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Effects of Angiotensin II on Schedule Dependent and Induced Behavior at Recovered Body Weight[1]

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WAYNER, M. J., A. D. MERKEL AND F. B. JOLICOEUR. *Effects of angiotensin II on schedule dependent and induced behavior at recovered body weight.* PHARMAC. BIOCHEM. BEHAV. 6(6) 701–704, 1977. – The effects of 4 doses of angiotensin II, 0.4, 0.8, 1.2 and 1.6 mg/kg, injected subcutaneously on schedule dependent lever pressing and schedule induced drinking and licking were studied in rats who had recovered body weight under ad lib eating conditions. Prior to this experiment rats had been maintained at 80% body weight and were tested under the usual conditions producing schedule induced drinking. Animals were then returned to the home cage and allowed to recover body weight. Results were analyzed for both the first 15 min of the 1 hr session and for the total 1 hr session. Data indicate that all four doses decreased lever pressing during the first 15 min and for the total 1 hr session. The three highest doses produced increased licking during the first 15 min. Only the highest dose augmented licking throughout the total 1 hr session. Water intakes were significantly increased by the three highest doses only during the first 15 min of the session. Peripheral effects of angiotensin II were discussed and a central pharmacological action on drinking was suggested.

Angiotensin II Schedule induced polydipsia Recovered body weight Schedule induced behavior Schedule dependent behavior Drinking Lever pressing Licking Stereotyped behavior Adjunctive behavior

BECAUSE angiotensin II produces drinking and the release of aldosterone and antidiuretic hormone in several species under a variety of conditions, it might be involved in the regulation of body fluids. However, a physiological role of peripheral endogenous angiotensin II in drinking has been questioned [1] because of the relatively high doses required. Peripheral administration of angiotensin II interacts with subcutaneous (SC) or intraperitoneal (IP) administration of hypertonic saline, polyethylene glycol, and also with water deprivation to enhance drinking [8,12]. These possible pharmacological actions of the hormone are interesting and justify further experimentation.

The effects of several doses of angiotensin II on schedule dependent lever pressing and schedule induced licking and drinking have been reported recently [12]. The effects were studied in rats at 80% normal body weight due to partial food deprivation and subjected to an FI 1 min schedule of food reinforcement. Results indicated decreases in lever pressing and increases in schedule induced drinking. These effects were of short duration and occurred immediately following SC injection of the hormone. Similar effects were observed in rats which had not been exposed to an intermittent schedule of food delivery but had been maintained at 80% body weight and subjected to identical testing procedures.

Schedule dependent lever pressing and schedule induced licking and drinking can be obtained in non-food deprived animals. The nature of these behaviors under these conditions and the methods involved to produce them have been described in detail elsewhere [10]. Essentially, animals who have developed schedule induced behaviors under food deprivation are allowed to recover body weight by ad lib eating and are then retested under the same experimental conditions. An obvious advantage of using this procedure is that drug effects can be assessed without the confounding effects of food deprivation.

The purpose of the present experiment was to examine the effects of SC injections of 0.4, 0.8, 1.2 and 1.6 mg/kg of angiotensin II on schedule dependent lever pressing, schedule induced drinking, and schedule induced licking in animals that had recovered body weight following a return to ad lib eating.

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METHOD

Animals and Apparatus

Four male hooded rats approximately 100 days old, weighing 310, 365, 405 and 450 g, were selected from our colony. These animals had been utilized in a previous experiment reported in detail elsewhere [12]. Briefly, the animals were adapted to individual living cages for ten days. Body weights were then gradually reduced to 80% of the Day 10 weight by gradually limiting food intake over a 7 day period. The rats were shaped to press a lever for 45 mg Noyes pellets over 3 days. Following this shaping period the animals were tested each day for 1 hr on a FI 1 min generator schedule, one which had produced schedule induced drinking. When the number of lever presses and schedule induced drinking stabilized a series of SC injections of angiotensin II and control vehicle was initiated. Therefore, prior to the present experiment these animals had been maintained at reduced body weight for 90 days, had been tested daily in a schedule induced drinking situation for the same period, and each had received 8 SC injections, 4 of control vehicle and 4 of angiotensin II, and each injection was separated by 2 days.

The testing apparatus consisted of a standard LVE 1469 medium sized test cage and matching sound attenuating cubicle with a lever and pellet dispensing mechanism. The food cup, delivery mechanism, test lights and lever were mounted on one wall as provided by the manufacturer. A modified glass insulated stainless steel ball point drinking spout fitted to a glass eudiometer tube was placed in the center of the back wall of the test chamber, 4.0 cm above the grid floor. The spout protruded 1.5 cm into the test cage.

Procedure

Following the last angiotensin II injection of a previous experiment [12], the rats were returned to the home cage for six days. During this period the rats were permitted to recover body weights. On Day 1 rats were given a quantity of Purina rat chow equal to the amount normally given to maintain 80% body weight, plus an additional 50% ration. On Day 2 the rats received the ration of Day 1, plus an additional 50%. This procedure was continued until the rats failed to consume their entire ration in 23 hr. At this time Purina rat chow in amounts exceeding daily intakes was placed in the cage after the remaining food from the previous day had been removed and weighed. Daily recordings of body weight, water intake (to the nearest ml), and food intake (to the nearest 0.1 g) were made at approximately the same time throughout the experiment.

On the 7th day following the initiation of the recovery period the animals were returned to the test chamber for the 1 hr daily test session at free feeding body weight. Number of lever presses, licks, and water intake were recorded for each of the four 15 min intervals and for the entire 1 hr sessions. Once lever presses, water intake, and licks had stabilized the series of SC injections was initiated.

Drug

Angiotensin II (Hypertensin – CIBA) was dissolved in 0.9% saline to obtain four doses – 0.4, 0.8, 1.2 and 1.6 mg/kg. The injection volume was 1 ml/kg body weight. Because angiotensin II contains approximately 17% ammonium acetate, control injection solutions of ammonium acetate alone were administered to test for possible osmotic effects. Therefore, each control injection contained amounts of ammonium acetate identical to that found in each drug dose, 0.068, 0.136, 0.204 and 0.272 mg/cc of 0.9% saline. These data constituted baseline to which drug injection results could be compared. Ammonium acetate and angiotensin II saline solutions were kept under refrigeration. The four control injections were administered SC every third day in a nonsystematic order immediately prior to the test session. Following the control injection sequence the drug injections were initiated. As with the control injections, the four drug doses were administered prior to the test session every third day in a nonsystematic order.

RESULTS

Data were analyzed separately for the first 15 min interval and the total 1 hr session. Previous results [12] indicated that no differences are produced by the addition of ammonium acetate in different amounts to the control injections; therefore, control data were collapsed and the effects of angiotensin II on licks, water intake, and lever pressing were assessed by means of individual one-way analyses of variance with repeated measures.

The effects of control and drug treatments on mean lever pressing performance are represented in Fig. 1 as a thin continuous line for the first 15 min interval (left panel) and the total 1 hr session (right panel). The statistical analysis of the first 15 min lever press data revealed a significant main effect, $F(4,12) = 13.07$, $p<0.01$. Subsequent comparison by means of a Dunnett's test indicated that all four drug doses significantly reduced lever pressing as compared to control injections ($p<0.01$). However, no single dose was more effective in producing this decrease. The decrease in lever pressing performance was also reflected in the 1 hr cumulative data, $F(4,12) = 9.57$, $p<0.01$. Further analysis of the data by a post hoc Dunnett's test indicated that all four doses of angiotensin II significantly depressed lever responding ($p<0.01$) when compared to the baseline. Individual comparisons between doses were not significant.

A significant main effect, $F(4,12) = 6.28$, $p<0.01$, was revealed by the one-way ANOVA performed on the 15 min lick data. A post hoc Dunnett's test indicated that only the three highest doses (0.8, 1.2, and 1.6 mg/kg) significantly increased the number of licks during the first 15 min when compared to baseline licking ($p<0.05$ for 0.8 and 1.2 mg/kg; and $p<0.01$ for 1.6 mg/kg). A trend analysis of the data was significant ($p<0.01$) indicating an increasing linear trend as a function of dose. Although the main drug effect was significant for the 1 hr lick data, $F(4,12) = 4.98$, $p<0.05$, analysis of the data by Dunnett's procedure revealed that only the highest dose of angiotensin II effectively increased the number of licks for the 1 hr session when compared to baseline ($p<0.01$). Further comparisons using a Tukey A test indicated that the number of licks produced by the 1.6 mg/kg dose were significantly greater than the number produced by the lowest (0.4 mg/kg) dose of the drug ($p<0.01$). However, further analysis for trends revealed that the total number of licks produced by each dose increased in a linear fashion ($p<0.01$). These effects are illustrated in Fig. 1 by means of solid circles connected by a dashed line where the mean

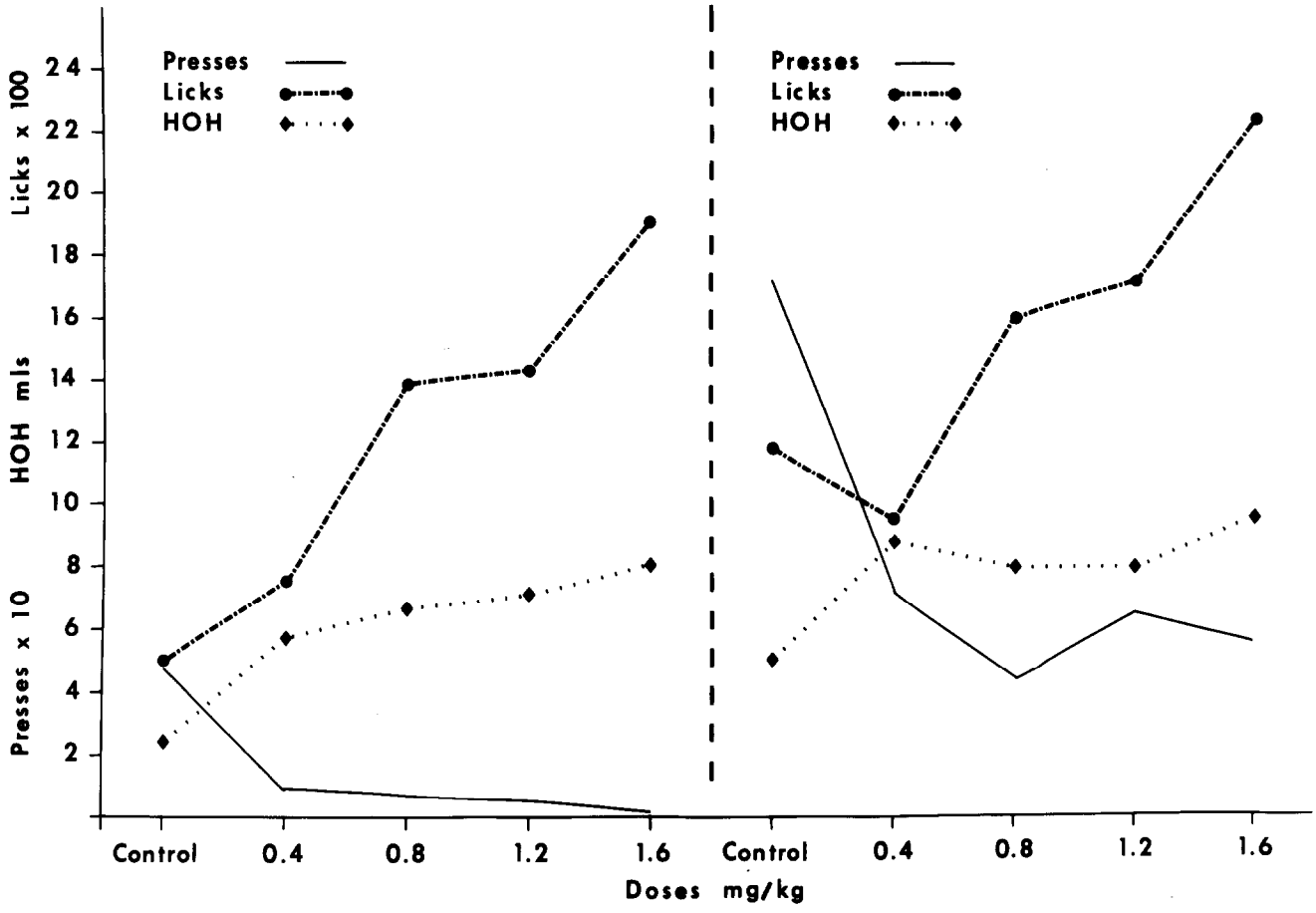

FIG. 1. Mean number of presses (continuous line), licks (dashed line), and water intake (dotted line) occurring during the first 15 min interval (Left panel) and during the total 1 hr session (Right panel) presented as a function of the dose of angiotensin II.

number of licks are presented for control and drug treatments for both the first 15 min interval and the total 1 hr session.

Mean water intakes during the first 15 min of the 1 hr session were significantly increased by angiotensin II administration, $F(4,12) = 4.01$, $p<0.05$. Comparisons of the drug doses to baseline by means of a Dunnett's test demonstrated that the 1.6 mg/kg dose, $p<0.01$, 0.8 and 1.2 mg/kg doses ($p<0.05$), significantly increased water intake. Although no specific dose effectively increased water intake over any other drug dose, a significant linear trend was found ($p<0.01$). There were no significant differences between the 1 hr water intakes for any of the experimental treatments. These results are illustrated in Fig. 1 with diamond symbols connected by a dotted line.

DISCUSSION

These results demonstrate differential effects of angiotensin II when administered peripherally on schedule dependent lever pressing and schedule induced drinking. Although four substantial doses were tested, a dose related effect was not observed. Schedule dependent lever pressing was decreased throughout the test session by all doses and schedule induced drinking was increased only during the first 15 min of the test session by the 3 highest doses. Because schedule induced drinking tends to be a post pellet phenomenon and lever pressing and drinking tend to be reciprocally related under these conditions, it is difficult to determine from the present results if the primary affect of angiotensin II is on schedule dependent lever pressing or on schedule induced drinking. Direct observation of the animals in the test environment indicates that when animals are injected with angiotensin II they begin to drink soon after placement in the test chamber. Consequently, lever pressing is decreased drastically during the initial portion of the test session when the animal is drinking. Initially, once animals begin to drink, licking persists in long bursts and then animals return to the more usual pattern of lever pressing, eating, and post pellet drinking.

Even though schedule induced drinking increased under present experimental conditions, it is difficult to speculate about the normal role of angiotensin II in the regulation of body fluids. The most plausible normal functions of angiotensin II appear to be the release of aldosterone involved in conservation of sodium and body fluid retention and an elevation in blood pressure due to a constriction of the peripheral vascular beds following a reduction in arterial renal pressure [5,7]. Because angiotensin II increases sodium efflux in smooth muscle [9] and constricts capillaries, it might also have a specific effect on the sodium ionic channel in excitable nerve cell membrane. Under these conditions the relatively large doses which were employed might have resulted in significant small amounts of angio-

tensin II crossing the blood brain barrier which enhanced the sodium sensitive mechanism of the lateral hypothalamus normally involved in drinking [11]. If this interpretation is correct, then angiotensin II has only peripheral normal effects and the increases in schedule induced drinking is an artifact due to the large doses administered and possible penetration into the lateral hypothalamus either through a leaky blood brain barrier or through the choroid plexuses and via the ventricular circulation. Recent evidence indicates that the direct administration of angiotensin II to nerve cells by microiontophoresis affects many different parts of the brain [11]. However, hypothalamic sodium sensitive cells which were examined seem to be the most sensitive. Because a large variety of sites was not sampled, the possibility exists that cells of other regions such as the subcommissural organ [6] and the lateral preoptic area [3] might be more sensitive. It is appealing to speculate that the subfornical organ also contains sodium sensitive cells which might be involved in the regulation of ventricular fluid composition and/or body fluid regulation just as the subcommissural organ and the lateral preoptic-lateral hypothalamic region have been so implicated. It is possible that many so-called sodium receptors which border on the ventricles exist [2]. Because angiotensin II definitely affects sodium permeability in excitable tissue [11], it can be expected to have differential effects on any of the sodium sensitive cells of the brain provided they are accessible to diffusion from the ventricles or by leaks or holes in the capillary blood brain barrier.

If these interpretations are correct, then the renin-angiotensin and related enzyme systems found in the brain [4] are normally involved only in vascular function. Consequently, angiotensin II induced drinking is due to a pharmacological action on drinking related brain neurons and the normal physiological action of angiotensin II on body fluid regulation is restricted to the regulation of kidney function.

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