with drinking in birds and mammals [16]. In the present studies, pBNP and ET-1 were dipsogenic in the Japanese quail. However, it is not known whether they are physiologically involved in thirst mechanisms. Further investigations are needed to clarify the problem not only with regard to pBNP and ET-1, but also with regard to the other peptides mentioned above.

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