GENERAL AND COMPARATIVE ENDOCRINOLOGY 26, 192–197 (1975)

Induction of Drinking in the White-Crowned Sparrow, *Zonotrichia leucophrys gambelii*, by Intracranial Injection of Angiotensin II[1]

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Accepted December 14, 1974

In the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*, single injections of 0.5 μg (ca. 500 pmoles) angiotensin II into the preoptic region, anterior hypothalamus, and lateral hypothalamus induce drinking. The minimum effective dosage is higher than in the white rat and the latency of effect is somewhat greater.

There is persuasive evidence from several species of mammals, but especially from rats (e.g., Epstein *et al.*, 1970), that a renin–angiotensin system causes drinking in response to an extracellular deficit of water (see Fitzsimons 1970, 1973, for résumés). Although such responses to angiotensin II have apparently been noted also in the pigeon (Fitzsimons, 1973), little appears to be known about this system in birds. We, therefore, report here the results of an investigation of the effect of administration of angiotensin II (mammalian) in *Zonotrichia leucophrys gambelii*.

MATERIALS AND METHODS

Male White-crowned Sparrows were captured with Japanese mist nets from migrating flocks in Kittitas County, Washington, in September and October 1973. They were held first in outdoor aviaries under natural conditions of temperature and photoperiod in Seattle until late February or March when, a week before the beginning of experiments, they were transferred into individuals cages in a room with constant conditions of temperature and photoperiod (20° and 8L 16D). Food (crumbled chick-starter pellets) and water (from inverted bottles with glass drinking tubes) were available ad lib.

Implantation of Guide Cannulae

A guide cannula was implanted for repetitive injections with a needle inserted in its lumen. The head of the bird was fixed without anesthesia in a stereotaxic apparatus. The scalp was incised dorsally in order to expose the skull. A stainless steel cannula (o.d., 0.7 mm; length, 6–12 mm) was mounted in the holder of the apparatus; frontal and lateral roentgenographs were made and the position of the cannula was adjusted thereby. Three holes, approximately in a diagonal line were made at appropriate positions in the dorsal wall of the skull, the middle one being for the subsequent insertion of the cannula. Stainless steel screws were inserted into the other two holes and fixed with dental cement. The cannula was inserted and adjusted to 1–2 mm above the desired position so that the top of the injection needle could be lowered to the desired position and was then fixed with dental cement to the skull and to the two screws.

[1] This investigation was conducted at the Department of Zoology, University of Washington, Seattle under Grant No. 5R040 from the Japan Society for Promotion of Science to Professor Kobayashi, and under Grant No. GF-33334, U.S.-Japan Cooperative Science Program of the National Science Foundation, and by Grant No. GB-28080X, also from the National Science Foundation, to Professor Farner.

Preparation of Test Solutions

Synthetic 5-valine-angiotensin II amide (Hypertensin CIBA) was dissolved in 0.9% NaCl solution in concentration of 0.1, 0.5, and 1.0 μg/μl for intracranial injections; an 0.9% NaCl solution was used for control injections. For intravenous administration angiotensin II was dissolved in 0.9% NaCl solution in concentration of 50 or 100 μg/0.1 ml.

Intravenous and Intracranial Injections of Angiotensin II

For intravenous injection, test or saline solutions of 0.1 ml were introduced through the jugular vein of intact birds.

For intracranial injection, test or saline solution was administered a week after implantation of the cannulae. A stainless steel needle (o.d., 0.3 mm; length, 8–14 mm) was inserted into the guide cannula connected to a Hamilton microsyringe with a polyethylene tubing (Intramedic^R PE-10, Clay Adams, Parsippany, NJ). The test solution of 1 μl was then injected in 10–20 sec. Immediately after the injection, the bird was released into the observation cage. Intervals between each injection in individual birds were greater than 2 days.

Observations

Drinking behavior was observed in an experimental cage in a paper box with a slit for observation. The cage was lighted from above in the box. Water was available to the test bird from a graduated cylinder with a glass drinking tube. As criteria for induced effects, the latency to the first drinking and number of pecks at the tap were recorded using a stopwatch. Total water intake was also recorded after the observation period. Observation ensued for 1 hr after intravenous injection and for 30 min for intracranial injection.

When observation following intracranial injection was completed the bird was decapitated and the brain fixed in Bouin's mixture, after removal of the tube with a part of the skull. The brain was then trimmed to an appropriate block, embedded in paraffin, sectioned at 10 μm, and stained with Toluidine Blue O. The location of the tip of the tube was determined and recorded on an enlarged brain chart.

RESULTS

Before the injection experiments were initiated, the rates of water consumption in intact birds, held two per cage, were recorded hourly throughout the day. During the hour immediately after the onset of light (at 0800) drinking was extensive, with the intake remaining at a relatively lower, almost constant rate through the remainder of the day (Fig. 1). Consequently the injection experiments were initiated between 0900 and 1600.

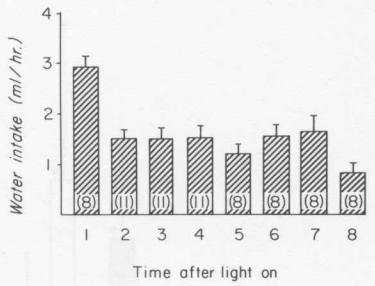

FIG. 1. Amount of water intake by White-crowned Sparrows housed in their original cages during each hour of the short photoperiod (8L 16D). Numbers in parentheses indicate number of observations using eight birds. Vertical bars on columns indicate standard errors of each mean.

Intravenous Injection of Angiotensin II

Intravenous injections of angiotensin II invariably induced pecks and water intake (Fig. 2). Intact and saline-injected birds showed a lower rate of pecking and drank relatively less. Induced drinking was almost invariably completed during the first 30 min. Latency in the intact or saline-injected birds ranged from approximately 23 to 48 min. In the birds injected with 50 μg angiotensin II latent periods were of the order of 2–10 min. However, latency after 100-μg injections was substantially greater, 7–14 min, with one case as great as 53 min. Although the number of pecks was greater in birds injected with 100 μg than in those injected with 50 μg, the intake of water was less in the former than in the latter (Fig. 2). The greater latency and lower water consumption of birds with 100 μg were perhaps due to another effect of angiotensin II since the birds stood relatively motionless on the floor of the observation cage for some time after the injection. From these results, we considered that the number of pecks at the tap is an

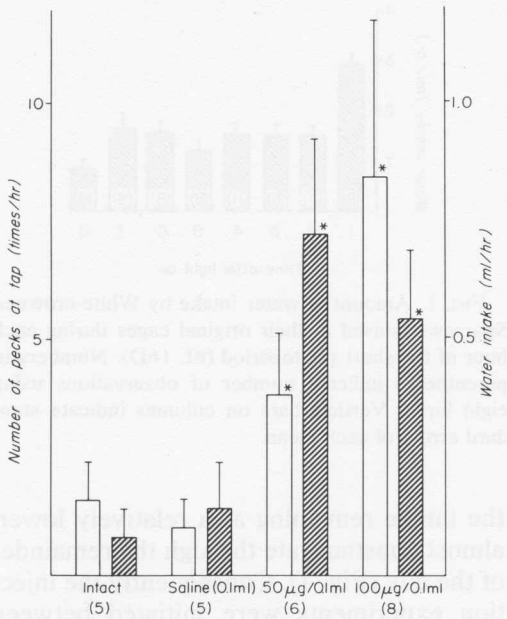

FIG. 2. Effect of intravenous injection of angiotensin II on drinking by White-crowned Sparrows. White columns indicate mean number of pecks and shaded columns indicate mean volume of water drunk. *Significant ($P < 0.05$) compared with saline control group tested by randomization test. Numbers in parentheses indicate numbers of birds tested. Vertical bars on each column indicate standard errors of means.

appropriate criterion of effects induced, and we therefore used it for the birds with intracranial injections.

Intracranial Injection of Angiotensin II

Cannulae were implanted in 27 birds and the sites of the tips were subsequently verified in histological sections. The principal effective locations were in the preoptic area, the anterior hypothalamus, and the lateral hypothalamus (Fig. 3). Initially 1 μg (ca. 1 nmole) in a volume of 1 μl was injected in order to obtain an indication of the extent of the response at each location. Responses were strong in the preoptic area and were also detected in the anterior and lateral hypothalamus (Figs. 3 and 4). In a single bird we found a very strong response from an injection in the *paleostratum augmentatum.*

Injections of 0.5 μg (ca. 500 pmoles) in a volume of 1 μl into the preoptic area also invariably induced responses (Figs. 4 and 5). In only one case was 0.1 μg (ca. 100 pmoles) effective in the same volume. In all locations control injections of physiological saline elicited little or no response (Fig. 4).

Injections of 0.5 μg angiotensin II in the anterior hypothalamus induced appreciable drinking in two of four birds, but 0.1 μg of angiotensin II or saline did not (Fig. 4). Injections of angiotensin II into the lateral hypothalamus yielded results similar to those in the anterior hypothalamus (Fig. 4). However, at two locations more dorsal in the anterolateral hypothalamic area

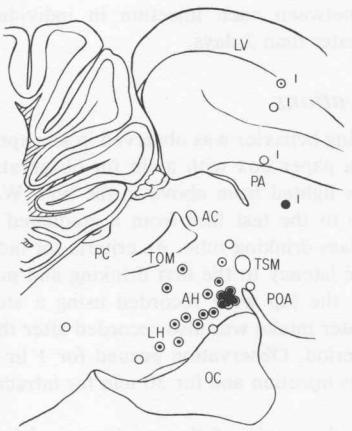

FIG. 3. Angiotensin sensitivity of the brain of the White-crowned Sparrows shown in parasagittal section. All of the placements are 0.7 mm lateral to the midline except those indicated by 1 which are 1.0 mm lateral. The central black discs indicate the degree of response to 1 μg angiotensin II; the strongest reaction is designated as 100% and the others are calculated and expressed in percentage. The percentage was given by an area of black circle occupying the white circle, for example (● = 100%), (◉ = 50%), (⊙ = 30%), and (○ = 0%). AC = anterior commissure, AH = anterior hypothalamus, LH = lateral hypothalamus, LV = lateral ventricle, OC = optic chiasma, PA = *paleostriatum augmentatum,* PC = posterior commissure, POA = preoptic area, TOM = tractus occipito-mesencephalicus, TSM = tractus septomesencephalicus.

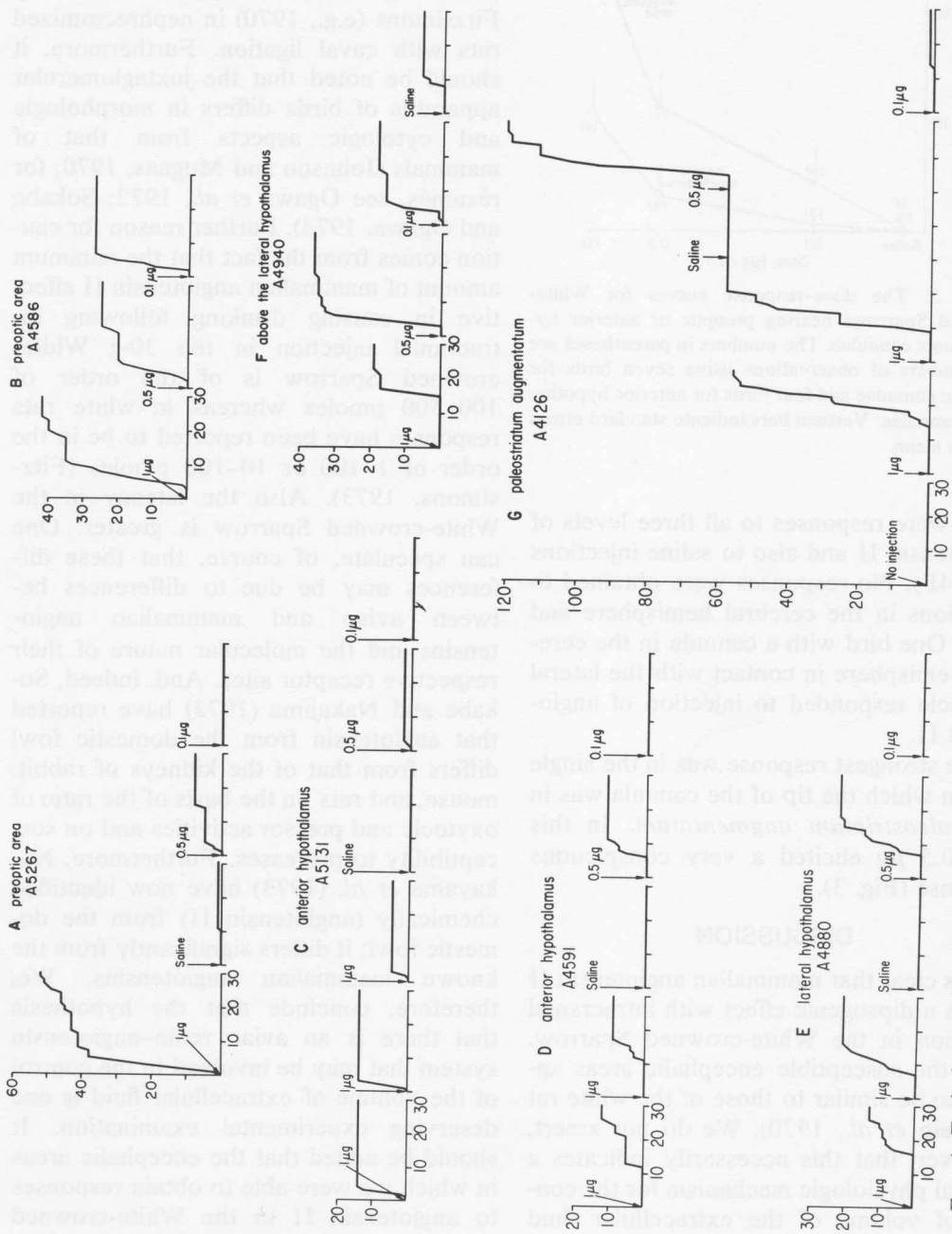

FIG. 4. Typical examples of cumulative number of pecks at the tap by White-crowned Sparrows after intracranial injection of angiotensin II. Doses are indicated in figures. Each injection was given on separate days unless otherwise explained. Location of the tips of injectors are indicated in Fig 3. Ordinates give the number of pecks and abscissa is the time after injection in minutes.

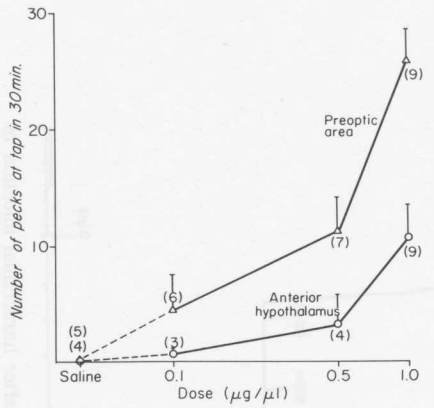

FIG. 5. The dose–response curves for White-crowned Sparrows bearing preoptic or anterior hypothalamic cannulae. The numbers in parentheses are the numbers of observations using seven birds for preoptic cannulae and four birds for anterior hypothalamic cannulae. Vertical bars indicate standard errors of each mean.

there were responses to all three levels of angiotensin II and also to saline injections (Fig. 4F). No responses were obtained to injections in the cerebral hemisphere and pons. One bird with a cannula in the cerebral hemisphere in contact with the lateral ventricle responded to injection of angiotensin II.

The strongest response was in the single bird in which the tip of the cannula was in the *paleostriatum augmentatum*. In this bird 0.5 μg elicited a very conspicuous response (Fig. 3).

DISCUSSION

It is clear that mammalian angiotensin II exerts a dipsogenic effect with intracranial injection in the White-crowned Sparrow. Also the susceptible encephalic areas appear to be similar to those of the white rat (Epstein *et al.*, 1970). We do not assert, however, that this necessarily indicates a normal physiologic mechanism for the control of volume of the extracellular fluid compartment as appears to be the case in mammals (Fitzsimons, 1973). Our caution in the interpretation of our results arises first from the lack of direct experimental demonstration of a role of the avian renal cortex in water intake in response to reduced pressure in the "low-pressure" side of the vascular system as demonstrated by Fitzsimons (e.g., 1970) in nephrectomized rats with caval ligation. Furthermore, it should be noted that the juxtaglomerular apparatus of birds differs in morphologic and cytologic aspects from that of mammals (Johnson and Mugaas, 1970; for résumés, see Ogawa *et al.*, 1972; Sokabe and Ogawa, 1974). Further reason for caution comes from the fact that the minimum amount of mammalian angiotensin II effective in causing drinking following intracranial injection in the 30-g White-crowned Sparrow is of the order of 100–500 pmoles whereas in white rats responses have been reported to be in the order of 1–100 or 10–100 pmoles (Fitzsimons, 1973). Also the latency in the White-crowned Sparrow is greater. One can speculate, of course, that these differences may be due to differences between avian and mammalian angiotensins and the molecular nature of their respective receptor sites. And, indeed, Sokabe and Nakajima (1972) have reported that angiotensin from the domestic fowl differs from that of the kidneys of rabbit, mouse, and rats on the basis of the ratio of oxytocic and pressor activities and on susceptibility to proteases. Furthermore, Nakayama *et al.* (1973) have now identified chemically (angiotensin II) from the domestic fowl; it differs significantly from the known mammalian angiotensins. We, therefore, conclude that the hypothesis that there is an avian renin–angiotensin system that may be involved in the control of the volume of extracellular fluid is one deserving experimental examination. It should be added that the encephalic areas in which we were able to obtain responses to angiotensin II in the White-crowned Sparrow correspond, at least generally, with those in the pigeon in which Åkerman *et al.* (1960) were able to elicit polydipsia by electrical stimulation.

Mammalian angiotensin II has also been demonstrated to have a tubular diuretic effect in the kidney of the domestic fowl (Cuypers, 1965; Langford and Fallis, 1966). Whether this represents a normal physiologic mechanism that is general in birds remains to be demonstrated. However, one can speculate that the drinking response demonstrated here in the White-crowned Sparrow and the induction of tubular diuresis demonstrated in the domestic fowl could be a mechanism that permits tubular excretion involving increased water loss for which there is simultaneous correction by increased water intake.

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