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Effect of Losartan Potassium and Deoxycorticosterone Acetate on Tail Skin Temperature Response to Acute Administration of Angiotensin II

MELVIN J. FREGLY[1] AND NEIL E. ROWLAND

Departments of Physiology and Psychology, University of Florida, Colleges of Medicine and Liberal Arts & Sciences, Gainesville, FL 32610

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FREGLY, M. J. AND N. E. ROWLAND. *Effect of losartan potassium and deoxycorticosterone acetate on tail skin temperature response to acute administration of angiotensin II.* PHARMACOL BIOCHEM BEHAV 43(1) 229-233, 1992.— The potent vasoconstrictor peptide, angiotensin II (AII) (200 μg/kg, SC), increases tail skin temperature (TST) and tail blood flow when acutely administered either peripherally (SC) or centrally (ICV) to rats. Colonic temperature declines with the increase in TST. These responses are apparently mediated by way of AII subtype receptor AT_1 because they are blocked by acute administration of the nonpeptide AT_1 receptor antagonist, losartan potassium (DuP 753) (10 mg/kg, SC). The responses were also blocked by the peptide AII receptor antagonist, saralasin, at 100 μg/kg SC. Chronic administration of the steroid deoxycorticosterone acetate (DOCA, 250-300 μg/day for 45 days) sensitized the response of TST to acute administration of AII. The increase in responsiveness resulting from chronic treatment with DOCA is consistent with the increase in dipsogenic responsiveness to AII under similar conditions. The latter was shown to be correlated with the upregulation of AII receptors in the diencephalon. While the location of the AII receptors mediating the increase in TST is not known with certainty, it is reasonable to suggest that they are also upregulated by chronic treatment with DOCA.

Tail skin temperature Colonic temperature Angiotensin II Losartin potassium DuP 753 Nonpeptide angiotensin II receptor antagonist Saralasin Peptide angiotensin II receptor antagonist Deoxycorticosterone acetate

IN addition to its well-known effect on blood pressure, vascular resistance, and drinking, administration of angiotensin II (AII) also induces hypothermia in rats (7). Thus, peripheral administration of graded doses of AII induced a dose-dependent hypothermic response characterized by vasodilation of the tail, reduction in rate of oxygen consumption, and a fall in colonic temperature (CT) (7). Thus, the physiological responses to acute administration of AII suggest that it is interpreted by the rat as an induction of a sudden heat load.

Additional studies from this laboratory have shown that peripheral administration of angiotensin I (AI) also induces a similar hypothermic response that is abolished by pretreatment with the AI-converting enzyme inhibitor, captopril (7). Further, the hypothermic response is elicited when AII is administered acutely by the ICV route (8). Pharmacological antagonism of either cholinergic, β-adrenergic, or opioid receptors or inhibition of prostaglandin synthesis was without effect on the AII-induced increase in tail skin temperature (TST) (7,8). Peripheral administration of angiotensin III failed to induce the hypothermic response characteristic of AII (8). Thus, the increase in TST appears to be mediated by AII receptors.

The purpose of the present study was to assess the effect of the new nonpeptide AII receptor antagonist, losartan potassium (DuP 753), as well as the peptide AII receptor antagonist, saralasin, on the hypothermic response to AII. Losartan preferentially engages the AT_1 subtype of AII receptor, while saralasin does not differentiate between AT_1 and AT_2 subtypes. The study is therefore designed to assess whether one or both subtypes are involved in the response of TST to AII. In addition, studies were carried out to determine whether chronic treatment of rats with the mineralocorticoid hormone, deoxycorticosterone acetate (DOCA), increased the responsiveness of TST to administration of AII. A similar regimen

[1] Requests for reprints should be addressed to Dr. Melvin J. Fregly, Department of Physiology, Box 100274, University of Florida, College of Medicine, Gainesville, FL 32610.

of DOCA upregulates the AII receptor (subtype unknown) in brain and enhances the drinking response to AII (2).

METHOD

General Procedures

Naive, male rats of the Sprague-Dawley (Harlan Industries, Indianapolis, IN) strain weighing 200–280 g were used. They were kept three per cage in a room maintained at 25 ± 1°C and illuminated from 0700–1900 h. All rats were allowed tapwater to drink and Purina Lab Chow (#5001, Ralston Purina Co., St. Louis, MO) to eat ad lib.

TST and CT were measured at an ambient temperature of 25 ± 1°C while rats were restrained in lucite tunnel-type cages. CT was measured with a copper-constantan thermocouple inserted 5 cm into the colon of each rat. An additional thermocouple was placed on the dorsal surface at the base of the tail for the measurement of TST. Both were secured to the tail with a small piece of adhesive tape. The thermocouples were connected to a recording potentiometer that recorded the temperature of each thermocouple at 6-min intervals. In all studies, measurements were made at the same time each day (9 a.m.) to minimize any circadian variation. In all experiments, rats were allowed 1 h to adjust to the restraining cages, and control measurements were recorded for 30 min. Although the protocols were similar, different drugs were administered in each of the experiments. Groups consisted of six rats each.

In the third experiment, in which DOCA was used to assess its effect on the TST response to AII, each rat in the treated group was implanted SC with a silastic tube (602-235, 50 mm long) containing crystalline DOCA. The tubes were sealed at both ends with silastic cement. These tubes have been shown to release DOCA at a rate of approximately 250–300 μg/day (5,9). Each rat in the second group was implanted SC with an empty silastic tube. At the time of the experiment, the tubes had been in place for 45 days.

AII (human sequence, A9525) was purchased from Sigma Chemical Co. (St. Louis, MO). Saralasin (Sar^1, Ala^8-AII, A8026) and DOCA (D7000) were also purchased from Sigma. Losartan potassium was the gift of Dr. Ronald D. Smith of the Du Pont Merck Pharmaceutical Co. (Wilmington, DE).

Statistical analysis was carried out by a repeated-measures analysis of variance (ANOVA). Comparison between individual means was made using the Newman-Keuls posthoc analysis.

RESULTS

Experiment 1: Effect of the Nonpeptide AII Receptor Antagonist, Losartan Potassium, on AII-Induced Hypothermia

Losartan potassium (10 mg/kg, SC) was administered 15 min prior to administration of AII (75 μg/kg, SC). This dose of losartan was chosen for use in this experiment because it completely antagonized the dipsogenic response to AII in an earlier study (3). Losartan alone had no significant effect on either TST or CT of rats during the first 15 min of the experiment (Figs. 1A and 1C). When AII was administered (at time 0 in Fig. 1), the group receiving saline 15 min prior to AII had an increase in TST of approximately 2.5°C. CT of this group decreased approximately 0.9°C by the end of the experiment (Figs. 1B and 1D). Administration of losartan prior to administration of AII prevented the rise of TST (Fig. 1A). It also attenuated the effect of AII on CT in that a decrease of approximately 0.5°C was observed by the end of the experiment (Figs. 1B and 1D).

Experiment 2: Effect of the Peptide AII Receptor Antagonist, Saralasin, on AII-Induced Hypothermia

This experiment was carried out to assess the ability of the peptide AII receptor antagonist, saralasin, to block the TST response to administration of AII. Four groups of rats were used. After a control period of 0.5 h, during which TST and CT of all rats were measured, group 1 was administered isotonic saline (1 ml/kg, SC), group 2 AII (200 μg/kg, SC), group 3 saralasin (100 μg/kg, SC), and group 4 both AII and saralasin. TST of the AII-treated group increased maximally approximately 4°C within 12 min after treatment (Figs. 2A and 2C). The group receiving saralasin increased their TST approximately 1°C within 12 min after treatment and this response was blocked by saralasin. TST of the saline-treated group decreased gradually during the course of the experiment.

CT of the saline-treated and saralasin-treated groups decreased gradually during the course of the experiment while CTs of the AII-treated and AII plus saralasin-treated groups decreased within 6 min after treatment and reached minimal levels approximately 30 min after treatment (Figs. 2B and 2D). During the first 30 min, coadministration of saralasin prevented the reduction in CT to the level observed for the group given AII alone. There were no differences among the groups thereafter. Thus, administration of saralasin in combination with AII attenuated the increase in TST and decrease in CT characteristic of administration of AII alone.

Experiment 3: Effect of Chronic Administration of DOCA on the Response of TST to Administration of AII

The objective of this experiment was to determine whether chronic treatment with DOCA increased the responsiveness of TST to acute administration of AII as had been observed previously for the dipsogenic response (2).

Two groups of six rats weighing between 400–450 g were used. Each rat in the first group had been treated with DOCA for 45 days prior to the experiment as described in the Method section. The second group consisted of untreated controls. Four separate studies were carried out. Each was similar to the others, but used a different dose of AII (50, 75, 100, and 200 μg AII/kg, SC). At least 3 days separated the succeeding studies.

The first study was carried out in the same fashion as those described above. At the end of the control period, both groups of rats were administered 50 μg AII/kg SC. The group pretreated with DOCA had a rise in TST of about 2.5°C within 18 min after treatment (Figs. 3A and 3B). The control group did not show a TST response to administration of AII. CTs of both groups decreased during the course of the experiment. That of the DOCA-treated group fell about 0.8°C while that of the control group fell about 0.4°C (Figs. 3C and 3D). There were no significant differences between the CTs of the two groups at any time during the course of the experiment.

Administration of graded doses of AII produced the expected dose-related increase in TST and decrease in CT. At each of the four doses of AII tested, DOCA-treated rats showed an increase in the effect of AII, with increased peak TST, duration of elevation of TST, and greater decrease in CT. The data for the 50- and 100-μg/kg doses are shown in Fig. 3 and 4, and a summary for all doses is given in Fig. 5,

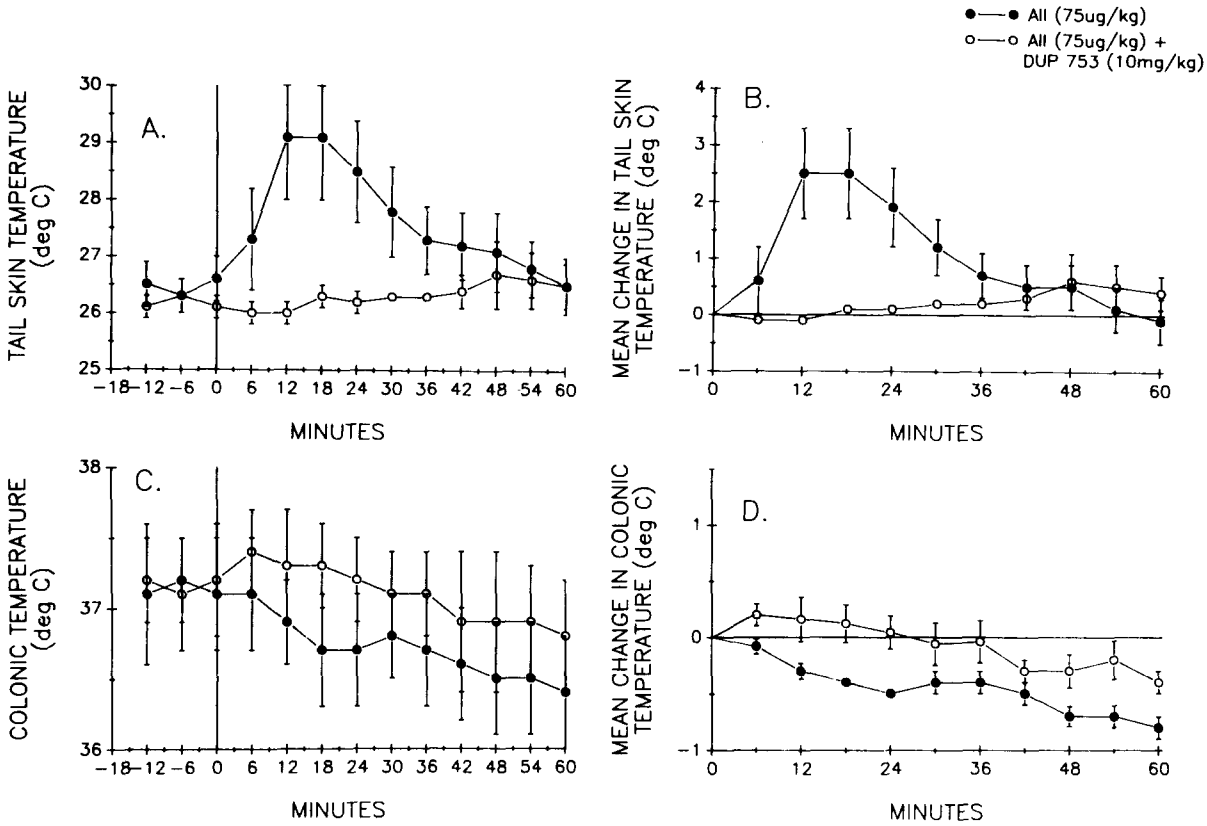

FIG. 1. Effect of losartan potassium (DuP 753) on the response of TST and CT of rats to administration of A II. TST (A), CT (B), change in TST (C), and change in CT (D) are shown. One SE is set off at each mean. The groups and doses of drugs are designated in the figure. A II was administered at time 0 while losartan potassium was administered 15 min prior to administration of A II.

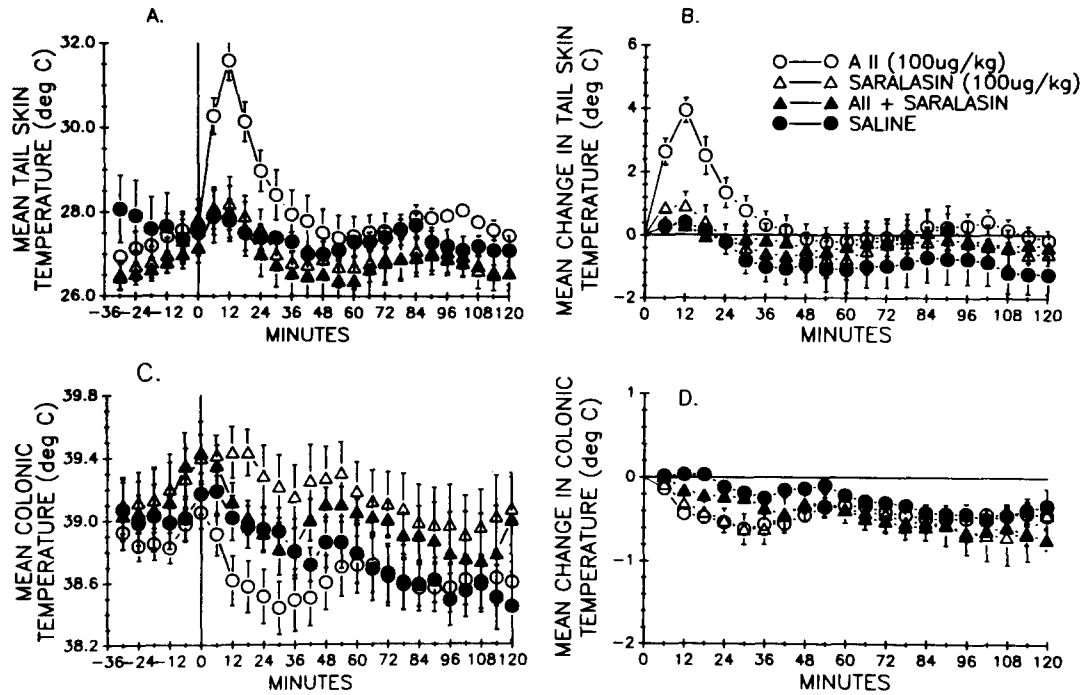

FIG. 2. Effect of administration of saralasin (100 μg/kg, SC) on TST and CT of rats. Other details as in Fig. 1.

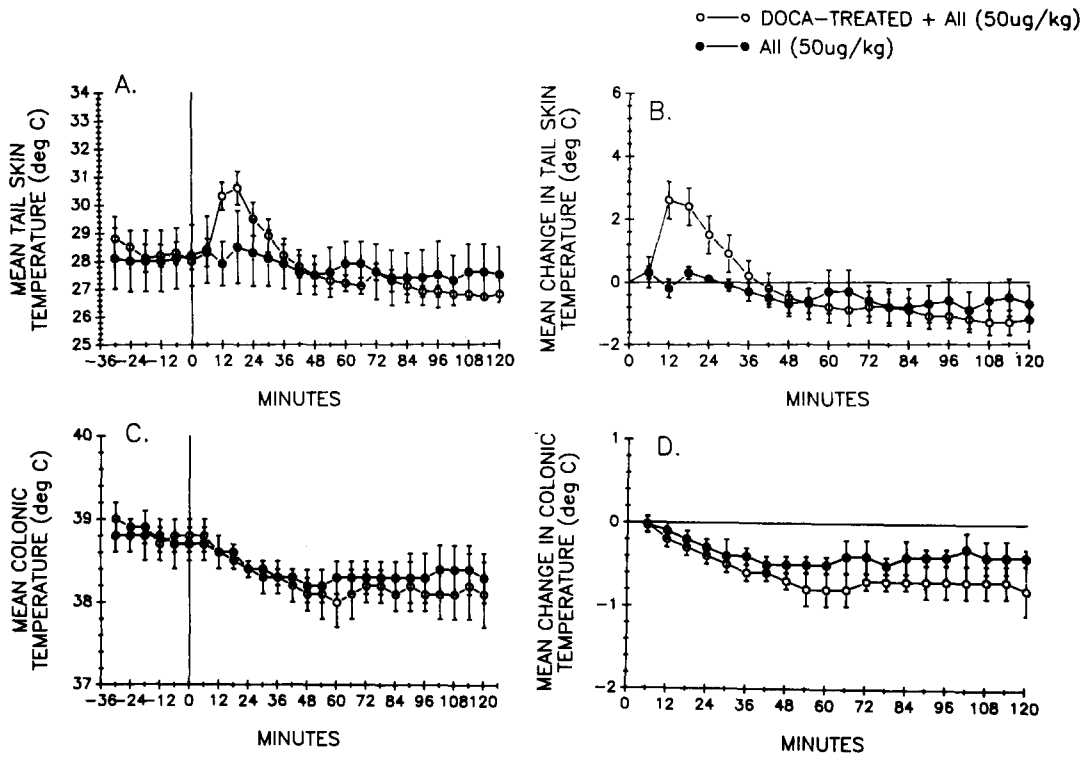

FIG. 3. Effect of chronic administration of DOCA on TST and CT responses to acute administration of A II (50 μg/kg, SC). Other details as in Fig. 1.

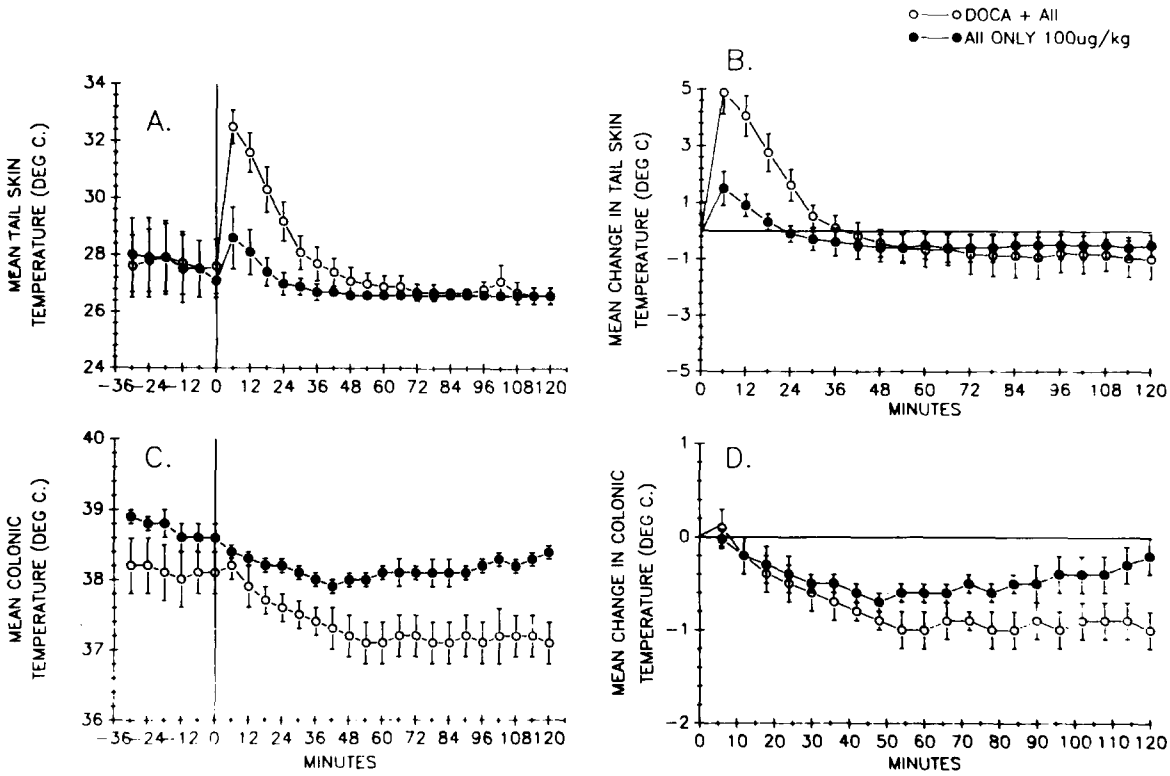

FIG. 4. Effect of chronic administration of DOCA on TST and CT responses to acute administration of A II (100 μg/kg, SC). Other details as in Fig. 1.

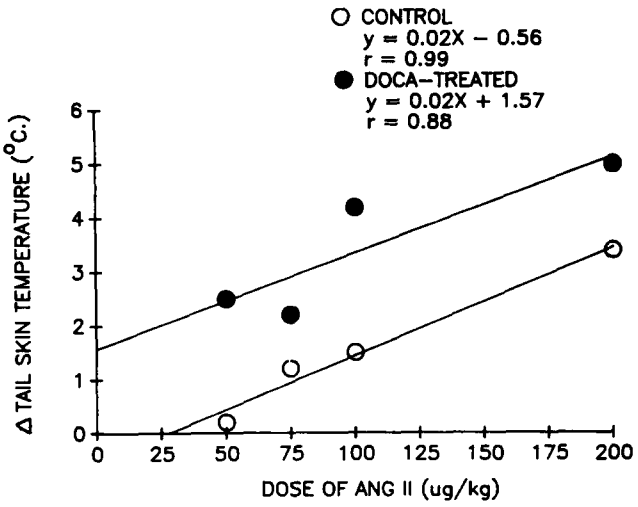

FIG. 5. Relationship between maximal increase in TST and dose of A II administered to DOCA treated and control groups. There was a direct linear relationship between the two variables. The equations of the lines and the correlation coefficients are shown for each group.

which shows the relationship between maximal change in TST and dose of AII administered to DOCA-treated and control groups. There is a positive linear relationship between change in TST and dose of AII for both groups, but the slopes do not differ significantly. However, there is a significant ($p < 0.05$) difference between the intercepts of the two lines. Thus, chronic treatment with DOCA increased the responsiveness of TST to administration of AII.

DISCUSSION

Vasodilation of the vasculature of the tail of the rat in response to administration of AII is a surprising response in view of the fact that it is known to be a potent vasoconstrictor agent. As mentioned initially, the rat responds to administration of AII as though it was subjected to a sudden heat load. Metabolic heat production is reduced, heat loss is increased via an increase in TST, and as a consequence CT is reduced (7). The effect on TST occurred within 6 min after SC administration of AII and was maximal by 12 min. The CT responded within a similar time frame. Further, the maximal increase in TST was dose dependent (Fig. 5).

The dependence of the response upon AII was shown by the fact that both the nonpeptide AII receptor blocker, losartan, and the peptide AII receptor blocker, saralasin, could prevent it (Figs. 1 and 3). These results suggest that the response is mediated by AT_1 receptors (3). Earlier studies showed that angiotensin III had no effect on TST (8). Hence, it seems unlikely that this metabolite of AII is associated with the increase in TST.

Further evidence that receptors for AII mediate the response is the fact that chronic administration of the steroid DOCA increased the responsiveness of TST to administration of AII (Figs. 4 and 5). Chronic treatment of rats with DOCA has been shown in earlier studies from this and other laboratories to increase both the drinking response to administration of AII and to upregulate specific receptors for AII in the diencephalon (4,6,9). Although not tested in the earlier study, it is likely that receptors for AII in other tissues and organs of DOCA-treated rats are also upregulated. It is not known with certainty at what organ(s) AII acts to induce the changes in TST. The most likely possibility is the hypothalamus because one of the known roles of TST is in the regulation of heat loss (1). The response elicited by administration of AII is consistent with a change in the set point temperature at which the hypothalamus regulates body temperature. An earlier study from this laboratory showed that ICV administration of AII can elicit an increase in TST, thus suggesting that receptors within the area of the brain to which the cerebroventricular fluid has access can initiate the response (7). Other studies showed that neither cholinergic-, adrenergic-, opioid-, nor prostaglandin-type activity plays a role in the increase of TST accompanying administration of AII because inhibitors of these agents were ineffective in preventing the response (8). Additional studies are needed to assess the mechanism beyond the AII receptor that may be responsible for the vasodilation of the tail and increase in TST induced by administration of AII.

These results are illustrative of short-term thermoregulatory responses to administration of AII in the rat. It would be of interest to determine the effect of chronic administration of AII on CT and TST of rats. An experiment to assess this remains to be carried out.

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