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Effect of Administration of Angiotensin II and Isoproterenol, Alone and in Combination, on Drinking and Tail Skin Temperature of the Rat

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FREGLY, M. J. AND N. E. ROWLAND. *Effect of administration of angiotensin II and isoproterenol, alone and in combination, on drinking and tail skin temperature of the rat.* PHARMACOL BIOCHEM BEHAV **51**(1) 83–88, 1995.– Both angiotensin II (AII) and the β-adrenergic agonist isoproterenol are well known to induce drinking and increase tail skin temperature (T_{SK}) in rats. Previous studies have shown that these two compounds have an additive, rather than interactive, dipsogenic effect when administered together. The present studies confirmed the additivity of the dipsogenic effect of the two compounds and attempted to extend these results to T_{SK}. The results reveal that at a series of dosage combinations of AII [100 and 125 μg/kg, subcutaneously (SC)] and isoproterenol (ISOP) (10, 12.5, and 25 μg/kg, SC) administered together, the increase in T_{SK} usually induced by each compound was canceled. This occurred at the two lower doses of ISOP in combination with AII. Thus, the differences between dipsogenic and thermoregulatory effects of combined doses of AII and ISOP suggest a difference in central integration. Important differences between the thermoregulatory responses to administration of the two compounds include increases in metabolic rate and colonic temperature when ISOP is administered and decreases in both when AII is administered. It is suggested that the effect of the two compounds to cancel each other with respect to T_{SK} is related to their opposite effects on metabolic rate.

Tail skin temperature Colonic temperature Drinking Rats Angiotensin II Isoproterenol

ISOPROTERENOL , a β-adrenoceptor agonist, is well known to increase metabolic rate, as well as tail skin (T_{SK}) and colonic (T_C) temperatures in the rat (1,3). Studies from this laboratory have shown recently that about half of the increase in T_{SK} induced by acute administration of isoproterenol (ISOP) is due to the release of renin and subsequent formation of angiotensin II (AII), a compound also well known to increase T_{SK} (5). This was shown both by AI converting enzyme inhibition (captopril) and AII receptor blockade (saralasin and losartan potassium) induced before administration of ISOP. Hence, the remaining half of the T_{SK} response to ISOP is independent of AII, suggesting separate mediation by β-adrenoceptors. The discovery that a two-component system responds to administration of ISOP and mediates the increase in T_{SK} raises a question regarding the interaction of β-adrenergic and AII systems in the increase in T_{SK} when both systems are active to varying degrees.

Although ISOP and AII are both well known to increase T_{SK}, their effects on T_C are opposite to one another, with ISOP increasing and AII decreasing it. Hence, an objective of the experiments described subsequently was to assess the responses of T_C and T_{SK} to administration of graded doses of these compounds, both alone and together. An additional objective was to compare the dipsogenic responses to the same dosage combinations of the two compounds to determine whether similarities existed between the dipsogenic and T_{SK}/T_C responses.

METHODS

General Procedures

Naive male rats of the Sprague–Dawley (Harlan Industries, Indianapolis, IN) strain weighing initially 300–350 g were used. They were kept three per cage in a room maintained at

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25±2°C and illuminated from 0700–1900 h. All rats were allowed tapwater to drink and Purina Laboratory Chow (5001; Ralston Purina Co., St. Louis, MO) to eat ad lib.

T_{SK} and T_C were measured at an ambient temperature of 22±2°C, whereas the rats were restrained in Lucite tunnel-type cages. The cages (01-280-10; Fisher Scientific Co., Pittsburgh, PA) allowed the rats to rest comfortably within, but restricted them from turning from head to tail. Numerous holes in the Lucite allowed free movement of air through the cage. 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 measured at 6-min intervals by a recording potentiometer. In all studies, measurements were started at the same time each day (0900 h) to minimize any potential time-of-day variation. In all experiments, rats were allowed 1 h to adjust to the restraining cages, following which baseline measurements were made for 30 min. Although the protocols were similar, different doses of ISOP and AII were administered in each of the experiments. All drugs administered to the rats were injected subcutaneously (SC) through a slot in the cage. Groups consisted of six rats each. ISOP (isoproterenol HCl, USP; Elkins-Sinn, Inc, Cherry Hill, NJ) and AII (human sequence, A9525; Sigma Chemical Co.) were purchased.

Statistical analyses of T_{SK}, T_C, and water intakes were carried out by a repeated-measures three-way analysis of variance (ANOVA). Comparison between individual means was made using the Newman-Keuls posthoc analysis.

Experiment 1: Effect of ISOP and AII, Alone and Combined, on Water Intake of Rats

Twenty-four rats were used in six separate studies. Each study was designed so that it could be analyzed by a two-way ANOVA and contained one group treated with ISOP, one with AII, one given both drugs at the same time, and a control group given an injection of the vehicle used to dissolve the drugs. In the first three studies, the dose of AII was the same (100 μg/kg, SC), whereas the doses of ISOP varied (10, 12.5, and 25.0 μg/kg, SC, respectively). In the last three studies, the dose of AII was also constant, but higher (125 μg/kg, SC), whereas the doses of ISOP varied as in the first three studies. Water intakes in each study were measured at 0.5, 1.0, and 2.0 h after treatment. At least 2 days elapsed between studies. The effects of AII on water intake have been shown to revert to control level within 2 days (7). Treatments were randomized in each of the studies.

Experiment 2: Effect of ISOP and AII, Alone and Combined, on Tail Skin and T_C of Rats

Six separate studies were performed using the same rats as in Experiment 1. Both the first and last three studies used doses of ISOP and AII identical to those used in Experiment 1. The methods used were those described in General Procedures. Treatments were again randomized in each of the studies.

RESULTS

Experiment 1

Administration of AII and ISOP together increased water intake in an additive fashion at submaximal doses (Fig. 1A, B). The results of the three-way repeated-measures ANOVA failed to reveal any significant interaction between AII and ISOP at any of the combinations of dosages used, although the responses to each individually were significant ($p < 0.05$) at all doses. Water intakes of control and AII-treated (100 μg/kg) groups remained relatively constant during the three studies shown in Fig. 1A. ISOP-induced water intake increased with increasing doses of ISOP, as did the water intake of the group given both ISOP and AII. In the case of the higher dose of AII (125 μg/kg), water intakes of the control group remained relatively constant during the three studies shown in Fig. 1B. Water intake of the ISOP-treated group tended to increase with increasing doses of ISOP, but the level was less than that of studies in Fig. 1A. The water intakes induced by AII (125 μg/kg) were more variable in this study than in that shown in Fig. 1A; the water intakes of the groups given both AII and ISOP increased with increasing doses of ISOP (Fig. 1B).

Figure 2 shows the combined results of all studies per-

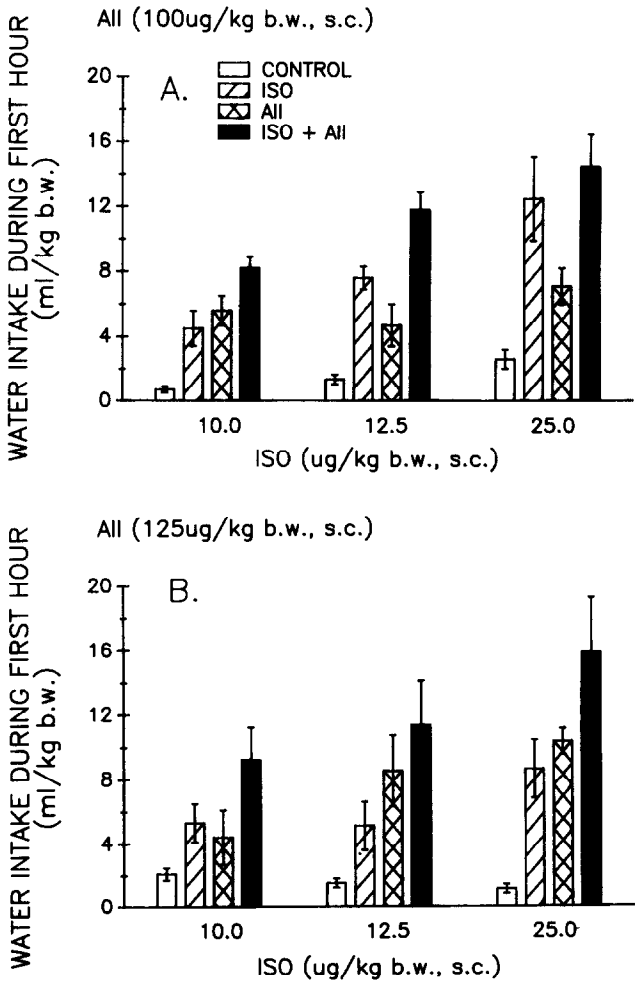

FIG. 1. Water intake during the 1st h after administration of isoproterenol (ISO), angiotensin II (AII), or a combination of both. Controls received isotonic saline. The dose of AII was constant at 100 μg/kg, SC, whereas the dose of isoproterenol was varied (10, 12.5, and 25 μg/kg, SC) (A). Means ± 1 SE are shown. (B) The dose of AII was constant at 125 μg/kg, SC, whereas the dose of isoproterenol was varied as in (A). The same animals were used in each study.

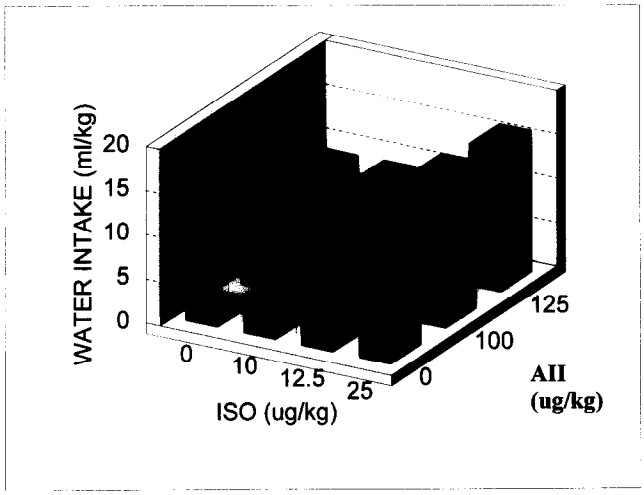

FIG. 2. The combined results of all dipsogenic studies performed in Experiment 1. See text for explanation.

formed in Experiment 1. To construct this figure, the data from the groups given ISOP alone and AII alone in the six studies shown in Fig. 1 were combined for each dose. Similarly, the data from the groups treated with the vehicle alone were combined. Figure 2 shows more clearly the dose-response relationships both within and among the dosage combinations used in the first experiment. An additive effect of the drugs at the lower doses can be seen in the figure.

Experiment 2

Figure 3 shows the characteristics of the responses of T_{SK} and T_C to administration of AII (125 μg/kg, SC), ISOP (10.0 μg/kg, SC), and the combination of the two. It may be noted in Fig. 3A that the increase in T_{SK} in response to administration of AII occurred within 6 min and was maximal at 12 min after treatment. In contrast, the increase in T_{SK} following administration of ISOP occurred much later (approximately 18 min) and reached a maximum at about 30 min after injection. At the dose administered, T_{SK} returned to control level by about 108 min after treatment with ISO. In contrast, the T_{SK} response to administration of AII returned to control level by about 24 min after treatment. In the case of ISOP, an increase in T_{SK} occurred following an increase in T_C. This was clearly not the case after administration of AII. In this study, the combination of the two compounds was not additive as it was in Experiment 1, but the effects of the two compounds given simultaneously appeared to cancel each other.

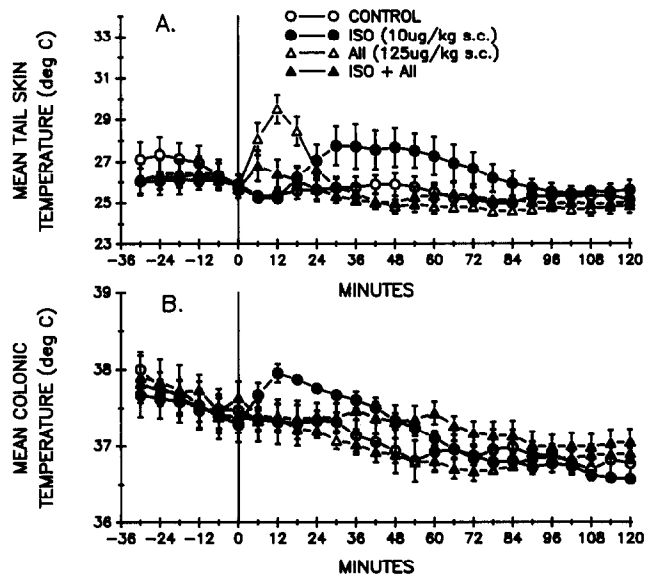

FIG. 3. Mean tail skin (A) and colonic (B) temperatures of rats administered isoproterenol (ISO) (10 μg/kg, SC) and angiotensin II (AII) (125 μg/kg, SC) alone and combined. Control rats were administered isotonic saline (1 ml/kg, SC). 1 SE is set off at each mean.

Figure 4 shows the mean change (Δ) in T_{SK} and T_C for the two studies in which AII was administered at a constant dosage (100 μg/kg, SC), whereas the dose of ISOP was varied (12.5 and 25 μg/kg, SC). The results indicate that at the dose used, AII was approximately at the threshold level for a response in T_{SK}, whereas even the lower dose of ISOP was clearly effective (Fig. 4A). At the lower dose of ISOP, the group receiving the combination of the two compounds had virtually no response; at the higher dose of ISOP, the group receiving the combination of the two compounds had a Δ T_{SK} response that was the same as that of the ISOP-treated group. Thus, there is no evidence of additivity with respect to Δ T_{SK} at this combination of doses.

With respect to Δ T_C, the lower dose of ISOP increased T_C within 6 min of treatment (Fig. 4B). Temperature returned to the level of the control group within 48 min. In contrast, administration of AII was accompanied by a reduction in Δ T_C to levels below the other three groups during the first 60 min of the experiment. The combination of the two compounds elevated Δ T_C to levels slightly, but not significantly, above those of the control group (Fig. 4B).

When a higher dose of ISOP (25 μg/kg, SC) was used in combination with the same dose of AII (100 μg/kg, SC), there was again no indication of additivity of Δ T_{SK} for the two compounds (Fig. 4C). The Δ T_{SK} of the group receiving the two compounds was not significantly different from that of the group receiving ISOP alone. The Δ T_{SK} of the AII-treated group was reduced to levels significantly below those of the control group.

In agreement with the results for Δ T_{SK} mentioned earlier, there was no significant interaction between ISOP and AII with respect to Δ T_C (Fig. 4D). Δ T_C of the group given the two drugs did not differ significantly from that of the group given ISOP alone. Δ T_C of the AII-treated group did not differ significantly from that of the control group throughout the experiment.

With a higher dose of AII (125 μg/kg, SC) in combination with either 10 or 12.5 μg ISOP/kg, SC, the failure of additivity of Δ T_{SK} was seen more clearly (Fig. 5A, C). Although Δ T_{SK} of the group administered the combined dose increased during the initial 18 min of the experiment, it did not reach the level of the group treated with AII alone. It also failed to increase to the level of the group treated with ISOP alone.

With respect to Δ T_C, it is also clear that there was no apparent additivity between the two compounds (Fig. 5B, D). However, it is clear that the combined treatment maintained Δ T_C at a more constant level throughout the two studies than either compound alone.

A striking difference occurred when the combination of 125 μg AII/kg and 25 μg ISOP/kg was administered (Fig. 6A,

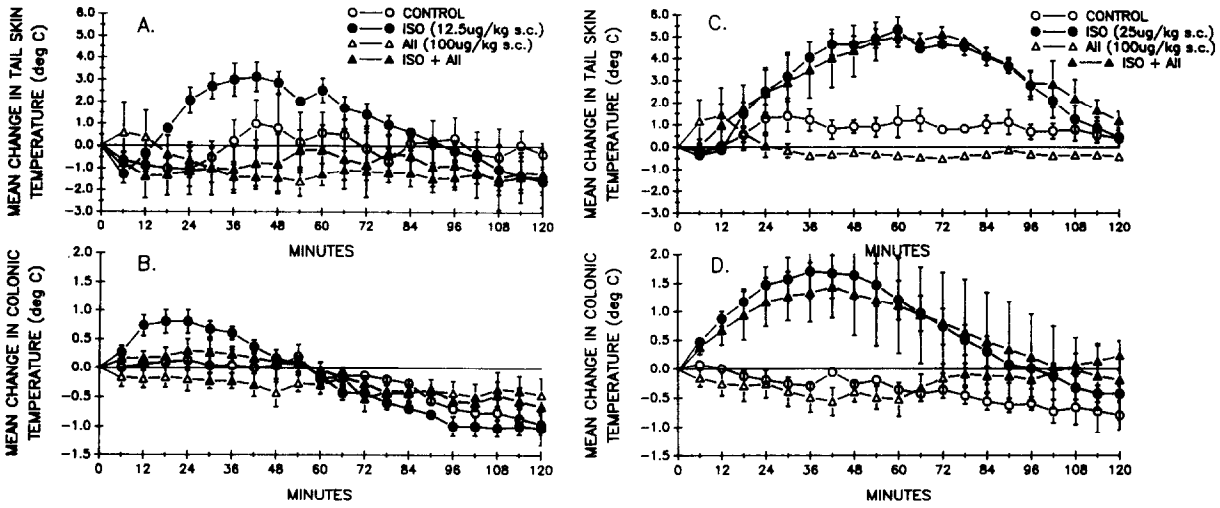

FIG. 4. Mean changes in tail skin (A) and colonic (B) temperatures from control period for the groups receiving 12.5 μg isoproterenol (ISO)/kg, SC, and 100 μg angiotensin II (AII)/kg, SC, alone and in combination, are shown. 1 SE is set off at each symbol. (C and D) The same changes for the groups receiving 25 μug ISO/kg, SC, and 100 μg AII/kg, SC.

B). With respect to T_{SK} and T_C, the group given the combined treatment closely paralleled the group given ISOP alone.

DISCUSSION

It is now well accepted that a key component of the brain concerned with fluid intake in response to administration of AII to rats is the lamina terminalis, consisting of neural tissue in the anterior wall of the third ventricle. It can be divided into three distinct segments: the subfornical organ (SFO) dorsally, and the organum vasculosum (OVLT) ventrally. These are highly vascularized and lack a blood-brain barrier. There is experimental evidence that they monitor the concentration of AII (SFO) and tonicity of the plasma (OVLT), both of which are important stimuli for drinking. Between the OVLT and SFO is the median preoptic nucleus (MnPO), which is considered to lie inside the blood-brain barrier, but is neurally connected with the SFO and OVLT. It is the final common pathway or relay site for both osmotic and AII signals. Neurons sensitive to a hyperosmotic stimulus project from the ventral portion of the SFO to the MnPO, and osmotically sensitive MnPO neurons project to the supraoptic nucleus (9). Further, the MnPO has reciprocal connections with structures within the preoptic-anterior hypothalamic area that are important in thermoregulation (2). Given the anatomic connections of the MnPO, it is reasonable to hypothesize that integration

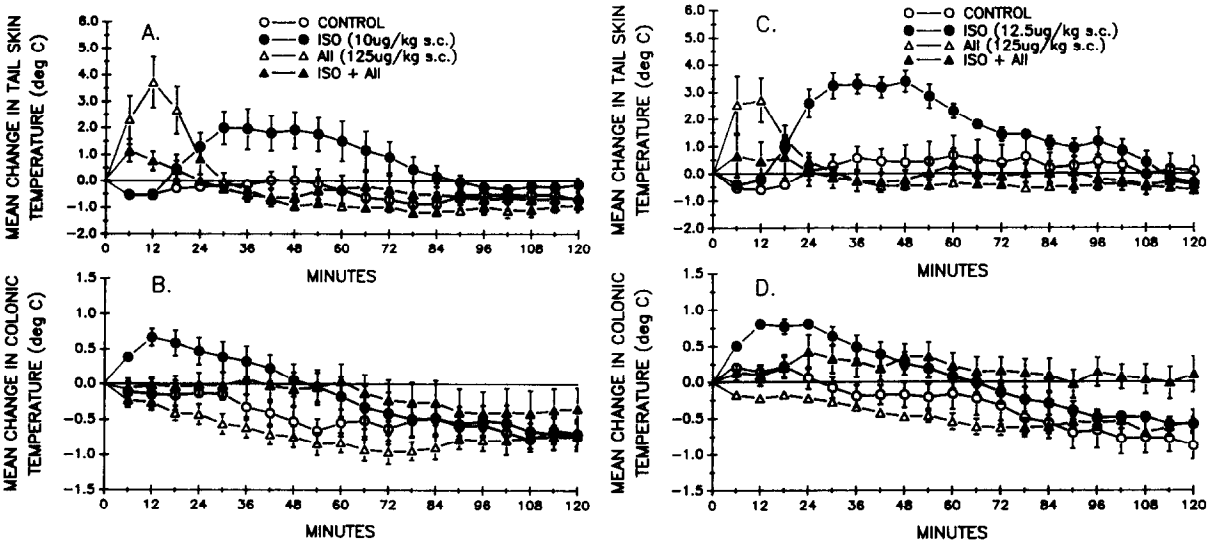

FIG. 5. Mean changes in tail skin (A) and colonic (B) temperatures from control periods for the groups receiving 10 μg isoproterenol (ISO)/kg, SC, and 125 μg angiotensin II (AII)/kg, SC, alone and in combination, are shown. 1 SE is set off at each symbol. (C and D) The same changes for the groups receiving 12.5 μg ISO/kg, SC, and 125 μg AII/kg, SC.

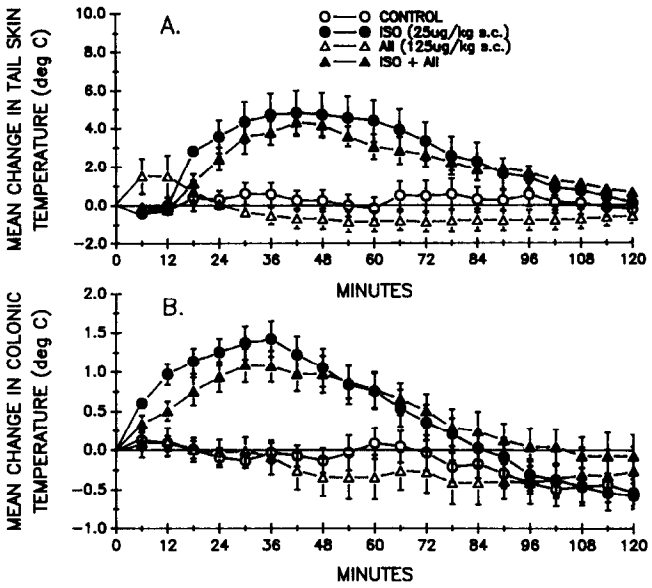

FIG. 6. Mean changes in tail skin (A) and colonic (B) temperatures from control periods for the groups receiving 25 μg isoproterenol (ISO)/kg, SC, and 125 μg angiotensin II (AII)/kg, SC, alone and in combination, are shown. 1 SE is set off at each symbol.

of information regarding blood pressure, blood volume, plasma osmolality, and temperature may take place within this nucleus. However, whether the MnPO neurons are sensitive to multiple stimuli, and how changes in temperature might influence the sensitivity of the MnPO neurons to AII, have not been well studied (11).

An earlier study from this laboratory reported an additive effect on water intake of simultaneous administration of ISOP and AII in combinations of submaximal doses of each (6). This was confirmed in the studies reported here (Fig. 1). Whether drinking following the administration of ISOP to the rat is related to direct effects of the compound to release renin from the kidneys or to indirect effects resulting from the reduction in blood pressure accompanying administration of ISOP has been the subject of controversy (4). The fact that the drinking response to the combined treatment is additive, and not interactive, suggests that there is a common mechanism mediating the drinking response to both compounds (6). This has been cited as circumstantial evidence that the drinking response to ISOP is mediated via AII. Recent studies from this laboratory have raised doubts that this could be the case (4). Thus, ISOP-induced drinking was not affected by blockade of AII receptors by either the AII (AT-1) nonpeptide receptor antagonist losartan potassium or the AII (AT-1 and 2) peptide receptor antagonist saralasin (4), although it could be blocked by β-adrenoceptor antagonists (4). This suggests that ISOP induces drinking by a mechanism independent of AII receptors. It is important to point out, however, that the AI converting enzyme inhibitor captopril, attenuated the drinking response to administration of ISOP (8). In view of the results of our recent studies with the AII receptor blockers, mediation of an additive drinking response to simultaneous administration of AII and ISOP is not clarified by the present experiments and will require additional study.

In contrast to the additivity of drinking responses to simultaneous administration of AII and ISOP, the increase in T_{SK} induced by each alone was neither additive nor interactive when the two compounds were administered simultaneously. When administered alone, each of these compounds has a dose-response relationship with T_{SK}. Care was taken to administer each compound at submaximal doses. Surprisingly, both doses of AII, in combination with the two lower doses of ISOP, canceled out the effect of each on T_{SK} (Figs. 3 and 4). The kinetics of the responses of T_{SK} to each compound are noteworthy. AII induces an increase in T_{SK} within 6 min. The increase is maximal within 12–18 min and has returned to control level within 24–30 min. In contrast, the increase in T_{SK} induced by ISOP is slow to develop, requiring about 24–30 min to increase above control level. The maximal increase in T_{SK} after administration of ISOP is maintained for a much longer period than after administration of AII. The longer latent period to increase T_{SK} after administration of ISOP is most likely related to the fact that a sequence of events must occur in which metabolic rate and T_C must increase maximally before an increase in T_{SK} (Figs. 3–5) (3). On the other hand, the responses to administration of AII appear to be reflexive in that a prompt increase in T_{SK}, a decrease in metabolic rate, and a consequent decrease in T_C occur (12). A long sequence of events is not required. The opposite effects of ISOP and AII on metabolic rate may be the basis on which the responses to the two compounds cancel each other.

There is an important difference between the latent periods for the induction of drinking and that for the increase in T_{SK} after administration of ISOP. Drinking occurs immediately after treatment, whereas the first increase in T_{SK} requires 24–30 min. Thus, drinking would appear to be a more direct response to administration of ISOP than the increase in T_{SK}. Because an increase in metabolic rate and T_C are required for the increase in T_{SK}, the latent period for an increase in either of these should correspond more closely with the latent period for induction of drinking. Perusal of Figs. 3–5 reveals that T_C is increased significantly above pretreatment level within 6 min after administration of ISOP. Thus, there do appear to be similar latencies between the increases in water intake and T_C under these conditions.

With respect to T_C, the results suggest that the groups treated with AII combined with the two lower doses of ISOP maintained T_C at a more constant level throughout the experiment than either compound alone (Figs. 3 and 4). At the lower doses of ISOP, administration of AII in combination with ISOP prevented the initial rise in T_C observed with ISOP alone (Figs. 3 and 4), although at the highest dose of ISOP used (25 μg/kg), no protection against this initial rise was afforded by simultaneous administration of AII (Fig. 5). This suggests that AII (100 and 125 μg/kg) may interfere with the increase in metabolic rate and heat production induced by ISOP at the lower doses used, but was unable to do so when the highest dose was given (Figs. 4C and 6A). This possibility remains for further study.

Earlier studies from our laboratories showed that about half of the T_{SK} response to administration of ISOP is due to the release of renin and formation of AII, whereas the remaining half is independent of AII receptor mediation (5). Hence, the dose of AII given exogenously may not represent the amount of this hormone interacting with the administered ISOP, because this compound is well known to induce the release of renin and the subsequent formation of AII (10). This suggests that the timing of the treatments in relation to each other may have important effects on the results observed, and advances the possibility that sequential administration of the two compounds, rather than simultaneous administration,

may show more clearly the additivity missing from the present studies. It is hoped that these plans will be carried out in the near future.

An earlier study from our laboratories compared the effect of administration of AII and ISOP, alone and in combination, on blood pressure measured directly in the rat (6). Following administration of AII (150 μg/kg, SC), mean systemic blood pressure of unanesthetized, chronically cannulated rats reached maximal levels within 5 min and returned to pretreatment control level by 60 min. Following administration of ISOP (25 μg/kg, SC), mean systemic blood pressure decreased within 5 min, was maximally depressed by 30 min, and had returned halfway to the pretreatment control level by 60 min. Simultaneous administration of ISOP and AII failed to induce a significant change in blood pressure, although water intake was increased significantly under these same conditions (6). These results are particularly interesting as they show that neither the pressor effect of AII nor the depressor effect of ISOP is essential for the induction of drinking under these conditions. These results also provide another set of physiologic variables that are not additive, and actually cancel each other, when AII and ISOP are administered simultaneously.

If it is assumed that the median preoptic area (MnPO) of the brain mediates both thermoregulatory and drinking responses, it is clear that its response to simultaneous administration of both AII and ISOP with respect to drinking (additivity) is different from that with respect to T_{SK} (cancelation). How and why this difference occurs remains to be determined in further study.

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