

Reduced Responsiveness to β -Adrenergic Stimulation in Renal Hypertensive Rats¹ (40205)

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When the β -adrenergic agonist, isoproterenol, is administered acutely to rats, an increase in both tail skin temperature and heat flow from the tail have been reported (1-3). The increase in tail skin temperature appears to be mediated by β -adrenergic receptors since the response can be blocked by propranolol, a β -adrenergic antagonist (2). Further, administration of the α -adrenergic agonist, phenylephrine, at several doses failed to influence tail skin temperature of rats (2). Thus, measurement of tail skin temperature following administration of graded doses of isoproterenol has been used to assess β -adrenergic responsiveness under a number of laboratory conditions; e.g., adaptation to cold (4); chronic administration of an estrogenic agent (1), and hypothyroidism (2, 5). Cold-adapted rats show an increased responsiveness to acute administration of isoproterenol while both estrogen-treated and hypothyroid rats have a reduced responsiveness.

Since the results of earlier studies from this laboratory suggested that rats made hypertensive by renal encapsulation with latex envelopes were hypothyroid relative to normotensive controls (6, 7), it seemed worthwhile to test the responsiveness of the tail skin temperature of renal hypertensive rats to acute administration of isoproterenol.

Methods. Twenty male rats of the Blue Spruce Farms (Sprague-Dawley) strain were used. The rats were housed in groups of 3 in hanging, stainless steel cages in a room maintained at $25 \pm 1^\circ$ and illuminated from 0600

to 1800 hours. Food (Purina Laboratory Chow) and tap water were provided *ad libitum*.

Bilateral renal encapsulation with latex envelopes was performed on 10 of the 20 rats while they were anesthetized with ether (8). Control rats were anesthetized and subjected to the same operative procedure except that the kidneys were not encapsulated. All rats were placed in individual cages immediately after the operation. Eleven weeks were allowed for the development of hypertension after which the studies described below were begun.

Systolic blood pressures were measured weekly by means of a Narco-Bio Systems transducer and polygraph, but without anesthesia as described by Fregly (9). Other investigators have also reported that this method correlates well with direct measurements of blood pressure obtained by arterial cannulation (10, 11).

These studies were performed during the 11th to 20th weeks after renal encapsulation. In all studies each rat was restrained in a tunnel-type cage containing a galvanized, mesh wire cover and a wooden floor (12). The cage was large enough to hold a rat comfortably but was adjusted to prevent movement of the rat from head to tail. The cages were made with an access port through which the rat could be injected without removal from the cage. Each rat had a copper-constantan thermocouple inserted 5 cm into the colon. It was held in place by a small piece of adhesive tape which bound it to the tail. The temperature of the dorsal surface of the skin of the tail, approximately 2 cm from the base, was measured by a second copper-constantan thermocouple. This thermocouple was woven into a single layer of a gauze sponge, 2.5 cm wide. A piece of adhesive tape was placed at the front and rear of the gauze pad to bind the thermocouple to the tail. The thermocouples were led off to a recording

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potentiometer which recorded both the tail skin and colonic temperatures of each rat at 1 minute intervals. After 30 min of restraint in a thermoregulated room maintained at $25 \pm 1^\circ$, each rat was randomly injected subcutaneously with *d, l*-isoproterenol (Isuprel hydrochloride, Winthrop Laboratories) at doses of 15, 25, 35, or 45 $\mu\text{g}/\text{kg}$ body weight. Each rat received all of the doses during the 8-week period of experimentation. Measurements of tail-skin and colonic temperatures continued at 1 min intervals for 2 hr after administration of isoproterenol at which time the rats were returned to their stock cages. An additional study was carried out in which both groups were administered only isotonic saline.

The data collected were analyzed statistically by means of the Student's *t* test (13). Significance was set at the 95% confidence limit.

Further evaluation of the dose-response

relationship between dose of isoproterenol administered and tail skin temperature response was assessed for each rat by determining the integrated area under the curve of tail skin temperature versus time. The baseline value about which the integration was computed was the tail skin temperature at time 0. Any temperatures below this value were considered negative while those above were considered positive. The algebraic sum of the integration over time is defined as the integrated tail skin temperature.

Results. Figure 1A illustrates the mean body weights of the two groups of animals during the course of the study. Renal encapsulation had no significant effect on body weight of the rats during this study. Survival rate, however, was reduced in the renal hypertensive group, resulting in the availability of only six animals at the time these studies were performed. These data lend support to

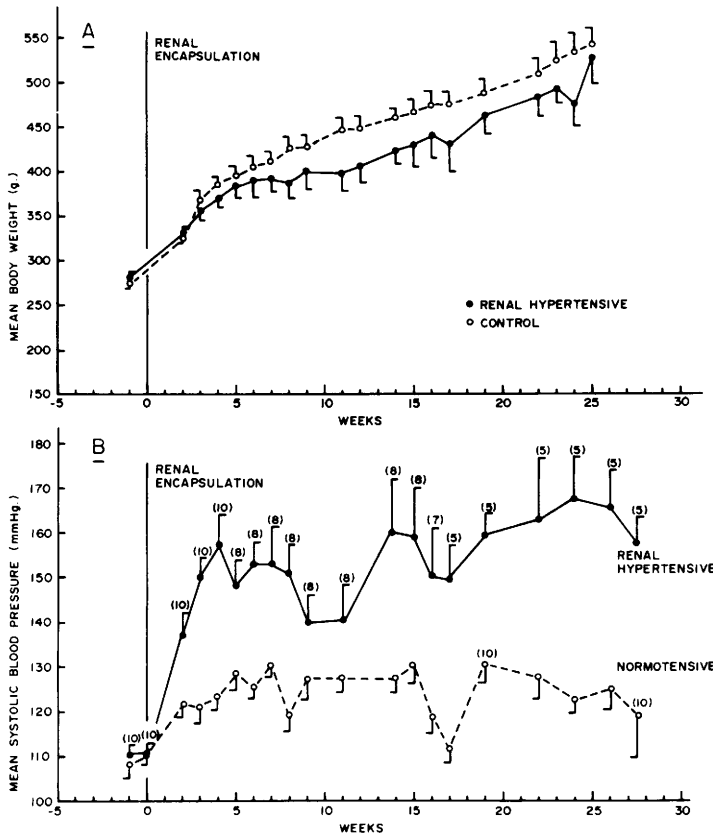


FIG. 1. Mean body weight (A) and mean systolic blood pressure (B) of normotensive control (O) and renal hypertensive (●) rats during the course of the experiment. One SE is set off at each mean. Numbers in parentheses represent number of surviving rats.

the earlier observations of Fregly (14) that renal encapsulation did not affect body weight but reduced survival.

Renal hypertensive animals had an increase in systolic blood pressure of 40 mmHg within 2 weeks after the kidneys were encapsulated (Fig. 1B). Mean systolic blood pressure of the renal-encapsulated group remained significantly ($P < 0.01$) higher than that of the control group throughout the 9 weeks of experimentation.

Administration of the lowest dose of isoproterenol (15 $\mu\text{g}/\text{kg}$) to either the control group or the renal hypertensive group resulted in a significant increase in mean tail skin temperature above the pretreatment level (Fig. 2A, B). Mean colonic temperatures of both groups also increased after administration of isoproterenol. The increase in colonic temperature in both groups occurred earlier than the increase in mean tail skin temperature. When the tail skin temperatures of the two groups were compared, those of the normotensive group were significantly ($P < 0.05$) elevated at 35–75 min after administration of isoproterenol. No significant differences between groups were observed in the case of colonic temperatures.

Administration of 25 μg of isoproterenol/kg body weight to both groups significantly ($P < 0.01$) increased their mean tail skin temperatures above their pretreatment control levels (Fig. 3A, B). The increases were greater than those observed when 15 μg isoproterenol/kg was administered. In the control group the increase in tail skin temperature remained significantly elevated above pretreatment levels throughout the 2-hr test. No significant differences between mean colonic temperatures of the 2 groups were observed throughout the experiment. However, a significant ($P < 0.05$) difference between mean tail skin temperatures of the 2 groups was observed at 95 to 120 minutes after administration of isoproterenol, with the control group being the higher.

When 35 μg of isoproterenol/kg body weight was administered to both the normotensive and the renal hypertensive groups significant increases in both mean tail skin ($P < 0.05$ – $P < 0.01$) and mean colonic temperatures above pretreatment control levels were observed (Fig. 4A, B). The two groups

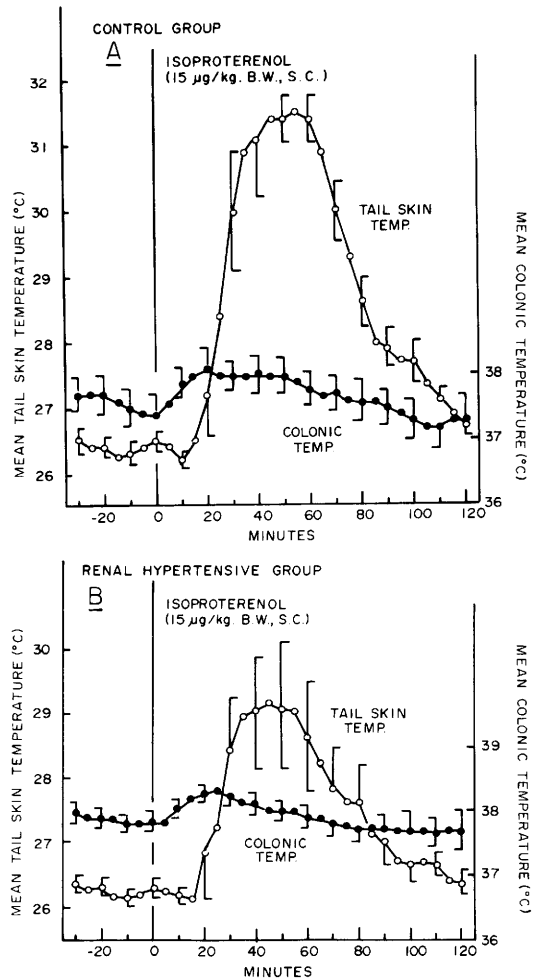


FIG. 2. Mean tail skin (left ordinate) and colonic (right ordinate) temperatures of normotensive control (A) and renal hypertensive (B) groups following subcutaneous administration of isoproterenol (15 $\mu\text{g}/\text{kg}$ body weight) at 0. One SE is set off at the means.

differed significantly ($P < 0.05$) from each other in the mean responses of their tail skin temperatures during 65 to 120 minutes after drug administration. No significant difference was noted between the mean colonic temperatures of the two groups throughout the experiment.

At the highest dose of isoproterenol administered (45 $\mu\text{g}/\text{kg}$), a striking increase above pretreatment, control level in maximal mean tail skin temperature (6.3 degrees) occurred in the normotensive group (Fig. 5A), compared with a maximal increase of 4.3 degrees in the renal hypertensive group (Fig. 5B).

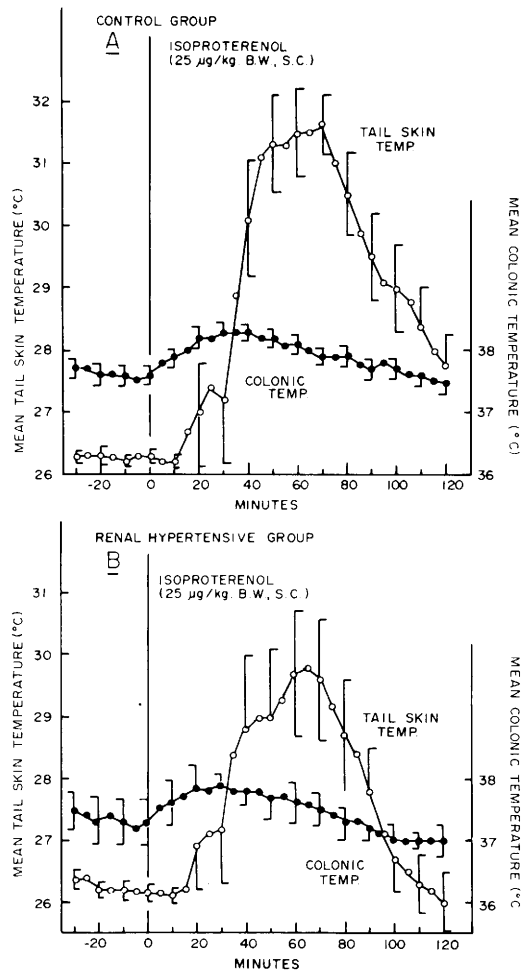


FIG. 3. Same as Fig. 2 except that 25 μg isoproterenol/kg was administered at 0.

Mean tail skin temperature of the renal hypertensive group did not differ from that of the control group prior to administration of isoproterenol. Following drug administration, the mean tail skin temperature of the control group was significantly ($P < 0.05$ – $P < 0.01$) higher than that of the renal hypertensive group from 60 to 120 min. No significant differences between mean colonic temperatures of the two groups were observed.

When isotonic saline was administered s.c. to both groups, no significant effect was observed on either tail skin or colonic temperatures in normotensive and hypertensive groups. During the course of the 2 hr experiment, tail skin temperatures of both groups declined approximately 0.5 degree. Colonic

temperatures of both groups declined by a similar amount.

The area under the tail skin temperature curve was integrated for all animals in both groups and at all doses of administered drug (Fig. 6). There was an increase in area observed with each increase in dose of isoproterenol. In all cases the renal hypertensive group was about 50% less responsive than the controls ($P < 0.01$).

Discussion. Induction of hypertension in rats by renal encapsulation was accompanied by an elevation of systolic blood pressure with no apparent alterations in body weight gain of the rats. However, renal hypertension was accompanied by a reduced responsive-

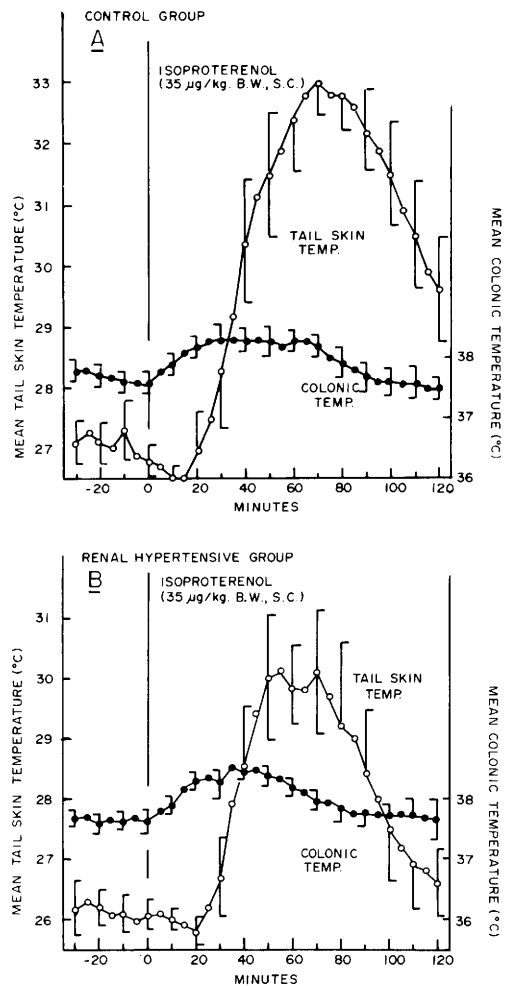


FIG. 4. Same as Fig. 2 except that 35 μg isoproterenol/kg was administered at 0.

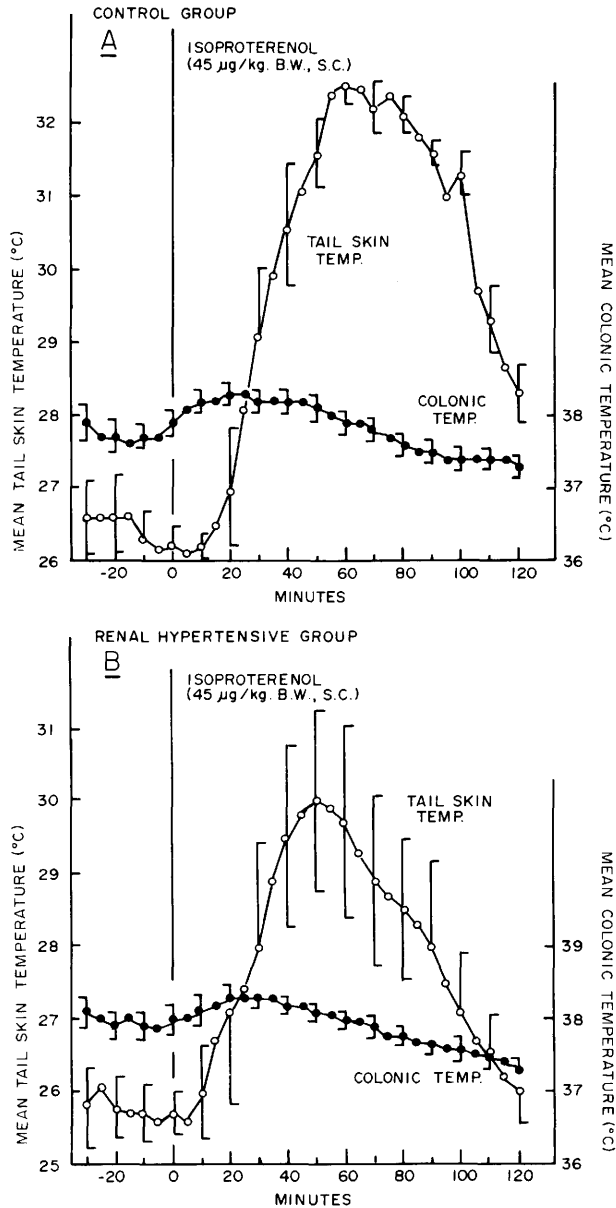


FIG. 5. Same as Fig. 2 except that 45 μg isoproterenol/kg was administered at 0.

ness of tail skin temperature to β -adrenergic stimulation.

The increase in tail skin temperature following administration of the β -adrenergic agonist, isoproterenol, was used in these studies as a test of β -adrenergic responsiveness. The increase in tail skin temperature in response to isoproterenol is inhibited by prior administration of the β -adrenergic antagonist, propranolol (2). Further, an increase in tail skin

temperature could not be induced by the α -adrenergic agonist, phenylephrine, at several different doses (2). Hence, the increase in tail skin temperature under these conditions appears to be mediated by β -adrenergic receptors. In addition, no differences were observed between the 2 groups with respect to mean colonic or tail skin temperatures either at the beginning of an experiment or in response to saline injection. This indicates that

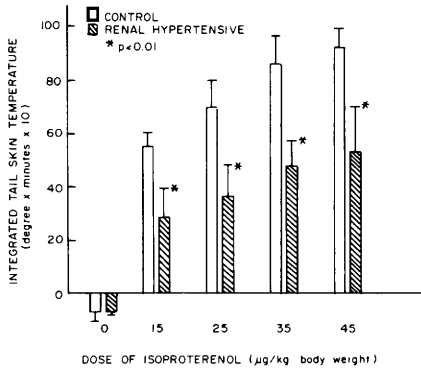


FIG. 6. Mean integrated tail skin temperature of normotensive control and renal hypertensive rats at each dose of isoproterenol. Open bars represent values for normotensive controls while cross-hatched bars represent values for hypertensives. One standard error is set off on each bar. The asterisk designates a significant ($P < 0.01$) difference between means.

the effects observed were drug-related and not an artifact of handling or injection.

Administration of graded doses of isoproterenol resulted in a graded increase in the mean tail skin temperatures of both groups; however, the response of the control group was higher and more prolonged than that of the renal hypertensive group. At the higher doses of isoproterenol (Figs. 3–5), mean tail skin temperature of the control groups did not return to baseline level 2 hr after administration of isoproterenol, whereas the renal hypertensive group returned to the baseline level in each case. At all four doses of isoproterenol administered, the response of the renal hypertensive group was significantly attenuated with respect to that of the control group (Fig. 6).

The tail serves the function of thermoregulation in the rat. When the rat is threatened with overheating, tail blood flow, skin temperature and heat loss increase in an effort to dissipate the excess heat (3, 15). The mechanism by which isoproterenol increases tail skin temperature is not yet clear. The increase in tail skin temperature could be indirect and occur secondarily as a result of an increase in heat production induced by isoproterenol. The increase could also be due to a direct effect of isoproterenol on the tail vasculature to induce vasodilation. The reduced responsiveness of tail skin temperature in hyperten-

sive rats may be the result not only of reduced β -adrenergic responsiveness but may also be the result of an increased α -adrenergic responsiveness. Field *et al.* (16) showed that the vascular reactivity of aortic rings of renal hypertensive rats to α -adrenergic stimulation was greater than that of normotensive controls.

It is also possible that the reduced β -adrenergic responsiveness of renal hypertensive rats may be related to the relative hypothyroid state reported for these animals (6, 7). Although not tested in the animals used here, the thyroid glands of renal hypertensive rats have been shown to manifest both the physiological (6, 7, 17, 18) and histological (7, 19) changes associated with hypothyroidism. These changes may be related to a goitrogen-like factor produced by the kidney, and possibly by certain other organs (20, 21). Hypothyroid rats are known to have a reduced metabolic responsiveness to β -adrenergic stimulation which is reversible by administration of thyroxine (4, 5, 22). In addition, Spaulding and Noth (23) reported that the peripheral vasculature shifts its responsiveness toward β - over α -adrenergic receptors in hyperthyroidism. Thus a reduced β -adrenergic responsiveness, coupled with a greater vascular reactivity to α -adrenergic stimulation, suggests an important role for catecholamines in the development and maintenance of renal hypertension. Whether the reduced β -adrenergic responsiveness of renal hypertensive rats observed here is induced by a relative hypothyroidism or by some other factors associated with elevation of blood pressure remains for further study.

Summary. Induction of hypertension in rats by encapsulation of both kidneys with latex envelopes was accompanied by a reduced responsiveness to β -adrenergic stimulation. Responsiveness was assessed by measurement of tail skin temperature during 2 hr following s.c. administration of the β -adrenergic agonist, isoproterenol. At doses of 15, 25, 35 and 45 μ g isoproterenol/kg of body weight, the responsiveness of tail skin temperature of rats with renal hypertension was significantly less than that of normotensive controls. The mechanism responsible for the reduced responsiveness in renal hypertensive rats remains speculative.

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