Role of Angiotensin II Receptors in Tail Skin Temperature Response to Isoproterenol

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Abstract. **The objective of these experiments was to assess the possibility that the increase in tail skin temperature (T_{SK}) accompanying administration of the β-adrenergic agonist isoproterenol (ISO) was mediated by angiotensin II (AngII) as a result of stimulation of renin release by ISO. Although AngII is known to be a potent vasoconstrictor in mammals, acute administration of this peptide to rats induces a vasodilation of blood vessels in the tail and an increase in T_{SK}. The objective was approached in several ways: (i) use of the nonpeptide AngII receptor antagonist losartan potassium (DuP 753); (ii) use of the peptide AngII receptor antagonist saralasin; (iii) use of the AngI-converting enzyme inhibitor captopril, and (iv) chronic administration of AngII. The rationale for these experiments was that blockade of AngII receptors (Experiments 1 and 2), inhibition of the enzyme that converts AngI to AngII (Experiment 3), or down-regulation of the AngII receptors (Experiment 4) would be expected to prevent any contribution to the ISO-induced increase in T_{SK} by AngII. The results of these experiments are consistent in revealing that the response of T_{SK} to ISO administration is due in part (approximately 55%) to a direct effect of ISO and in part (approximately 45%) to an indirect effect resulting from the ISO-stimulated release of renin from the kidneys and the formation of AngII in the blood.** [P.S.E.B.M. 1993, Vol 203]

Administration of isoproterenol (ISO) to rats increases tail skin temperature (T_{SK}) in a dose-related fashion (1, 2). While ISO, a β-adrenergic agonist, is a vasodilator and could act directly to increase T_{SK} via increased blood flow to the tail, it might also act via the release of renin, with subsequent formation of angiotensin II (AngII). ISO is well known to stimulate the release of renin in the rat (3). While AngII is known generally as a vasoconstrictor agent, its administration to rats induces a dose-related vasodilation of the tail (4, 5). The response is mediated by AngII (type AT_1) receptors (6). Thus, at least a portion of the vasodilatory effect of ISO could occur by way of increased formation of AngII. Hence, the objective of the experiments to be described was to assess this possibility.

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Materials and Methods

General Procedures. Naive male rats of the Sprague-Dawley (Harlan Industries, Indianapolis, IN) strain, weighing 350–400 g, were used. They were kept three per cage in a room maintained at 25 ± 2°C and illuminated from 0700 to 1900 hr. All rats were allowed tapwater to drink and Purina Laboratory Chow (No. 5001, Ralston-Purina Co., St. Louis, MO) to eat *ad libitum*.

T_{SK} and colonic temperatures (T_C) were measured at an ambient temperature of 25 ± 2°C while the rats were restrained in Lucite tunnel-type cages. The cages allowed the rats to rest comfortably within them, but restricted them from turning from head to tail. T_C 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 T_{SK}. Both were secured to the tail with a small piece of adhesive tape.

The temperature of each thermocouple was meas-

ured at 6-min intervals by a recording potentiometer. In all studies, measurements were made at the same time each day (0900 hr) to minimize any potential time-of-day variation. In all experiments, rats were allowed 1 hr to adjust to the restraining cages, after which baseline measurements were made for 30 min. Although the protocols were similar, different drugs were administered in each of the experiments. All drugs administered to the rats were injected subcutaneously through a slot in the cage. Groups consisted of six rats each.

ISO, AngII, and saralasin were purchased from Sigma Chemical Co. (St. Louis, MO). Captopril was the gift from E. R. Squibb Co. (Princeton, NJ), and losartan potassium (DuP 753) was the gift of Dr. Ronald D. Smith of the DuPont Merck Pharmaceutical Co. (Wilmington, DE).

Statistical analyses of T_{SK} and T_C were carried out by a repeated measures analysis of variance (ANOVA). Comparison between individual means was made using the Newman-Keuls *post-hoc* analysis.

Experiment 1: Effect of the Nonpeptide AngII Receptor Antagonist Losartan Potassium on the Response of T_{SK} to Administration of ISO. Four groups of six rats were used. One group was administered losartan potassium (DuP 753; 20 mg/kg, sc) 15 min before the administration of ISO (50 μg/kg, sc). A second group was administered isotonic saline (1 ml/kg, sc) 15 min before the administration of the same dose of ISO. The third group received losartan potassium 15 min before the administration of isotonic saline, while the fourth group received isotonic saline 15 min before the administration of a second dose of isotonic saline (control group). After the second injection of either ISO or saline, T_{SK} and T_C were measured at 6-min intervals for approximately 2 h.

Experiment 2: Effect of the Peptide AngII Receptor Antagonist Saralasin on the Response of T_{SK} to Administration of ISO. This study was carried out in the same way as Experiment 1. Four groups, each containing six rats, were used. The first group was given saralasin (100 μg/kg, sc) 15 min before the administration of ISO (50 μg/kg, sc). The second group was given saralasin 15 min before the administration of isotonic saline (1 ml/kg, sc). The third group was given isotonic saline 15 min before the administration of ISO, while the fourth (control) group was given saline 15 min before the administration of a second dose of saline. Measurements of T_{SK} and T_C continued for 2 hr after the administration of ISO. The dose of saralasin used had been shown in a previous study to inhibit the increase in T_{SK} induced by administration of AngII (4).

Experiment 3: Effect of the AngI-Converting Enzyme Inhibitor Captopril on the Response of T_{SK} to Administration of Isoproterenol. Two groups (six rats each) were used. The first group received captopril (35 mg/kg, ip) 15 min before the administration of ISO (50 μg/kg, sc). The second group was given isotonic saline (1 ml/kg, ip) 15 min before the administration of ISO. The remainder of the protocol was carried out identically to that of Experiment 2 and will not be described in detail.

Experiment 4: Effect of a Chronic Infusion of AngII on the Response of T_{SK} to Administration of Isoproterenol. Two groups (six rats each) were used. One group was anesthetized with penthrane and implanted subcutaneously with an Alzet osmotic minipump (No. 2001; Alza Corp., Palo Alto, CA) containing the octapeptide AngII (7.5 mg/ml) dissolved in saline. The mean nominal amount of AngII released daily from each minipump was 360 μg/kg body wt. The control group was also implanted with an osmotic minipump containing isotonic saline. The rats were infused with AngII for 6 days before this study. At the beginning of the study, the rats were placed in individual tunnel-type cages with thermocouples attached, as described above. After a 1-hr equilibration period, a 0.5-hr period of control measurements of T_{SK} and T_C was begun. At the end of this time, both groups were given ISO (50 μg/kg, sc). The T_{SK} and T_C of each rat were measured at 6-min intervals for a 2-hr period thereafter.

Results

Experiment 1. Administration of ISO (50 μg/kg, sc) induced a maximal increase of 6.0°C in T_{SK} and 0.7°C in T_C (Fig. 1). T_{SK} increased within 18 min after the administration of ISO and reached a maximal level within 42 min (Fig. 1B). It began to decrease from this peak 78 min after treatment with ISO and reached the level of the control group by 132 min. T_{SK} was significantly ($P < 0.01$) greater than that in the untreated control group from 42 min onward, throughout the experiment (Fig. 1B). Both the untreated control group and the group treated with losartan (DuP 753) had no significant change in T_{SK} throughout the experiment (Fig. 1, A and B). The group treated with both losartan and ISO had an increase in T_{SK} that was significantly elevated above that in the control group within 54 min and remained significantly ($P < 0.01$) above that in the control group until 126 min after the injection of ISO (Fig. 1B). The maximal change in T_{SK} achieved by this group was 3.5°C. Thus, administration of losartan decreased the response of T_{SK} to ISO.

The repeated measures two-way ANOVA of T_{SK} revealed a significant ($P < 0.01$) effect of treatments ($F[3, 21] = 15.0$), a significant ($P < 0.01$) effect of time ($F[22, 682] = 25.4$), and a significant ($P < 0.01$) group × time interaction ($F[66, 682] = 9.3$).

In the case of T_C, administration of ISO alone induced an increase within 6 min, which reached a maximum within 36 min (Fig. 1, C and D). By 57 min

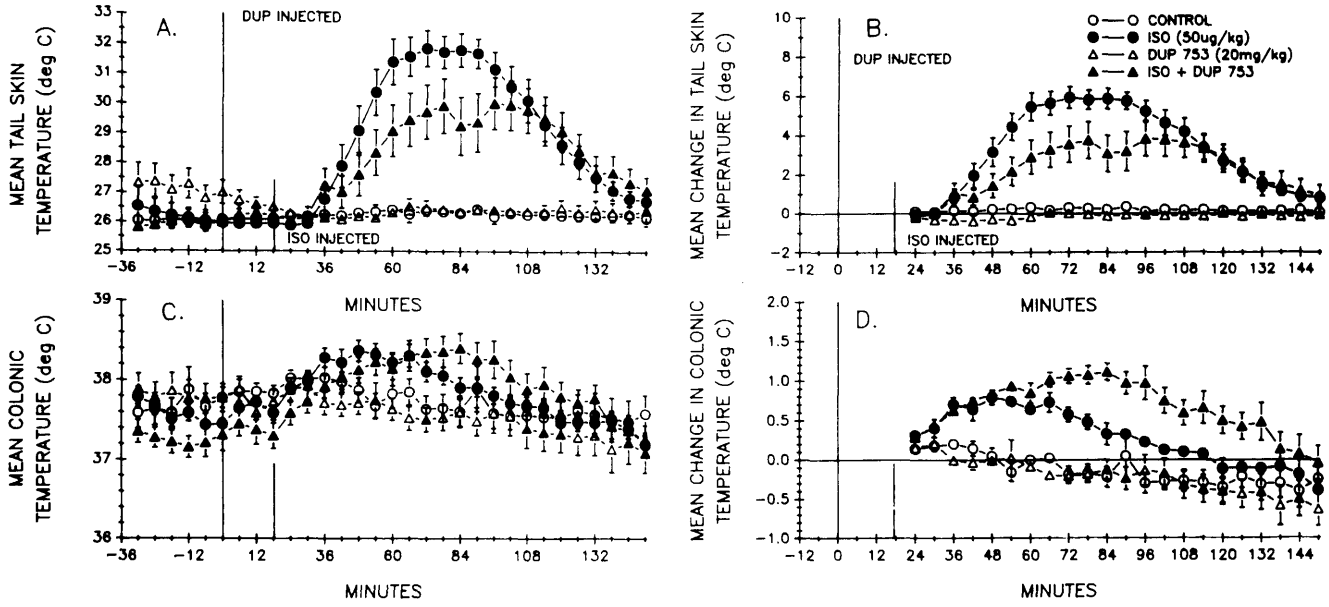

Figure 1. (A) Comparison of the responses of T_{SK} to ISO administration after pretreatment with losartan potassium (DuP 753). The T_{SK} values in the groups administered isotonic saline and losartan potassium alone are also shown. The groups are designated in the figure. Values are the mean ± SE. When SE is not shown, it falls within the symbol. (B) Changes in T_{SK} for the same groups. (C) T_C values in the four groups during the course of the experiment. (D) Changes in T_C values in the four groups.

after the administration of ISO, T_C began to decrease toward the control level, which was reached by 100 min. The T_C values of both control and losartan-treated groups decreased during the course of the study to reach levels 0.4 and 0.6°C below their pretreatment level by the end of the experiment (Fig. 1D). The group administered both losartan and ISO also had an increase in T_C to a level higher (1.1°C) than that observed in the group treated with ISO alone (Fig. 1D). The higher T_C of this group was maintained for a longer period than was observed in the ISO-treated group and returned to the level in the control group at approximately the same rate of decline as in the ISO-treated group (Fig. 1D). Thus, the net result of treatment with losartan and ISO was to increase T_C to a greater level than that achieved with ISO alone and to maintain the higher level for a longer period of time.

The repeated measures two-way ANOVA of change in T_C revealed a significant ($P < 0.01$) effect of treatments (F[3, 32] = 16.6), a significant ($P < 0.01$) effect of time (F[21, 672] = 17.18), and a significant ($P < 0.01$) group × time interaction (F[63, 672] = 2.99).

Experiment 2. The administration of 100 μg saralasin/kg inhibited the increase in T_{SK}, but not T_C, in response to treatment with ISO (Fig. 2, A–D).

The repeated measures two-way ANOVA for change in T_{SK} revealed a significant ($P < 0.01$) effect of treatment (F[3, 17] = 9.77), a significant ($P < 0.01$) effect of time (F[8, 136] = 8.18), and a significant ($P < 0.01$) treatment × time interaction (F[24, 136] = 7.17). The two-way repeated measures ANOVA for change in T_C revealed a significant ($P < 0.01$) effect of time and a significant ($P < 0.01$) treatment × time interaction.

Experiment 3. Administration of captopril attenuated and delayed the ISO-induced rise in T_{SK} (Fig. 3A). A rise in the T_{SK} of the captopril-treated group did occur, but appeared much later (24–30 min) than that in the group given only ISO (12 min; Fig. 3A). The maximal change in T_{SK} (Fig. 3B) was approximately the same for the two groups, possibly due to the difference in T_{SK} before the administration of ISO. The striking feature, however, is the attenuated increase in T_{SK} that accompanied administration of captopril. T_C rose for both groups, but to a greater extent in the captopril-treated group than in the control group (Fig. 3C). This was due, in part at least, to the failure of T_{SK} to rise in the captopril-treated group. The change in T_C was greater and lasted longer in the captopril-treated group than in the control group (Fig. 3D).

Experiment 4. After 1 week of AngII infusion by osmotic minipump, the infused group and their controls were given ISO as in the studies described above. Chronic infusion inhibited the increases in T_{SK} (Fig. 4, A and B) and T_C (Fig. 4, C and D) accompanying administration of ISO.

The repeated measures ANOVA of change in T_{SK} revealed a significant ($P = 0.05$) effect of treatment (F[1, 8] = 4.14), a significant ($P < 0.01$) effect of time (F[19, 152] = 19.34), and a significant ($P < 0.05$) treatment × time interaction (F[19, 152] = 1.66). In the case of change in T_C, the analysis revealed a significant ($P < 0.05$) effect of treatment (F[1, 9] = 5.10), a significant ($P < 0.01$) effect of time (F[21, 189] = 8.95),

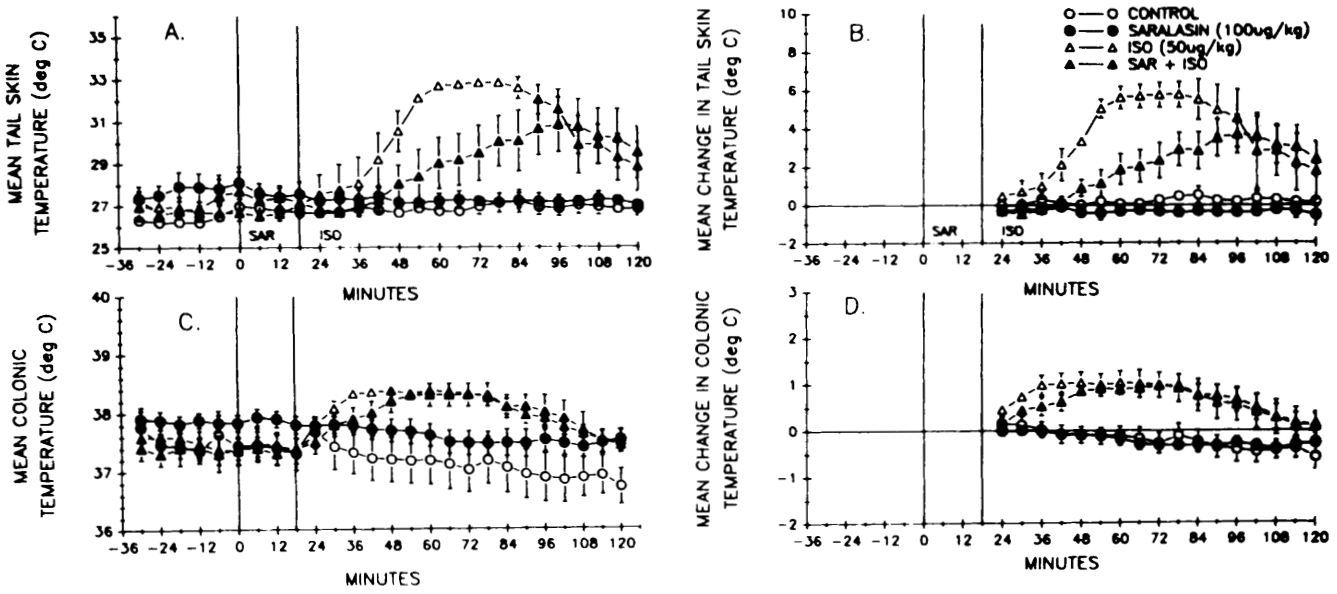

Figure 2. Comparison of the responses of T_{SK} (A), change in T_{SK} (B), T_C (C), and change in T_C (D) to ISO administration to rats pretreated with 100 μg saralasin/kg, sc. The groups are designated in the figure. Means ± SE are shown.

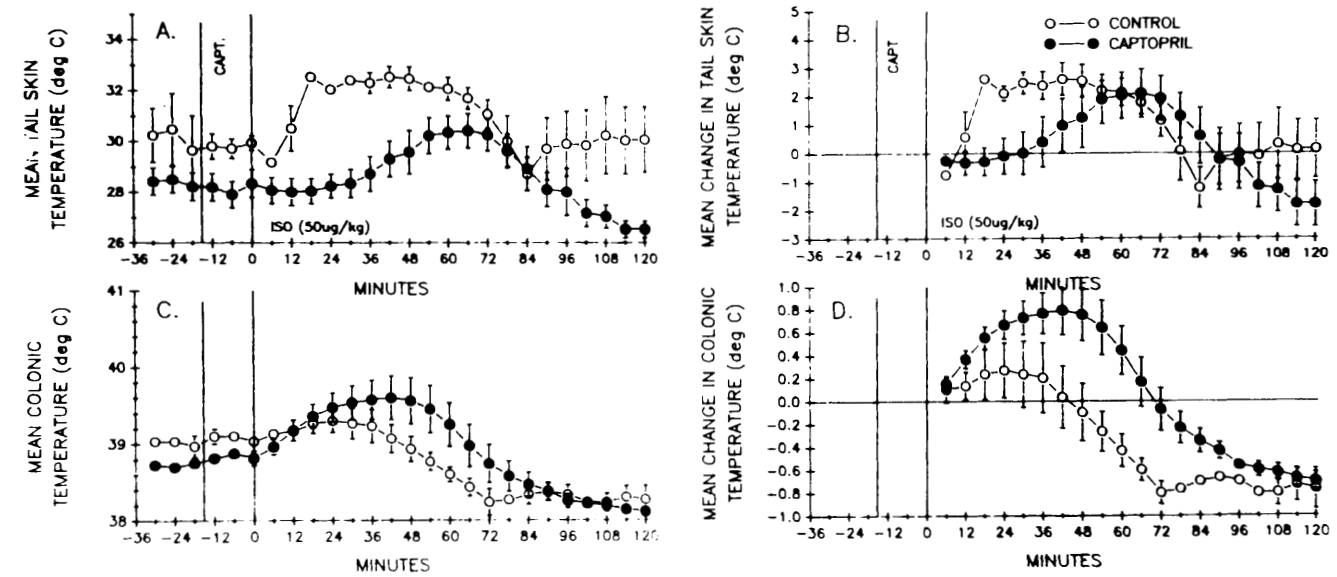

Figure 3. Comparison of the responses of T_{SK} (A), change in T_{SK} (B), T_C (C), and change in T_C (D) to ISO administration to rats pretreated with captopril (35 mg/kg, ip). The groups are designated in the figure. Means ± SE are shown.

and a significant ($P < 0.01$) treatment × time interaction (F[21, 189] = 2.12).

Discussion

The objective of these experiments was to assess the possibility that the increase in T_{SK} accompanying ISO administration was mediated by AngII as a result of stimulation of renin release by ISO. This objective was approached in several ways: (i) use of the nonpeptide AngII receptor antagonist losartan, (ii) use of the peptide AngII receptor antagonist saralasin, (iii) use of the AngI-converting enzyme inhibitor captopril, and (iv) chronic administration of AngII. The rationale for these experiments was that blockade of the AngII receptors would be expected to prevent any contribution to the ISO-induced increase in T_{SK} by AngII. A comparison of the areas under the curves in Fig. 1B revealed that blockade of the AT_1 receptors resulted in approximately a 30% reduction in responsiveness (Fig. 1B). Hence, under the conditions of this experiment, it would appear that the T_{SK} response is about 70% attributable to stimulation of β-adrenergic receptors and 30% attributable to stimulation of AngII (AT_1) receptors. Previous studies from this laboratory showed

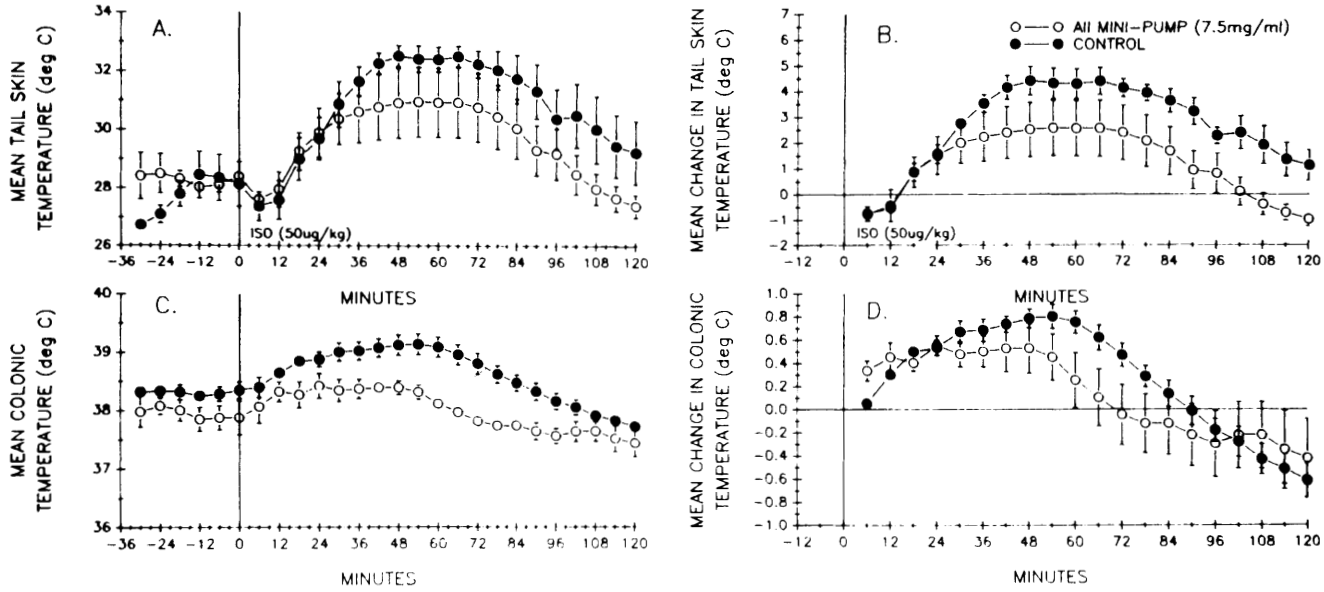

Figure 4. Comparison of the responses of T_{SK} (A), change in T_{SK} (B), T_C (C), and change in T_C (D) to ISO administration to rats infused with AngII for 6 days. The groups are designated in the figure. Means ± SE are shown.

that the response of T_{SK} to ISO administration was completely blockable by prior administration of β_1-, but not β_2-, adrenoceptor antagonists (7).

The greater increase in T_C in the losartan-treated group accompanying administration of ISO compared to that in the group receiving only ISO illustrates the importance of the tail of the rat in the dissipation of heat (8) (Fig. 1B). Thus, the increase in heat production induced by ISO could not be dissipated as readily by the losartan-treated group because the response of T_{SK} was attenuated. A comparison of the areas under the curves in Fig. 1D revealed that blockade of AngII receptors by losartan resulted in an approximately 67% increase in T_C above that in the group treated with ISO alone.

Since losartan potassium inhibited the T_{SK} response to ISO, an attenuation of this response by saralasin would also be expected, as it is known to inhibit both AT_1 and AT_2 receptors for AngII. Thus, treatment with saralasin (100 μg/kg) in Experiment 2 resulted in a 46% reduction in the T_{SK} response to ISO administration. There was also a 14% reduction in the response of T_C to ISO administration when the rats were pretreated with saralasin. The results suggest that a greater reduction in the T_{SK} response to ISO administration was achieved with saralasin, an AT_1 and AT_2 receptor blocker, than with losartan, an AT_1 receptor blocker. These results suggest that the AT_2 receptor may also contribute to the increase in TST induced by ISO. However, confirmation of this suggestion must await the results of studies in which additional doses of losartan and saralasin as well as an AT_2 receptor blocker are used.

A more striking difference between the studies in which losartan and saralasin were used is seen in a comparison of the T_C responses (Figs. 1D and 2D). The T_C in the saralasin-treated group failed to remain elevated above the level in their control group, whereas that in the losartan-treated group did. The greater and longer elevation of T_C in the losartan-treated group was explained above as being due to the inability of this group to dissipate heat as readily as controls, as T_{SK} failed to rise as high as that in controls. This explanation assumes that losartan did not affect the ability of ISO to increase heat production. The failure of the saralasin-treated group to manifest an elevation in T_C above that in its control was unexpected. These results suggest that either AT_2 receptors can influence T_C under these conditions or the increase in heat production induced by ISO was attenuated by saralasin. Additional studies will be required for a better understanding of these possibilities.

Blockade of the AngI-converting enzyme by captopril attenuated the increase in T_{SK} characteristically accompanying administration of ISO, again suggesting that the release of AngII contributes to the increase in T_{SK} under these conditions. A comparison of the areas under the curves of change in T_{SK} (Fig. 3B) for captopril-treated and control groups indicated that treatment with captopril induced a 45% reduction the response of T_{SK} to administration of ISO. This is similar to that induced by treatment with saralasin in Experiment 2.

The greater increase in T_C in the captopril-treated group (290%) accompanying administration of ISO compared to that in the group receiving only ISO again illustrates that the increase in heat production induced by ISO could not be dissipated as readily by the capto-

pril-treated group because the response of T_{SK} was attenuated.

The rationale for the use of an infusion of AngII was based on the supposition that an increase in the blood concentration of AngII would downregulate both of its receptor subtypes. The net result of this would be a reduced responsiveness of T_{SK} to ISO administration, assuming that AngII and its receptors play a role in the response. This assumption appears to be correct, at least in part, because the responsiveness of T_{SK} to ISO administration was reduced in the AngII-infused group compared to that in the control group (Fig. 4B). Comparison of the areas under the curves of change in T_{SK} and change in T_C in Fig. 4, B and D, indicates that chronic infusion of AngII resulted in 45% and 40% decreases, respectively, in responsiveness to ISO. The percent change in T_{SK} was similar to that observed when either saralasin or captopril was administered before ISO, but was a greater reduction than that observed with the use of losartan. The discrepancy may be accounted for by the possibility that either the dose of losartan used was not high enough to block all AT_1 receptors or blockade of both AT_1 and AT_2 receptors is required for maximal effects. Within this limitation and in spite of the quantitative differences, the results of these experiments are consistent in revealing that the response of T_{SK} to ISO administration is due in part (approximately 55%) to a direct effect of ISO and in part to an indirect effect (approximately 45%) resulting from the ISO-stimulated release of renin from the kidneys and the formation of AngII in the blood.

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