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The Effects of Central and Peripheral Angiotensin on Hypertension and Nociception in Rats

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IRVINE, R. J. AND J. M. WHITE. *Effects of central and peripheral angiotensin on hypertension and nociception in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 37–41, 1997.—Spontaneously hypertensive rats (SHRs) rats have been reported to have decreased sensitivity to pain, but as yet a mechanism has not been identified. This study investigated the effects of subcutaneous and intracerebroventricular (ICV) infusions of angiotensin II on blood pressure, locomotor activity, and tailflick and hot plate latencies in the Wistar–Kyoto (WKY) and outbred Wistar rat. Peripheral but not central administration of angiotensin II (567 μg/kg/day) increased hot plate latencies in WKY and Wistar rats to a level equivalent to that observed in the SHR. Peripheral administration of norepinephrine (50 and 100 mg/kg/day) to WKYs increased blood pressure but had no effect on hotplate latency. ICV administration of losartan (1&3 mg/kg/day) to SHRs had no effect on blood pressure or nociception. The results indicate that angiotensin II has a role in the altered pain perception observed in the SHR and that its site of action is peripheral. © 1997 Elsevier Science Inc.

Spontaneously hypertensive rat Wistar–Kyoto rat Analgesia Angiotensin Losartan

GENETICALLY hypertensive rats have been reported to have decreased sensitivity to pain (23,14). Although a common mechanism underlying the strain differences in pain sensitivity and blood pressure has not been identified, the renin-angiotensin system may play an important role (10,11). Treatment of spontaneously hypertensive rats (SHRs) with the angiotensin converting enzyme inhibitor captopril during the development phase of hypertension has been reported to prevent the onset of hypertension (13,4). Captopril also decreases latencies of SHRs in the hot plate test (11). The AT-1 receptor antagonist losartan has similar effects in the SHR, but hydralazine, an anti-hypertensive agent acting independently of the renin-angiotensin system, does not influence the increased latencies of the SHRs (11).

One hypothesis to explain the action of ACE inhibitors such as captopril on pain sensitivity is that they influence endogenous opioid systems by virtue of their ability to inhibit enzymes such as neutral endopeptidase 24.11 (6,15). However, the observation that losartan, a specific AT-1 receptor antagonist, has a similar effect to captopril on nociception suggests that the effect on pain described above is in some way mediated by angiotensin II acting at AT-1 receptors. In addition, angiotensin itself has an analgesic effect when acutely administered intracerebroventricularly (ICV) in rats and rabbits and this is blocked by the nonselective angiotensin antagonist saralasin (10,7).

In view of the possibility that increased levels of angiotensin are responsible for the increased hot plate latencies in the SHR, we investigated the effect of angiotensin infusions in the normotensive Wistar–Kyoto (WKY) rat and in the outbred albino Wistar strain. If angiotensin plays a role in altered pain responses in the SHRs, then infusion of angiotensin in the normotensive rat strains should mimic the pattern of nociceptive responses seen in the hypertensive SHR strain.

All components of the renin-angiotensin system have been found in the brain as well as in peripheral tissues (16). In view of the central analgesic actions of angiotensin described above (10,7), and the suggestion that the central renin-angiotensin system is the primary site for the cardiovascular actions of ACE inhibitors and angiotensin antagonists in SHRs (20,19), central as well as peripheral administration of angiotensin was evaluated to clarify the site of action.

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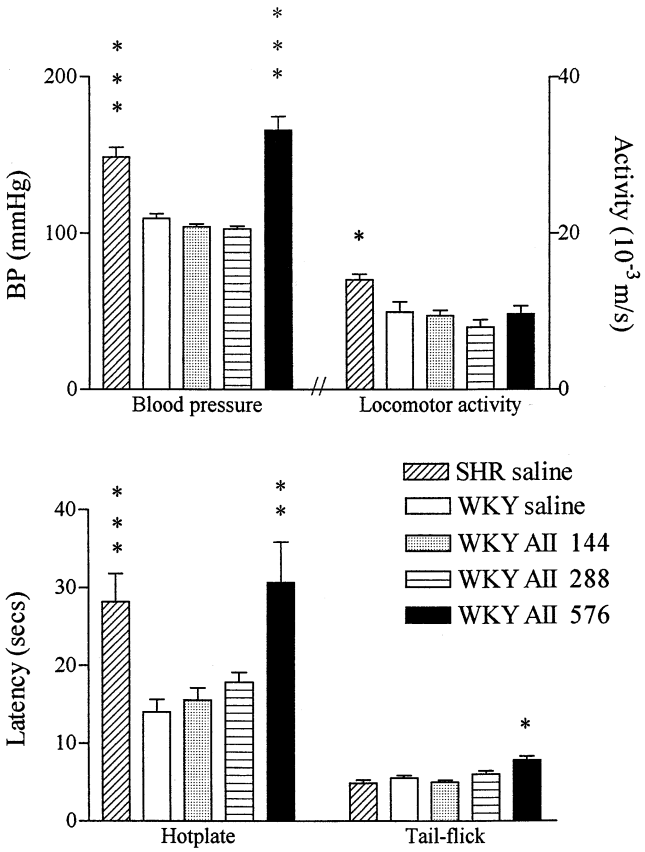

FIG. 1. Means and SEM for systolic blood pressure, locomotor activity, hotplate and tailflick latencies in WKY and SHR rats. Effect of angiotensin II in μg/kg/day for 10 days SC. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to WKY saline, n = 6–8.

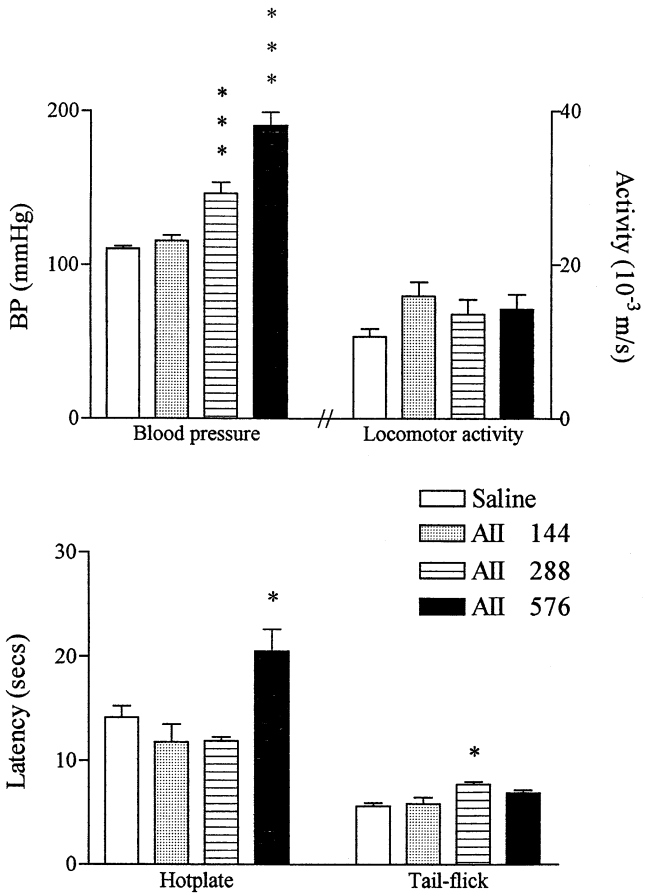

FIG. 2. Means and SEM for systolic blood pressure, locomotor activity, hotplate and tailflick latencies in Wistar rats. Effect of angiotensin II in μg/kg/day for 10 days SC. $*p < 0.05$, $***p < 0.001$ compared to Wistar saline, n = 7–11.

The central effect of the angiotensin AT-1 receptor antagonist losartan in the hypertensive SHR strain was also investigated, as this drug has been shown to have an effect on blood pressure and nociception in SHRs. The effect of losartan is presumably mediated via a direct effect on AT-1 receptors, but controversy exists as to whether or not losartan has central actions (2,17). The purpose of the present study was to confirm the earlier findings and to determine whether the effect of losartan was centrally or peripherally mediated.

As a control, a hypertensive agent which acts independently of the renin-angiotensin system, norepinephrine, was used to demonstrate that any change in nociceptive response was not secondary to a change in blood pressure.

Tail flick was used as a measure of reflex response to pain and the hot plate test as a response which involved supraspinal sensory processing. Locomotor activity was measured as it has been found to be elevated in genetically hypertensive rats (12,21), but it also served as an index of any general psychomotor depressive or stimulant effects of the drugs used. An increase in water consumption is observed in rats when very low levels of angiotensin II are administered ICV (9), and this was used throughout these experiments to provide a sensitive indication of the access of angiotensin to the cerebral ventricles. Osmotic minipumps were utilised in order that chronic dosing could be undertaken without inducing handling stress associated with repeated injections.

METHODS

Adult male, WKY and SHR rats of the Okamoto-Aoki strain and albino Wistar rats weighing 300–400 g, age-matched were used. They were housed individually in a normal light/dark cycle with food and water ad lib. The guidelines on ethical standards for investigation of experimental pain in animals (24) were followed.

Losartan was provided by Du Pont-Merck Pharmaceuticals, Wilmington, Delaware, USA. Angiotensin II was obtained from Auspep Pty Ltd., Parkville, Australia. L-norepinephrine was obtained from Sigma Chemical Co., Castle Hill, NSW, Australia. Losartan and angiotensin were dissolved in normal saline and l-norepinephrine in ascorbic saline. These solutions were inserted in osmotic minipumps (Alzet model 2002), which deliver at 0.5 μl/h for 14 days. The pumps were primed by incubation in sterile saline for 4 h at 37°C immediately prior to use.

Animals were anaesthetised with Brietal/Nembutal 9:1, 5ml/kg and the osmotic minipumps were inserted subcutaneously between the scapulae under aseptic conditions. For ICV infusions, a brain infusion kit (Alza) was utilised to allow transport of the drug from the pump to the brain. The needle was positioned 4mm deep (skull surface), 1mm lateral and 3 mm posterior relative to Bregma and fixed in place with dental

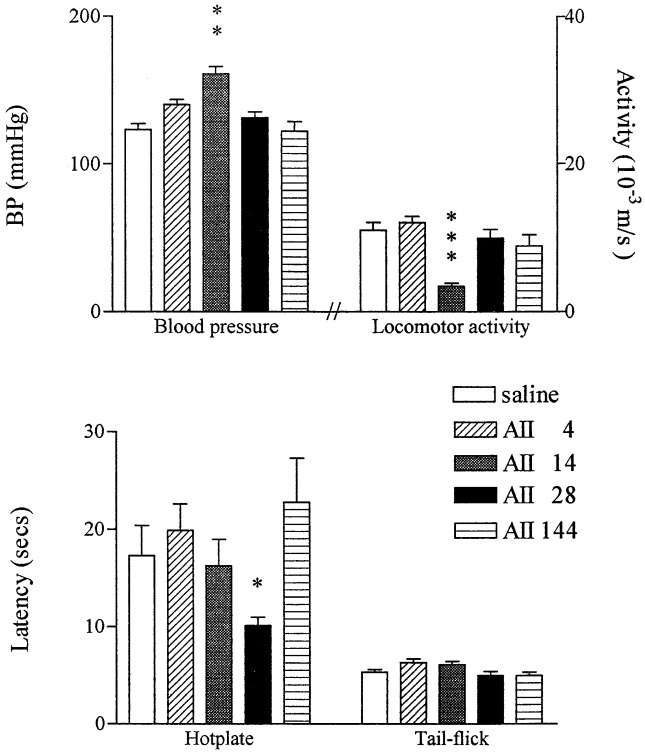

FIG. 3. Means and SEM for systolic blood pressure, locomotor activity, hotplate and tailflick latencies in WKY rats. Effect of angiotensin II in μg/kg/day for 10 days ICV. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to WKY saline, n = 6–8.

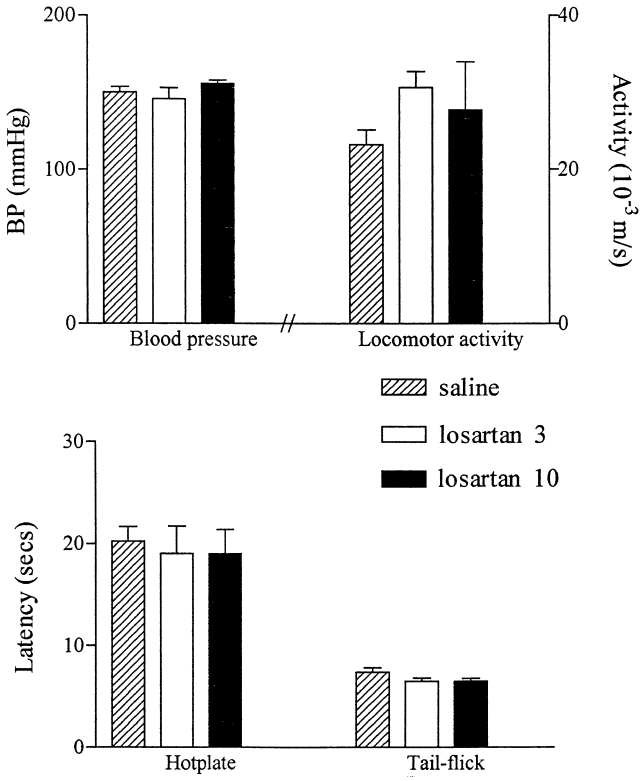

FIG. 4. Means and SEM for systolic blood pressure, locomotor activity, hotplate and tailflick latencies in SHR rats. Effect of losartan mg/kg/day for 10 days ICV. n = 6–8.

cement. The wounds were closed with suture clips and the animals allowed to recover. Testing took place 10 days subsequently. The position of the needle in the ventricles was confirmed post mortem.

Systolic blood pressure was measured by the tail–cuff technique (3). Animals were placed in a 100mm diameter restraint tube and heated to an air temperature of 32°C. Blood flow in the caudal artery was detected by a ultrasonic flowmeter while tail cuff pressures were read on a mercury manometer. Locomotor activity was measured in a perspex activity chamber (30 × 30 cm) utilising infrared detectors linked to a personal computer. Activity was measured for a period of 20 min between the hours of 0900 and 1100 on each experimental day. Tail flick latencies were measured using a Peltier diode as a heat source applied to the ventral surface of the tail. Hot plate latencies were measured on a 52°C plate surrounded by a 10cm high barrier. The end point was the time when the animal licked a foot or jumped over the barrier. Water consumption was measured from day 5 to day 10 of drug treatment.

For each drug treatment the effects of various doses were compared to the effect of saline treatment using one-way ANOVA followed by Dunnet's test; $p < 0.05$ was taken as significant.

RESULTS

The untreated SHR when compared to untreated WKY animals showed higher blood pressures, higher locomotor activity, no difference in tail flick latencies and higher hotplate latencies (Fig. 1).

Subcutaneous (SC) angiotensin infusions in the WKY had no effect on any of the parameters measured at 144 and 288 μg/kg/day (Fig. 1). The highest dose of angiotensin in the WKY produced a pattern similar to the untreated SHR. Blood pressure increased to the same level as the SHR, and hotplate latency increased to a similar level. There was no effect on locomotor activity and a slight increase in tail flick latency.

The results from Wistar rats exposed to identical treatment conditions are shown in Fig 2. Wistar rats showed an increased blood pressure at 288 and 576 μg/kg/day. There was no effect at any dose for locomotor activity, an increase in hotplate latency at the highest dose and a small increase in tailflick latency at a dose of 288 μg/kg/day. The overall pattern of responses was very similar to that seen in Fig. 1 for the WKY strain of rat administered angiotensin.

When angiotensin was infused icv in WKY rats there was an increase in blood pressure only at a dose of 14 μg/kg/day. Blood pressures following administration of angiotensin at 28 and 144 μg/kg/day were not different from saline (Fig. 3). There was a substantial fall in locomotor activity at 14 μg/kg/day with no effect at lower or higher doses. Hot plate latencies were not significantly modified by angiotensin except at a dose of 28 μg/kg/day where a decrease was seen. No effect on tail flick latency was observed for any dose of the drug.

ICV infusions of losartan at doses of 3 and 10 mg/kg/day had no effect on blood pressure, locomotor activity, hot plate or tail flick in the SHR strain (Fig. 4).

Norepinephrine at 50 and 100 mg/kg/day SC raised blood pressure, but did not affect hot plate or tail flick latencies. There was a decrease in locomotor activity at the highest dose (Fig. 5).

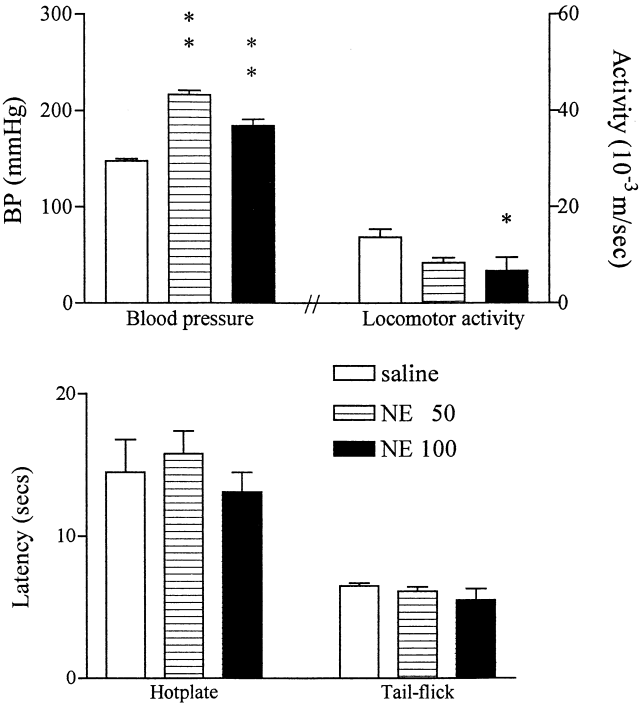

FIG. 5. Means and SEM for systolic blood pressure, locomotor activity, hotplate and tailflick latencies in WKY rats. Effect of norepinephrine mg/kg/day for 10 days SC. *$p < 0.05$, **$p < 0.01$ compared to WKY saline, $n = 8$.

Water consumption was unaffected by 576 μg/kg/day SC infusion of angiotensin (Fig. 6), but was increased 3–5 fold by icv infusions at all doses tested. A slight increase in water consumption was observed for both doses of losartan in the SHRs but was not statistically significant.

DISCUSSION

The results of this study confirmed previous findings (14,23,12,21,11) suggesting that the SHR has a lower sensitivity to pain in the hotplate test and higher locomotor activity. To date, the mechanisms underlying each of these differences have not been clearly defined. However, the present results show that peripheral infusions of angiotensin at 567 μg/kg/day to Wistar and WKY rats increased both blood pressure and hot plate latencies to values comparable to those achieved by untreated SHRs. A small, but significant increase in tail-flick latency was also observed, but there was no change in locomotor activity. Indeed, the only effect of the drugs on locomotor activity in these studies occurred following administration of angiotensin at 14 μg/kg/day ICV to the WKY, where a considerable decrease in activity was observed. The marked increase in hotplate latency in the WKY animals with subcutaneous angiotensin infusion suggests that increased angiotensin levels may be responsible for the increased latencies in the hypertensive animals. From these results it appears that the altered nociceptive response of SHRs, but not their altered activity, may be due to elevated angiotensin.

There was a possibility that the peripherally administered angiotensin was acting at a central site to cause analgesia, but this hypothesis was not supported when the data from the icv infusions were considered. Although blood pressure was raised by ICV angiotensin at 14 μg/kg/day, there was no ob-

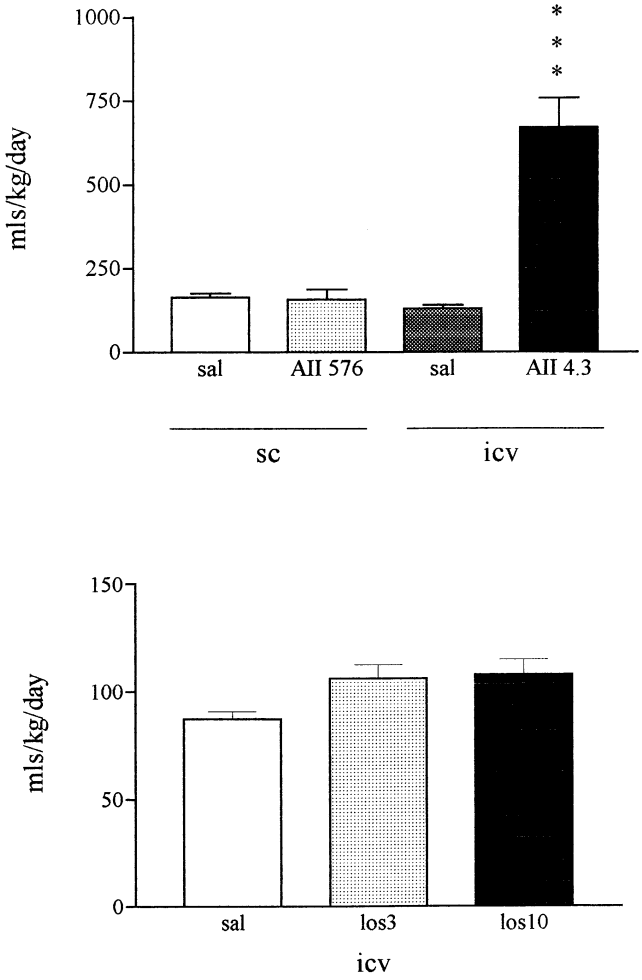

FIG. 6. Means and SEM for water consumption in WKY rats top panel and SHR rats bottom panel. Effect of angiotensin II in μg/kg/day and of losartan mg/kg/day for 10 days, ***$p < 0.01$ compared to saline, $n = 7$–8.

served increase in hotplate or tail-flick latencies at this or any dose, as was evident when it was administered subcutaneously. Thus, chronic ICV infusion of angiotensin at a range of doses in WKY rats cannot mimic the altered nociceptive responses seen in the SHR.

The dipsogenic effect of angiotensin on the subfornical organ is well known (9) and it is clear from our water consumption data that the ICV route of administration was effective. The lack of dipsogenic effect when angiotensin was administered peripherally in the WKY indicates that it is unlikly that the drug was entering the CNS from the periphery to a significant extent, and this cannot be an explanation for the effects on nociception and blood pressure. The minimal increase in water consumption observed with ICV losartan treatment in the SHR also indicates that increased central angiotensin levels are not an explanation for the altered pain perception in these animals.

The lack of change in any of the behavioural parameters when blood pressure was increased with peripheral norepinephrine infusions suggests that the changes observed with angiotensin treatment are not simply a consequence of increased blood pressure. Norepinephrine's effect on locomotor

activity at the highest dose and an observed loss of body weight in these animals indicates that this dose was indeed very high and that increased sympathetic outflow of norepinephrine is an unlikely explanation for the changes following angiotensin administration.

Losartan has been reported to be capable of entering the brain from the peripheral circulation to block central angiotensin receptors (17), but no effect of the drug on blood pressure or nociception was observed here in spite of the chronic dosing at high concentrations directly into the ventricles. The lack of effect on blood pressure following chronic ICV administration of losartan to the SHR is consistent with an earlier study in which the drug was given acutely by the same route (5). Together, these results do not support a role for central AT-1 receptors in the hypotensive effects of losartan nor in its effect on nociception in this model of hypertension.

Although angiotensin is clearly involved in regulation of nociception in these animals, the results do not support a role for centrally located angiotensin. This is not in accordance with the findings of other workers who have demonstrated analgesic effects of ICV angiotensin in the rat and rabbit (10,7). This discrepancy may be explained in terms of dosing. The experiments described here used chronic infusions over 10 days prior to testing. This would allow diffusion of the drug from the site of injection to more distant structures in the CNS. In addition, a constant concentration of drug in the animal for 10 days may result in some adaption. Down-regulation of receptor numbers or changes in feed back circuits may have occurred. The experiments mentioned above (10,7), measured acute effects of bolus injections of angiotensin.

Naloxone blocks the increased hot-plate latency observed in the SHR (22), indicating that endogenous opioid systems are involved in the altered nociceptive response described here. Therefore, it is possible that peripheral angiotensin increases the release of endogenous opioids from sympathetic ganglia for example, or in some other way facilitates the effectiveness of endogenous opioid systems. There is some evidence to support this. Peripherally administered angiotensin II has been shown to influence the release of β-endorphin and ACTH in Holzman rats (18). In addition, there have been reports (8,1) of a decreased expression of POMC in the intermediate and anterior lobes of the SHR compared to the WKY. Thus, it is possible that detailed investigation of the influence of peripheral angiotensin on hypothalamo-pituitary release of stress hormones may provide a clearer understanding of the mechanisms involved in the decreased pain sensitivity of SHRs.

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