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Attenuation of a β-Adrenergic Response in Rats Treated Chronically with Ethynyl Estradiol[1] (39981)

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Chronic administration of combinations of the estrogenic agent, ethynyl estradiol, and the progestational agent, norethynodrel, to intact female rats reduced their responsiveness to the β-adrenergic agonist, isoproterenol. In these studies responsiveness was assessed by measurement of the increase in heart rate and water intake following acute administration of isoproterenol (1, 2). An additional test that has also been used to assess β-adrenergic responsiveness is the increase in tail skin temperature following acute administration of isoproterenol (3).

The objective of the present experiment was to use this test to study the responsiveness of tail skin temperature to acute administration of graded doses of isoproterenol to ovariectomized rats treated chronically with several different doses of ethynyl estradiol.

Methods. Twenty-four female rats of the Blue-Spruce Farms (Sprague-Dawley) strain weighing 210 to 260 g were used. They were kept in hanging, galvanized mesh-wire cages in an animal room maintained at 24 ± 1° and illuminated from 0600 to 1800 hr. All rats were ovariectomized while anesthetized with sodium pentobarbital (40 mg/kg body weight) to avoid any contribution of endogenously produced ovarian hormones to the observed results. One week after ovariectomy, a Silastic tube containing crystalline ethynyl estradiol was implanted subcutaneously between the shoulder blades. Although it was originally intended that there should be three different doses of ethynyl estradiol, the first 12 rats were implanted in error with the same Silastic tubing (No. 602-281, 0.0315-in. wall thickness, 10 mm long). A second group of six rats was also implanted with Silastic tubing (No. 602-231, 0.0095-in. wall thickness, 10 mm long). The remaining six rats were implanted with Silastic tubing (602-231) which contained no steroid, and served as the control group. Prior to implantation, the sealed tubes were placed in a vacuum desiccator for 48 to 72 hr and weighed on an analytical balance. The tube in each rat was palpated at weekly intervals to be certain it was still in place.

Dimethylpolysiloxane (Silastic) tubing has been shown to allow diffusion of certain crystalline steroids into various media at a constant rate over relatively long periods of time (4, 5). Previous experience in this laboratory has indicated that this method of steroid administration provides a reliable means of achieving reasonably constant drug release for periods of up to 6 months.

During the fifth and sixth weeks after implantation of the Silastic tubes, each rat was lightly restrained in a Lucite, tunnel-type cage large enough to hold it comfortably while preventing it from turning from head to tail. The cages were provided with access ports through which the rat could be injected during the experiment without removing it from the cage. A copper-constantan thermocouple was inserted 5 cm into the colon and held in place by a 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 its base, was measured by a second copper-constantan thermocouple. This thermocouple was held in place by weaving it into a single layer of gauze sponge 3 cm long. The two ends of the sponge were bound to the tail with adhesive tape. The thermocouples were led off to a recording potentiometer which recorded the skin and

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colonic temperatures of each rat at 1-min intervals. After 30 min of restraint in a room maintained at 24 ± 1°, each rat was injected sc with either 50, 100, or 200 μg of *l*-isoproterenol (Isuprel hydrochloride, Winthrop Laboratories, New York)/kg body weight. Measurements of colonic and tail skin temperatures continued for an additional 2 hr, after which the rats were returned to their stock cages. Three to five days were allowed between studies.

Statistical analyses of the data were made by means of an analysis of variance (6). Significance was set at the 95% confidence limit.

At the end of the experiment, the tubes containing ethynyl estradiol were removed from their subcutaneous sites and dried for 48 hr in a vacuum desiccator. The tubes were then weighed on an analytical balance and daily drug dose was calculated from the weight loss of the tube and the mean body weight of the rat during the period of drug administration. The tubes that were removed never contained fluid inside them.

Results. When the control rats were administered 50 μg of isoproterenol/kg sc, tail skin temperature increased from 25 to 31.6° within 30 min (Fig. 1A). An increase in tail skin temperature was detectable within 10 min after drug administration. Tail skin temperature returned to control level within 100 min after administration of isoproterenol. No change in tail skin temperature was seen in either of the ethynyl estradiol-treated groups in response to this dose of isoproterenol. Initial colonic temperatures of both groups treated with ethynyl estradiol were significantly ($P < 0.05$) less than the temperature of the control group prior to administration of isoproterenol. Colonic temperatures of all three groups fell during the course of the experiment (Fig. 1B). However, the colonic temperatures of both ethynyl estradiol-treated groups remained significantly lower ($P < 0.05$) than the temperatures of the control group following administration of isoproterenol.

Administration of 100 μg of isoproterenol/kg sc was accompanied by an increase in tail skin temperature from 24.8 to 32.0° within 30 min (Fig. 2A). The time course of change in tail skin temperature for the

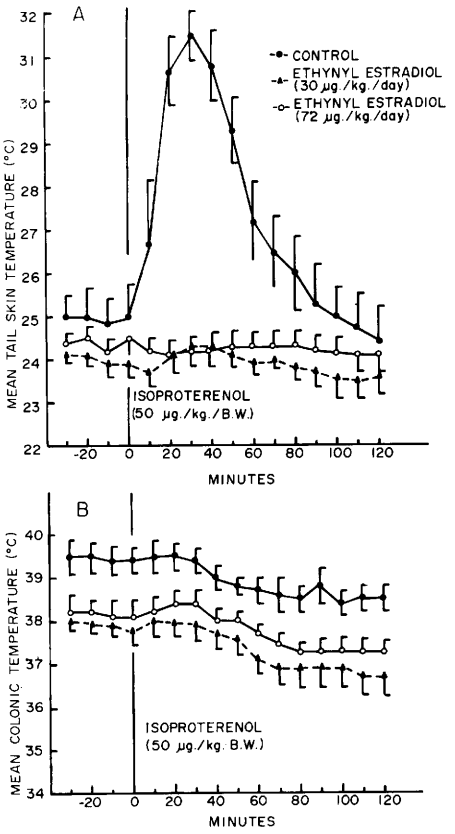

FIG. 1. (A) Mean tail skin temperatures of control and ethynyl estradiol-treated groups prior to, and following, administration of 50 μg of *l*-isoproterenol/kg body weight, sc, at time 0. Designation of each group is shown in the figure. One standard error is set off at the means. (B) Mean colonic temperatures of the same three groups during the same experiment are shown.

control group was very similar to that described above for the lower dose of isoproterenol, although tail skin temperature returned to control level only after 120 min following drug administration. In the case of the ethynyl estradiol-treated groups, the higher dose of isoproterenol used in this study increased tail skin temperature 2.8° in the group receiving the lower dose of the estrogenic agent and 1.1° in the group receiving the higher dose (Fig. 2A). Colonic temperatures of the three groups decreased during the course of the experiment (Fig. 2B). The initial colonic temperatures of the group receiving the lower dose of ethynyl estradiol were significantly lower than those

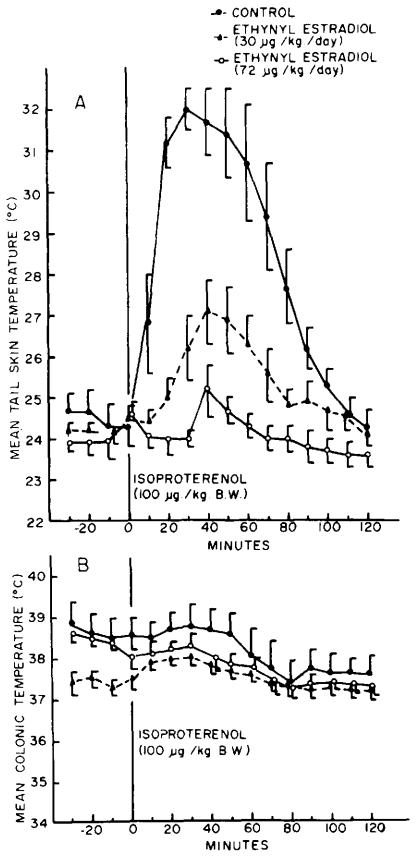

FIG. 2. (A) Mean tail skin temperatures of control and ethynyl estradiol-treated groups prior to, and following, administration of 100 μg of *l*-isoproterenol/kg body weight, sc, at time 0. Designation of each group is shown in the figure. One standard error is set off at the means. (B) Mean colonic temperatures of the same three groups during the same experiment are shown.

of the control group. All three treated groups showed changes in colonic temperature that approximated the time course of changes in tail skin temperature.

Administration of 200 μg of isoproterenol/kg to control rats was accompanied by an elevation of tail skin temperature from 24 to 32° (Fig. 3A). The maximal temperature was not different from that observed after administration of the two lower doses but was maintained for a longer period. Administration of this dose of isoproterenol to the ethynyl estradiol-treated groups resulted in an increase in tail skin temperature that was much attenuated compared to that of the control group (Fig. 3A). The group receiving the lower dose of ethynyl estradiol had an increase in tail skin temperature of 3.0°, while the group receiving the higher dose had an increase of 2.0°. Colonic temperatures of all three groups decreased during the course of the experiment (Fig. 3B). Both treated groups had lower colonic temperatures than controls during the control period. The lower colonic temperatures were maintained throughout the experiment.

The area under the curve of tail skin temperature versus time was determined by integration for each rat. The baseline value about which the integration was computed was the tail skin temperature at time 0. Any temperatures below this value were

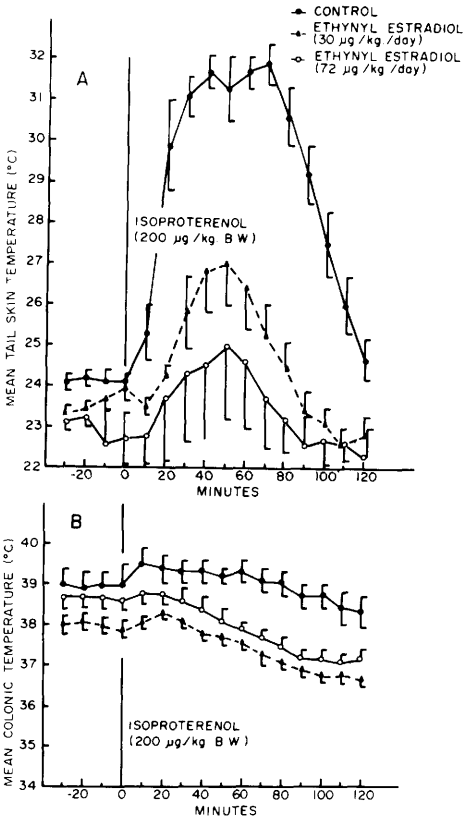

FIG. 3. (A) Mean tail skin temperatures of control and ethynyl estradiol-treated groups prior to, and following, administration of 200 μg of *l*-isoproterenol/kg body weight, sc, at time 0. Designation of each group is shown in the figure. One standard error is set off at the means. (B) Mean colonic temperatures of the same three groups during the same experiment are shown.

considered negative, while those above were considered positive. The algebraic sum of the integration over time is defined as the integrated tail skin temperature. The mean integrated tail skin temperature of the control group increased with increasing doses of isoproterenol (Fig. 4). The integrated tail skin temperatures of the two treated groups at each dose of isoproterenol failed to differ from one another, but each was significantly less ($P < 0.01$) than that of the control group. Apparently the attenuation of responsiveness to administration of isoproterenol was near its maximal level in the group receiving the lower dose of ethynyl estradiol.

At the end of the 17th week of the experiment, each rat was anesthetized with ether and the tube containing the drug was removed from its subcutaneous site. After cleaning, drying, and weighing each tube as described above, the group receiving the thicker-walled tube had a mean weight loss of 6.8 ± 0.7 μg/day, while the mean weight loss of the thinner-walled tube group was 16.4 ± 0.5 μg/day. When calculated on the basis of mean body weight during the experiment, weight losses of 30 and 72 μg of ethynyl estradiol/kg/day occurred from the tubes.

Discussion. The tail of the rat, like the ears of the rabbit, appears to act as a thermal radiator. When the body of the rat is threatened with overheating, tail blood flow, tail skin temperature, and heat loss increase in an effort to dissipate the excess heat (7). Acute administration of the β-adrenergic agonist, isoproterenol, produced a striking elevation in tail skin temperature of rats (Figs. 1-3) (8, 9). Whether this response is due to a direct action of the β-adrenergic agonist on tail vasculature or indirect via effects on metabolism is not as yet clear. It is clear, however, that this response is not produced by administration of isotonic saline (9). Furthermore, the response to isoproterenol can be blocked by the β-adrenergic antagonist, propranolol (9), and attenuated by chronic treatment with ethynyl estradiol (Fig. 4). Other studies have shown that tail skin temperature is not affected by the α-adrenergic agonist, phenylephrine (9). The time course for development of the attenuation was not studied here but was studied earlier in rats receiving combinations of ethynyl estradiol and norethynodrel (3). Reduced β-adrenergic responsiveness appeared from 3 to 5 weeks after initiation of steroid treatment and was present in all treated groups at 5 weeks (3). However, the earlier results may not be applicable here because of differences in experimental protocol. Earlier studies used different combinations of norethynodrel and ethynyl estradiol and the rats were not ovariectomized.

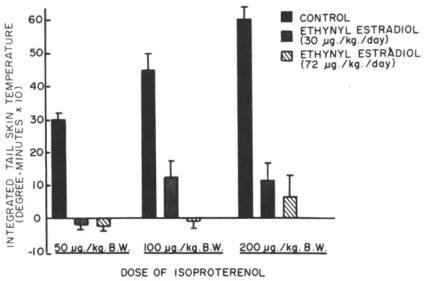

FIG. 4. The mean integrated tail skin temperatures (areas under the curves in Figs. 1-3) for control and ethynyl estradiol-treated groups following administration of 50, 100, and 200 μg/kg of *l*-isoproterenol are shown. The groups are designated in the figure. One standard error is set off at the means.

In all three studies described here, the control rats had initial colonic temperatures that were greater than those of the treated groups. The reduced colonic temperature of the treated rats suggests that the ability either to produce heat or to conserve it, or both, was affected by chronic treatment with ethynyl estradiol. The lower colonic temperatures of the treated groups may reflect a reduction in the contribution of the β-adrenergic system to maintenance of metabolic rate (heat production) under normal conditions. The ability of estrogen-treated rats both to produce and to conserve heat is currently under study in this laboratory.

The results of the present study do not provide clues regarding the mechanisms by which chronic administration of ethynyl estradiol attenuates the response to acute administration of isoproterenol. Whether one of these factors is a reduced responsiveness of the β-adrenergic receptor, or a site beyond, is also not clear at present and will require additional studies.

Summary. Chronic treatment of ovariectomized female rats with ethynyl estradiol (30 and 72 μg/kg/day) attenuated the increase in tail skin temperature characteristically accompanying sc administration of *l*-isoproterenol (50, 100, or 200 μg/kg). The mechanism by which the attenuation occurs awaits additional studies.

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