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The Drinking Response of the Chicken to Peripheral and Central Administration of Angiotensin II

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SNAPIR, N., B. ROBINZON AND M. GODSCHALK. *The drinking response of the chicken to peripheral and central administration of angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 5(1) 5–10, 1976. – Intravenous injection of Ang II (val^5 angiotensin II amide) elicited an immediate drinking response in the domestic fowl which lasted at least 20 minutes. The minimal dosage needed was 300 μg. Intracranial injection of 10 μg Ang II through cannulas implanted in the anterior diencephalon caused a significant increase in water intake. The minimal intracranial dosage of Ang II which evoked drinking was 2.5 μg. Intracranial injection of isotonic KCl inhibited the drinking response induced by intravenously injected Ang II when administered simultaneously. This suggests that drinking caused by both intravenous or intracranial injection of Ang II is activated through identical brain regions. The positive drinking response of the chicken to repeated consecutive intracranial injections of Ang II declined from the first injection through the following ones.

Angiotensin II Chicken Drinking response

ADIPSIA has been produced in the domestic fowl by placing bilateral electrolytic lesions in the area located dorsolateral to the hypothalamic ventromedial nuclei (VMH) [18,19]. Bilateral electrolytic lesions located below the VMH and mammillary nuclei, just above the optic chiasma elicited a state of polyuria-polydipsia [17]. In the pigeon, polydipsia could be produced by lesions in the preoptic region which frequently impinged on the borders of the third ventricle [34], or by electrical stimulation of the anterior hypothalamus-preoptic region [1].

Intracranial injections of carbachol, particularly into the hypothalamus, failed to induce drinking responses in the dove [21] and in the domestic fowl (authors' unpublished data). Except in the rat [14,15] and in the rabbit [32], no drinking response could be produced by intracranial cholinergic stimulation in other species of mammals tested [20]. In contrast, intracranial stimulation with Angiotensin II (Ang II) elicited drinking responses in a much wider variety of mammals as reviewed by Fitzsimons [10, 11, 12, 13].

Although a drinking response to intracranial injection of Ang II, has, in fact, been noted in the pigeon [13], the information gathered about this system in avian species is scanty. It was the purpose of the present investigation to secure more information on the effect of Ang II on the domestic fowl, when administered both intravenously and intracranially.

METHOD

Animals

Four-month-old White Leghorn cocks were used for the experiments. The birds, kept in individual cages, were fed and watered ad lib. Commercial breeder mash was used as the standard diet. The water was available to the birds through individual automatic drinkers connected with plastic tubes to graduated cylinders. The birds were subjected to 14 hours light daily.

Surgery

Some of the birds were implanted with a double cannula constructed according to Grossman's design [15]. The birds were anesthetized by intravenous (IV) injection of 0.6 ml sodium pentobarbital (Sombital 6% diluted with 10% Ethanol, 20% Propylene Glycol, and 70% bidistilled water, Rafa, Israel) and held in a specially designed stereotaxic instrument, as described previously [8]. The procedure and technique described in the x-ray atlas of the chicken diencephalon [29] was routinely used for implanting the cannulas.

Procedure

Ang II (val^5 angiotensin II amide) dissolved in 0.9%

NaCl was used in the present experiments. Daily water intake was recorded 30 days post surgery, before starting any of the experimental procedures. At the end of each experiment the cannulated birds were killed by decapitation, their brains were immediately removed and fixed in 10% neutral buffered formalin. After embedding in gelatin, frozen frontal serial sections of 25 μm thickness each, were prepared and stained with thionin. The sections were subjected to microscopical observation for localization of the cannula tip. For further verification of the location of the cannula tip, postmortem intracannula marker injection was performed prior to pulling out the cannula. The statistical evaluation of the results was carried out using Student *t*-test [30].

RESULTS

Experiment 1

The purpose of this experiment was to investigate whether Ang II induces a drinking response in the chicken when administered intravenously.

Method. Eight birds were used. They were administered via brachial vein with 2.5 ml saline or 2.5 ml saline containing various dosages of Ang. II in an increasing order. Single injection per day only was carried out, using an alternate day regime of injections.

Immediately after injections, the birds were placed in their cages and observed for their drinking behavior. Water intake was continuously measured up to the point, when the birds that exhibited drinking response stopped drinking. However, in each case, at least a 20 min period of measuring was allowed. This whole procedure was repeated 4 times for each experimental bird.

Results. Table 1 presents the averages of water intake following the injections. In each case where drinking response was elicited, it lasted for a maximum of 20 min. It should be emphasized that in spite of the low amount of water intake in response to the 40 μg Ang II dosage, all birds injected with this dosage showed some behavioral acts of drinking. This was reflected particularly by approaching the water cups and exhibiting the typical drinking motions of a chicken.

TABLE 1

WATER INTAKE DURING THE FIRST 20 MIN POSTINTRAVENOUS INJECTION OF 2.5 ML SALINE OR VARIOUS DOSAGES OF ANG II DISSOLVED IN THE SAME VOLUME

Type of Injection	N	(Means ± S.E.) Water Intake (ml)
Saline	8	0(a)*
30 g Ang II	8	0(a)
40 g Ang II	8	2.1 ± 1.0(a)
300 g Ang II	8	32.9 ± 10.7(b)
500 g Ang II	8	40.0 ± 8.3(b)

*Figures which are not marked by the same letter are statistically different from each other ($p<0.05$).

Experiment 2

This experiment was carried out in order to study the effects of central administration of Ang II on water intake and drinking behavior.

Method. Each of 29 cocks were stereotaxically implanted with a single cannula. The cannulas were located along the brainstem, from the anterior commissure to the posterior commissure, as shown schematically in Fig. 1. Using a 10 μl syringe (Hamilton), 5 μl of saline (0.9% NaCl) or various dosages of Ang II dissolved in 5 μl of saline were injected through the implanted cannulas. Each bird was injected once a day with a single dose at 9:00 a.m. and then successively on alternate days. On every day of injection a different dose was used until each bird had received one injection of each of the following doses of Ang II and in the order given: 0.025; 0.05; 0.25; 5.0; 10.0 and 20.0 μg. After the injections the birds were immediately returned to their cages and observed for their drinking behavior. Water intake was determined for 30 min postinjection; preliminary observations having shown very little abnormal behavior after this time. The whole procedure described, was repeated for at least 4 times per bird.

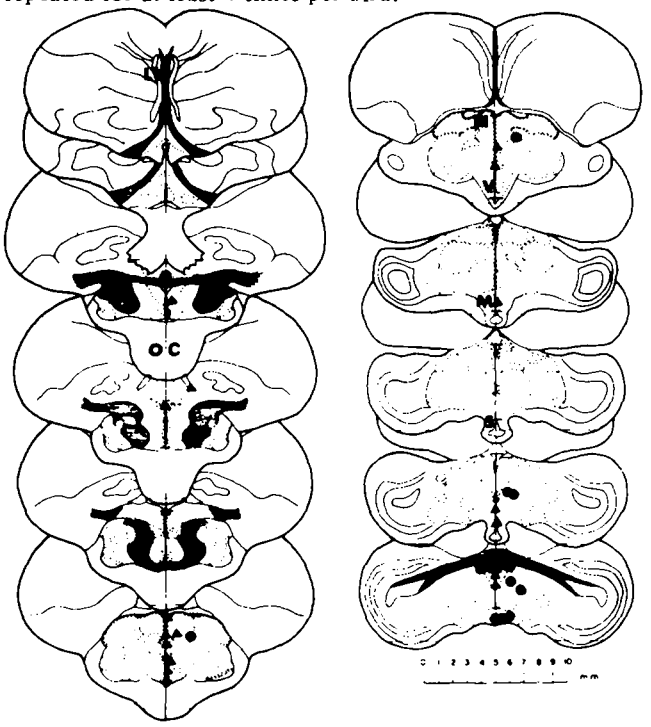

FIG. 1. Schematic drawings of frontal sections of the chicken diencephalon [29] showing locations of the tips of the cannulas in those birds showing positive (▲) or negative (●) drinking response to intracranial Ang II injection. III = third ventricle; V = hypothalamic ventromedial area; M = dorsal and ventromedial mammillary nuclei; S = pituitary stalk; OC = optic chiasma; LV = lateral ventricles.

Results. The birds that had their cannulas implanted in various locations of the anterior diencephalon or in the ventricles (nineteen altogether), showed a positive drinking behavior to Ang II administration. The location of the cannula tips of these birds are marked by triangles in Fig. 1. On the other hand, the birds that had their cannulas located in other areas of the brain stem (marked as circles in Fig. 1), showed no response to any of the Ang II dosages administered. The dose-response relationship of responding birds is presented in Fig. 2. Although no increase in water intake could be detected following the 2.5 and 5.0 μg Ang II injections – as compared to the saline injections – a definite drinking behaviour was expressed by the birds approaching drinking cups and pecking.

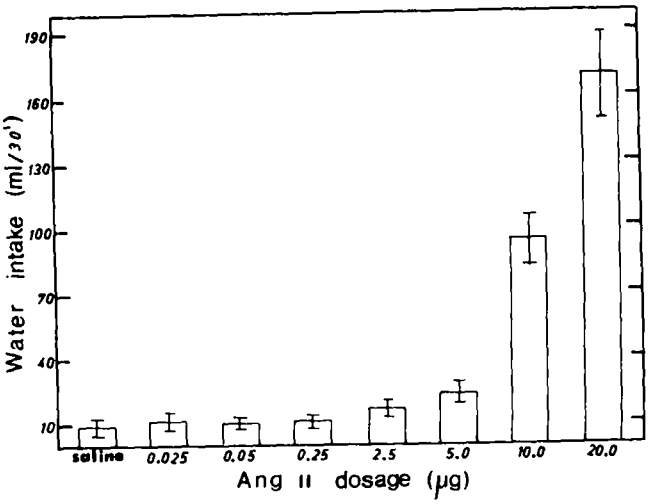

FIG. 2. Dose response diagram for the 19 W. Leghorn birds that responded with drinking to intracranial injection of Ang II. (Vertical bars indicate S.E. of the means). The water intake caused by the 10 and 20 μg dosages are significantly higher than the rest ($p<0.05$); the water intake due to the 20 μg dosage is significantly higher than that of 10 μg ($p<0.05$).

Experiment 3

The purpose of this experiment was to clarify whether the drinking induced by peripheral injection of Ang II could be inhibited by a central depression of those regions in the brain, which when stimulated with Ang II induced drinking response. For this, intracranial administration of isotonic KCl was used.

Method. Based on the previous experiment, cannulas were stereotaxically implanted in 5 birds in the rostral part of the diencephalon, where it was previously demonstrated that Ang II administration induced drinking. After proving positive drinking response due to intracranial injection with Ang II, the following procedure was undertaken: The birds were first injected with 5 μl of saline into the cannula, followed by intravenous injection of 500 μg Ang II. This procedure was repeated after 2 days, except that instead of saline, isotonic KCl (1.13%) was injected into the cannula. Water intake and drinking behavior were recorded for the first 30 minutes post injections. The procedure described above was repeated four times for each bird.

Results. Figure 3 shows the locations of the cannula tips of the experimental birds. The water intake of the individual birds is shown in Table 2. It is evident that the injection of the KCl caused inhibition of the drinking response induced by peripheral administration of the Ang II. No other visualized behavioral effect could be detected in the KCl-injected birds.

Experiment 4

The following experiment was conducted in order to study the immediate drinking response of the chicken due to repeated intracranial injections of Ang II.

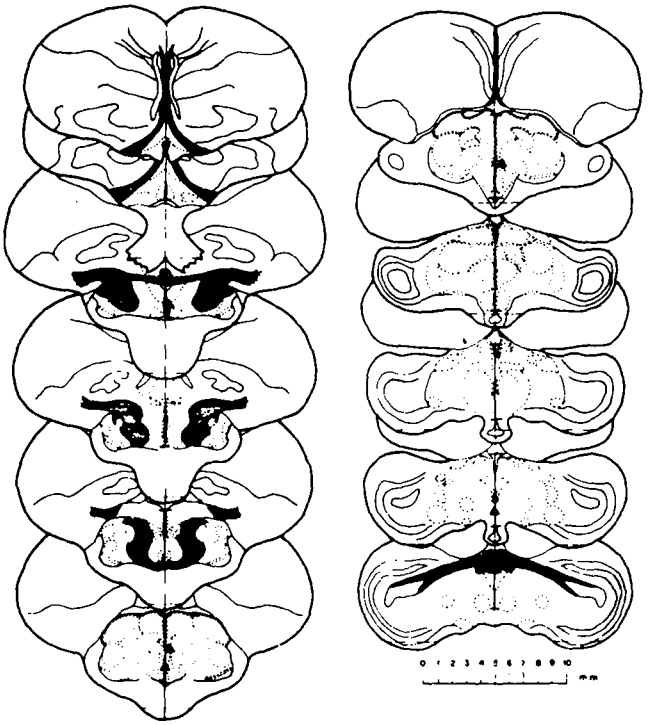

FIG. 3. Schematic drawings of frontal sections of the chicken diencephalon [29] showing locations of the tips of the cannulas (▲) in birds injected simultaneously with Ang II IV, and KCl or saline, intracranially.

TABLE 2

INDIVIDUAL WATER INTAKE DURING THE FIRST 30 MIN POSTINTRAVENOUS INJECTION OF 500 G OF ANG II AND SIMULTANEOUSLY GIVEN INTRACRANIAL INJECTION OF SALINE, OR ISOTONIC KCl

Bird No.	Water intake (ml) after i.v. injection of Ang II and i.c. injection of saline	Water intake (ml) after i.v. injection of Ang II and i.c. injection of KCl
2	65	0
3	50	0
12	53	5
32	25	10
35	25	10
Means ± S.E.	46.3 ± 8.0*	5.0 ± 2.2†

* x †$p<0.002$.

Method. The locations of the cannula tips in the eight birds used in this experiment are schematically presented in Fig. 4. Twenty μg of Ang II dissolved in 5 μl saline were administered intracranially to all birds in each of the individual injections hereunder described. The first injection to each and every bird was performed at 9:00 a.m. Repeat injections were carried out whenever a bird ceased drinking to a total of four consecutive injections per bird. Water intake of each experimental bird was measured during the injection period up till 24 hours after the first injection. After two days, the birds were injected intracranially and repeatedly with 5 μl saline, at time intervals comparable to those between Ang II injections. This total procedure was repeated four times for each bird.

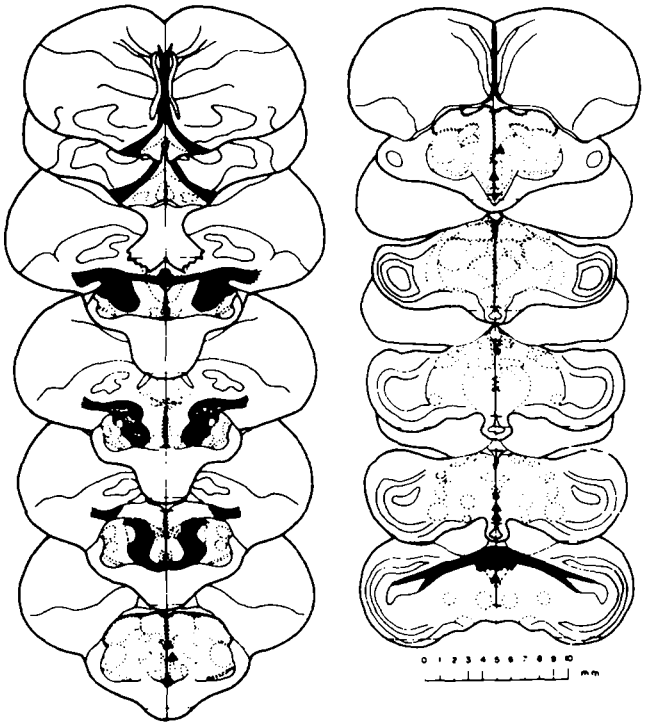

FIG. 4. Schematic drawings of frontal sections of the chicken diencephalon [29] showing locations of the tips of the cannulas (▲) in the birds with repeated intracranial injections of Ang II.

Results. Figure 5 presents the average cumulative water intake of the eight birds used, as response to the repeated Ang II or saline injections. The average amount of water intake after each of the separate injections is shown in Fig. 6. As seen from these figures, a progressive decline in water intake between the consecutive intervals of Ang II injections was exhibited by the birds. Table 3 presents averages of water intake during the first 3 hours, where the birds were repeatedly injected intracranially with saline or Ang II, and the water intake following the next 21 hours. It is evident from this data that an almost normal amount of water was drunk by the birds during the rest of the day – after the Ang II injections – as compared to the amount drunk in the parallel period, after the saline injections. However, a significant negative correlation was found between the amount of water drunk by the birds during the Ang II injection period (3 hr) and the water drunk during the following 21 hours, where no injections were given.

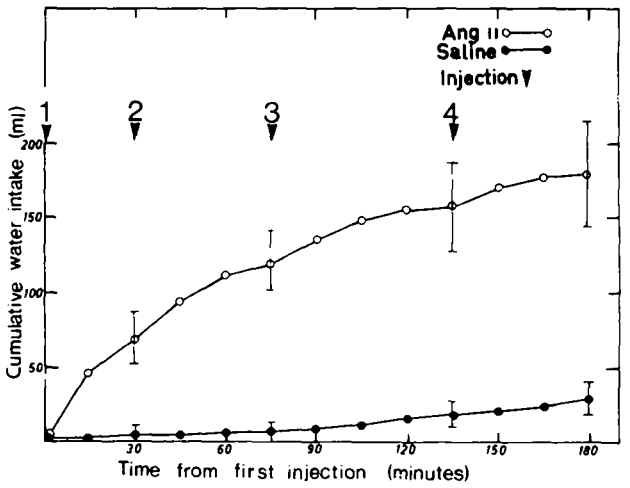

FIG. 5. Average of cumulative water intake in birds (n = 8) with repeated intracranial injections of Ang II or saline (vertical bars indicate S.E. of the means).

TABLE 3

AVERAGE WATER INTAKE DURING THE 3 HR OF THE REPEATED INTRACRANIAL INJECTIONS OF ANG II OR SALINE, AND DURING THE FOLLOWING PERIOD OF 21 HR DURING WHICH NO INJECTIONS WERE GIVEN (MEANS ± S.E.)

	Hr	Water intake (ml) of saline injected birds (n=8)	Water intake (ml) of Ang II injected birds (n=8)
Injection period	0-3	28.1 ± 7.1*	177.5 ± 32.0†
No injection	3-24	148.8 ± 10.8‡	117.5 ± 34.1§
Total period	0-24	176.9 ± 16.1¶	295.0 ± 16.5#

† x §: r = −0.890 ($p<0.001$).
* x †: $p<0.001$.
‡ x §: N.S.
¶ x #: $p<0.001$.

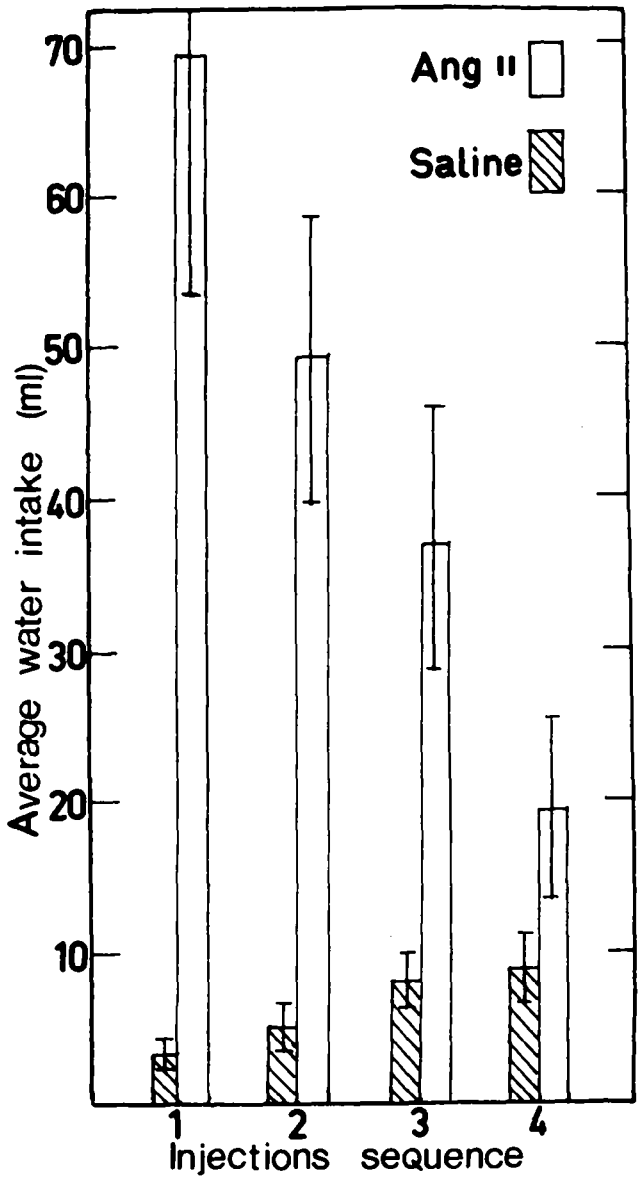

FIG. 6. Average water intake in the intervals between each of the repeated consecutive intracranial injections of Ang II or saline (vertical bars indicate S.E. of the means).

DISCUSSION

It was clearly demonstrated in the present investigation that similar to many mammals [10, 11, 12, 13] the domestic fowl responds with immediate drinking to both intravenous and intracranial injections of Ang II. However, in contrast to the relatively low dosages needed in order to cause the drinking response in rats [5,24], cats [3,4], monkey [22,28] and goats [2], the dose eliciting drinking in the domestic fowl was much higher (x 10-100). The differences between the chemical structure and physiological activity of the Ang II of mammals and that of the domestic fowl [23,31], may partly explain the needs for higher dosages of Ang II (Hypertensin) to induce drinking in the fowl. There is still no information on the effect of chicken source Ang II on drinking in the fowl.

The dipsogenic effect due to intracranial injection of Ang II was obtained in those birds, where the cannula tips were located in the anterior diencephalon or in the cerebral ventricles (see Fig. 1). These results are in agreement with those reported in mammals [6, 7, 9, 25, 26, 27, 33] in which intracranial injection of Ang II into similar locations caused increase in water intake. It has been recently postulated [16] that those areas which respond to stimulation with angiotensin by eliciting drinking are located not in the diencephalon but rather in the lining of the cerebral ventricle. It was also shown [16] that the major determinant of angiotensin sensitivity of a given intracranial injection site is the extent to which delivery of angiotensin in the ventricular system is inserted. Our results may support the hypothesis presented, since all the effective sites obtained in the present investigation were either intraventricular or located in tissue closely bordering the cerebral ventricle (Figs. 1, 3, and 4). Furthermore most of the implanted cannulas have passed through the ventricles. In view of this and of the relatively high volume (5.0 μl) injected, it is quite possible that the injected angiotensin seeped back along the cannula to the ventricle. The production of polydipsia by electrical stimulation of the anterior diencephalon in the pigeon [1] cannot be ruled out and may be partly substantiated by the findings of this study.

An inhibition of drinking response to intravenous injection of Ang II caused by central depression of those brain regions in which Ang II administration normally elicited drinking, was demonstrated in the present investigation. This supports the suggestion that the drinking caused by an intravenous or an intracranial injection of Ang II is activated through identical brain regions.

As seen from Figs. 5 and 6, the birds responded to the first three consecutive intracranial injections of Ang II with significant higher water intake as compared to the response induced after saline injection. The water intake after the fourth injection of Ang II was not significantly different from that caused by saline. Based on the negative correlation found between the amount of water ingested during the period of the consecutive injections of Ang II and the water drunk during the rest of the 24 hours, it is suggested, that the progressive lowering of water intake in the injection period may be due to overhydration caused by the compulsive drinking and not by habituation to Ang II.

ADDENDUM

During the last stages of preparation of the present manuscript, an investigation by Wada, M., H. Kobayashi and D. S. Farner: "Induction of drinking in the White-Crowned Sparrow, Zonotrichia Leucophrys Gambelli, by intracranial injection of Ang II", *Gen. Comp. Endocrinol.* **26**: 192–197, 1975, was published. Our results are in agreement with the abovementioned study in respect with the finding that higher dosage of intravenous and intracranial administration of Ang II are required to induce drinking in the avian species, as compared to mammals. In addition, similar brain locations, in which administration of Ang II was effective, were found in both studies.

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