Increased heat loss in ovariectomized hypothyroid rats treated with estradiol

MARK L. LAUDENSLAGER, HARRY J. CARLISLE, AND STEVE E. CALVANO
Department of Psychology, University of California, Santa Barbara, California 93106

LAUDENSLAGER, MARK L., HARRY J. CARLISLE, AND STEVE E. CALVANO. *Increased heat loss in ovariectomized hypothyroid rats treated with estradiol.* Am. J. Physiol. 243 (Regulatory Integrative Comp. Physiol. 12): R70–R76, 1982.—The role of the thyroid in the mediation of an estrogen-associated change in thermal balance was studied in thyroidectomized and in propylthiouracil-treated ovariectomized rats. Prior to propylthiouracil treatment, estrogen-treated ovariectomized rats and intact female rats had higher rates of heat production and dry heat loss at −5°C than ovariectomized rats. Heat production of estrogen-treated and intact female rats was well below their rates of dry heat loss without an alteration in the absolute rate of heat loss in the hypothyroid condition. Heat production exceeded heat loss only in the hypothyroid ovariectomized group not receiving estrogen. Ovariectomized rats without estrogen maintained thermal balance, whereas rectal temperatures fell in both intact and estrogen-treated ovariectomized rats during cold exposure. Increased heat loss unbalanced by heat production was also observed in surgically thyroidectomized estrogen-treated ovariectomized rats tested at −5°C. These results suggest that an estrogen-induced increase in heat loss, which is compensated by an increase in heat production in the euthyroid but not the hypothyroid condition, is one mechanism responsible for estrogen-associated changes in thermal balance during cold exposure.

dry heat loss; gradient-layer calorimetry; hypothermia; ovarian cycle; oxygen consumption; propylthiouracil; rectal temperature; temperature regulation; thyroxine

THE RELATIONSHIP BETWEEN CHANGES in levels of ovarian steroids and concomitant changes in body temperature is not well understood. Although progesterone is generally associated with increased thermogenesis (6), the effects of estrogen are highly variable and difficult to characterize (1, 8, 14, 16). Rectal temperature is paradoxically depressed in estrogen-treated ovariectomized rats even though behavioral heat intake is increased during cold exposure (21). This suggests that either heat production is reduced or heat loss is increased by estrogen treatment. Since both heat production and dry heat loss are elevated in estrogen-treated rats at ambient temperatures between 2.5 and 30°C (13), it would seem that the primary effect of estrogen treatment is to increase heat loss rather than heat production. However, the primary effects of estrogen are not easily separated in steady-state calorimetry studies such as those described (13). Ratios of dry heat loss to heat production are similar for both estrogen-treated and untreated animals. Thus estrogen might increase heat production, perhaps through thyroid activation, with heat loss increasing either passively or as a compensatory response. Alternatively, estrogen might increase heat loss, with heat production increasing as a compensatory mechanism. Because estrogen administration influences various aspects of thyroid function (4, 5, 9, 23), perhaps the factors affecting thermal balance associated with estrogen administration may be better separated in the hypothyroid rat (7). The present study investigates this possibility. If estrogen increases only heat production via thyroid activation, then administration of estrogen to thyroid-deficient rats should have little or no effect on heat production or dry heat loss. However, if estrogen increases heat loss, then estrogen-treated rats should demonstrate higher rates of heat loss regardless of thyroid condition. The first study investigated the influence of propylthiouracil treatment on heat production and dry heat loss in intact, ovariectomized, and ovariectomized estrogen-treated rats. To verify that the effects noted in the first experiment were not due to side effects of the propylthiouracil treatment, a similar study was performed with surgically thyroidectomized rats.

MATERIALS AND METHODS

The experimental apparatus consisted of a sealed 15.2 × 15.2 × 15.2 cm (inner dimensions) gradient-layer calorimeter (model SEC A 060, Thermonetics). The calorimeter was maintained within a modified chest-type freezer equipped with forced-air cooling and heating. The refrigeration system ran continuously, and heater voltage was regulated by a proportional controller (RFL Industries) to ensure a constant temperature. Temperature control provided by this arrangement maintained the outside calorimeter wall within ± 0.05°C of any desired temperature between −10°C and 45°C. The calorimeter output was calibrated by applying voltage to a Nichrome wire coil of known resistance placed inside the calorimeter. The output of the calorimeter was converted to energy loss per unit mass (W/kg) and termed dry heat loss (DHL).

Dried air was drawn through the calorimeter at a fixed rate (range 1.6–2.0 l/min). A portion of the effluent air was redried, passed through a Beckman 755 paramagnetic oxygen analyzer, recombined with the remaining effluent air, and passed to a dry-gas test meter (American Instruments). The oxygen analyzer was calibrated by the

partial pressure method (2). Metabolic heat production (HP, in W/kg) was determined from the average rate of oxygen consumption (STPD) assuming a respiratory quotient of 0.8 and 10 ml O_2 consumed/min as equivalent to 3.38 W. Air temperatures (T_a) in the calorimeter and environmental chamber and wall temperatures inside and outside the calorimeter were measured with 36-gauge copper-constantan thermocouples referenced to a 0°C electronic reference (Wescor). In addition, a thermistor probe (Yellow Springs Instrument) connected to a Wheatstone bridge circuit measured air temperature in the calorimeter. The thermocouple outputs were recorded on a 12-point recording potentiometer (Leeds and Northrup). The analog signals from the oxygen analyzer, gradient-layer calorimeter, and Wheatstone bridge were processed by an analog-to-digital converter for signal averaging and on-line data conversion by a minicomputer (PET 2001 series, Commodore Business Machines). Each input was sampled every 5 s, averaged over a 2.5-min interval, converted to W/kg (HP and DHL) or °C (T_a), and printed on a line printer. Overall averages, summed over preceding intervals, could be obtained at any time. Rectal temperatures (T_{re}) were measured before and after all tests with a telethermometer (model 46 TUC, Yellow Springs Instrument) with the vinyl probe inserted 6 cm.

Experiment 1. Propylthiouracil treatment of estrogen-treated and nontreated ovariectomized rats and intact female rats. Eighteen adult female Sprague-Dawley rats, weighing 190–218 g at the time of ovariectomy, were caged individually in an animal colony room maintained at 24 ± 2°C. The rats were maintained on a reversed day-night cycle (LD 12:12) and tested at approximately the same time during the dark portion of the cycle. Purina laboratory chow and water were freely available except that food was withheld for approximately 15 h prior to all tests. Twelve rats were ovariectomized at 75 days of age. Six of these received a single subcutaneous intrascapular Silastic capsule implant (2 mm long; 3.2 mm OD; 1.6 mm ID, ends sealed with wood applicator sticks) containing estradiol-17β (E_2) (Sigma Chemical). Capsules of these dimensions produce average plasma E_2 concentrations of approximately 30 pg/ml (13), which compare favorably with endogenous levels noted in the intact rat at proestrus (11). The remaining ovariectomized rats received empty capsules of the same dimensions. The intact rats received no capsule. Thus three groups were tested, an ovariectomized group receiving an E_2 capsule, an ovariectomized group receiving an empty capsule, and an intact group receiving no capsule. Three weeks following ovariectomy, all rats were tested in the calorimeter at a T_a of 15°C for 90 min. Average HP and DHL were determined during the final 60 min of each test. At least 2 days elapsed before a 6-h test at −5°C was made. For this test, each rat was maintained during the first 4.5 h at −5°C in a wire-mesh cage (24.5 × 18 × cm) identical to their home cage but without food and water. At the end of this time the rat was removed from this cage, T_{re} was measured, and the rat was quickly placed in the calorimeter (equilibrated at −5°C) for 90 min. Average HP and DHL were determined during the last 60 min.

Following all tests at 15 and −5°C, blood samples were taken for thyroxine (T_4) radioimmunoassay. Home-cage water was then substituted with a 0.05% solution of propylthiouracil (PTU) (Sigma Chemical) and distilled water. No other water was available. The laboratory chow was replaced with a special low-iodine purified diet (lot 1145, 5859C6FV, Ralston Purina). Blood samples were taken at 2-wk intervals for T_4 radioimmunoassay. A rat from both the estrogen-treated ovariectomized group and the ovariectomized group not receiving estrogen died within 4 wk following initiation of PTU treatment. Consequently, one intact rat was randomly deleted from further study in order to maintain equal group sizes. After 6 wk, the remaining rats (n = 15) were retested at 15°C as before. However, PTU-treated rats could not tolerate the original design of the 6-h test at −5°C. Following 4.5 h at −5°C, the first two rats tested were severely hypothermic (T_{re} of 25.1 and 24.5°C) before we could determine HP and DHL. The testing procedure was altered, and rats were placed in the calorimeter from the start of exposure to −5°C. Average HP and DHL were determined at 1-h intervals. HP of hypothyroid rats declined over time during the test at −5°C. Consequently, the rats were left in the calorimeter for 3 h or until HP fell below 6 W/kg. The rats were then removed from the calorimeter, T_{re} was measured, and they were placed on a heating pad for rewarming if T_{re} was less than 35°C. Blood samples were taken again for T_4 determinations. The PTU-adulterated water was then replaced with a 0.9% saline solution made with iodized table salt and distilled water, and standard Purina chow was returned. After 3 wk fresh water was returned, and blood samples were taken again for T_4 radioimmunoassay. Four weeks after the removal of the PTU solution and low-iodine diet, the rats were tested a third time at 15 and −5°C as described above. Thus rats were tested before, during, and after recovery from PTU treatment.

Experiment 2. Thermal balance in thyroidectomized E_2-treated ovariectomized rats. This experiment replicated the general features of *experiment 1*, with the exception that thyroid function was disrupted surgically rather than chemically. Twelve adult female Sprague-Dawley rats, weighing 185–210 g at the time of ovariectomy, were used. All rats were surgically thyroidectomized by the supplier (Charles River). They were maintained on a solution of 0.5% $CaCl_2$ drinking water. Otherwise maintenance was as in *experiment 1* prior to PTU treatment. Blood samples were taken prior to ovariectomy for T_4 determinations to ensure total thyroidectomy.

Two weeks after thyroidectomy, all rats were ovariectomized and assigned to either an estrogen-replacement or no-replacement group. Rats received Silastic capsules as in *experiment 1*. Two weeks after ovariectomy, 10 rats whose T_4 levels fell below 1.0 μg/100 ml were tested at 15 and −5°C as in *experiment 1*. At the conclusion of tests, blood samples were taken again for T_4 determinations.

Thyroxine radioimmunoassay. Blood (300 μl) was collected into heparinized hematocrit tubes by tail tip incision. No anesthesia was employed. Following centrifugation, plasma was stored at −20°C. Plasma T_4 levels were determined by radioimmunoassay (17) using anti-

serum generated in rabbit by immunization with porcine thyroglobulin (Sigma Chemical). Cross reactivities were <0.2% for mono- and diiodotyrosine and 7% for triiodothyronine. High-specific-activity (1,200 μCi/μg) ^{125}I-T_4 (New England Nuclear) was used to maximize assay sensitivity for small volumes (5 μl plasma for euthyroid rats and 15 μl for hypothyroid rats). Assay parameters were as follows: sensitivity, 0.6 μg/100 ml; T_4-free plasma blank, 0.5 μl/100 ml; intra-assay coefficient of variation (CV), 7.9%; and interassay CV, 14.8%.

Statistical analysis. The effects of experimental treatments were tested by an analysis of variance (22). A posteriori comparisons were made with Duncan's multiple-range tests when main effects were found to be significant. When appropriate, *t* tests for differences between two means were used.

RESULTS

Experiment 1. The estrogen-treated ovariectomized rats had the highest rates of HP and DHL at 15°C, regardless of thyroid condition (Fig. 1). The low-iodine diet combined with the PTU treatment successfully reduced plasma T_4 levels as shown along the *x*-axis of Fig. 1. Plasma T_4 levels returned to normal when regular chow and iodine-supplemented water were given to all groups. For tests at 15°C, the lower plasma T_4 levels were associated with only slightly lower rates of HP and DHL; this decrease was not statistically significant. Comparisons of all groups for tests of 15°C indicated that the rates of HP and DHL of the estrogen-treated ovariectomized rats were always higher than the ovariectomized rats not receiving E_2 replacement ($P < 0.05$). Rates of HP and DHL for the intact female rats were intermediate to the other two groups but were not statistically different from either the estrogen-treated or untreated ovariectomized groups.

Rectal temperatures were not different between groups for tests at 15°C either before or after tests (Table 1). T_{re} increased from pre- to posttest in all groups regardless of thyroid condition or estrogen treatment ($P < 0.005$). The reduced plasma T_4 levels during the PTU treatment were associated with lower pre- and posttest T_{re} ($P < 0.001$).

Thus the tests at 15°C revealed no effect of disrupting thyroid function on HP and DHL in estrogen-treated ovariectomized rats, ovariectomized rats not receiving estrogen replacement, or intact female rats. The estrogen-treated rats maintained the highest rates of HP and DHL at 15°C regardless of thyroid status. In contrast, extended tests at −5°C revealed differences between the groups as a function of thyroid status and estrogen treatment. First, the 6-h test at −5°C was tolerated well by all groups prior to PTU treatment. Average rates of HP and DHL during the final hour at −5°C are given in Fig. 2. As observed at 15°C, HP and DHL of the estrogen-treated ovariectomized rats exceeded both the intact female rats and the ovariectomized rats not receiving estrogen replacement ($P < 0.005$). Rates of HP ($P < 0.01$) and DHL ($P < 0.05$) of the intact female rats were also significantly greater than the ovariectomized rats not receiving estrogen replacement. Regardless of these differences in rates of HP and DHL between groups, thermal balance was approximately equivalent across groups, and T_{re} did not change from pre- to posttest (see Table 2).

Ratios of DHL to HP reflect energy balance, and ratios that exceed 1.0 are indicative of uncompensated heat loss resulting in a fall in body temperature. Prior to PTU treatment, there was a close correspondence of DHL/HP for the estrogen-treated ovariectomized rats (0.84 ± 0.05),

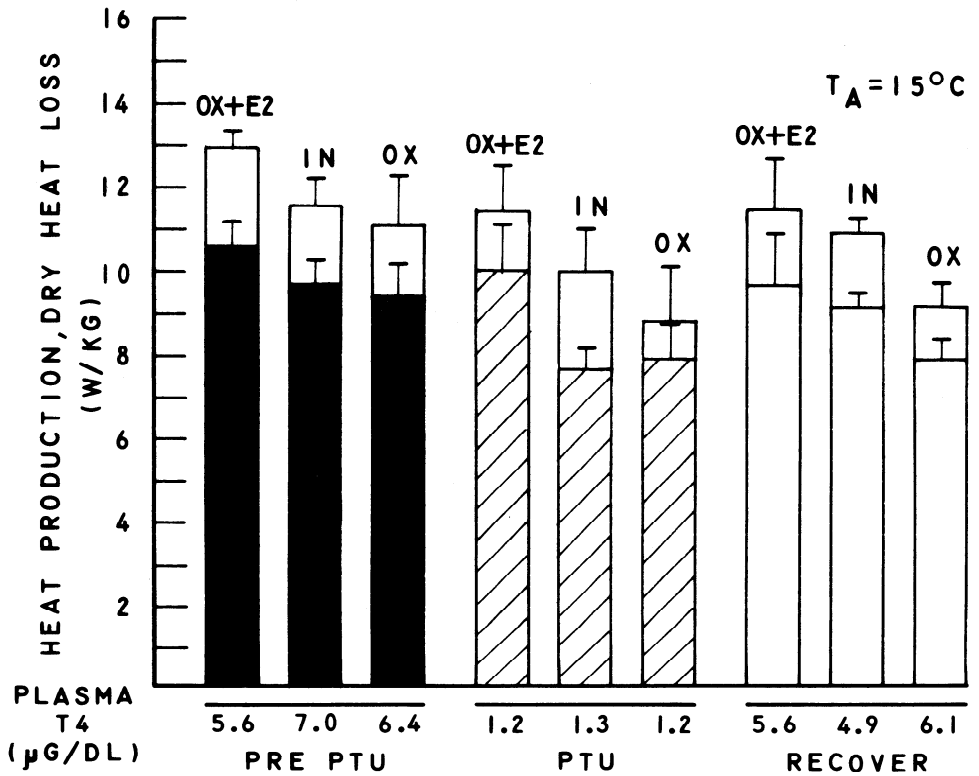

FIG. 1. Effect of PTU treatment on heat production (*upper bar*) and dry heat loss (*lower bar*) at 15°C. Plasma T_4 concentrations are indicated along *lower axis* for all groups in *expt 1. Solid bars* indicate results observed prior to PTU treatment; *hatched bars* indicate hypothyroid results; *open bars* indicate observations made following recovery from PTU. *Bracket* encloses 1 SD. Intact animals are indicated by IN; ovariectomized animals are indicated by OX; estrogen-treated ovariectomized animals are indicated by OX+E_2.

TABLE 1. *Pretest and posttest rectal temperatures for 15°C tests in expt 1*

Thyroid Condition	Group		
	IN	OX + E_2	OX
Euthyroid, pre-PTU			
Pre	37.7 ± 0.7	38.0 ± 0.2	37.7 ± 0.8
Post	38.0 ± 0.3	38.1 ± 0.2	37.8 ± 0.7
Hypothyroid, PTU-treated			
Pre	36.9 ± 0.4	37.1 ± 0.4	36.6 ± 0.4
Post	37.1 ± 0.4	37.1 ± 0.6	37.4 ± 0.7
Euthyroid, PTU-recovered			
Pre	37.8 ± 0.5	37.5 ± 0.7	37.1 ± 0.1
Post	38.1 ± 0.2	37.8 ± 0.5	38.1 ± 0.2

Values are means ± SD in °C; n = 5. Groups: IN, intact; OX + E_2, ovariectomized estrogen-treated; OX, ovariectomized.

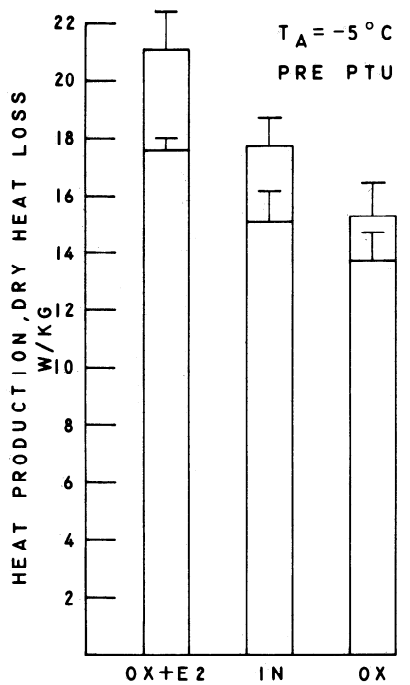

FIG. 2. Heat production and dry heat loss during last hour of a 6-h exposure to −5°C for all groups prior to PTU treatment. Heat production is indicated by *upper bar* and dry heat loss is indicated by *lower bar*. Note that heat production always exceeds heat loss for all groups. *Bracket* encloses 1 SD. Groups are indicated as in Fig. 1.

the ovariectomized rats not receiving estrogen replacement (0.89 ± 0.06), and the intact female rats (0.86 ± 0.09). However, in the hypothyroid condition, DHL/HP exceeded 1.0 in both the intact female rats and the estrogen-treated ovariectomized rats during the 1st and subsequent h at −5°C. As a consequence, body temperature declined rapidly in hypothyroid rats with either endogenous or exogenous sources of estrogen but not in ovariectomized rats that did not receive estrogen replacement (Fig. 3). The influence of this thermal imbalance on T_{re} during PTU treatment is shown in Table 2. The absolute rate of DHL of the estrogen-treated ovariectomized rats was greater than the DHL of the intact female rats, which in turn exceeded the DHL of the ovariectomized rats not receiving estrogen ($P < 0.005$). The maximal absolute rate of HP noted at any time during the 1st h at −5°C in the hypothyroid condition for the estrogen-treated ovariectomized rats (17.8 ± 2.2 W/kg), the intact female rats (16.5 ± 1.2 W/kg), and the ovariectomized rats not receiving estrogen (16.4 ± 1.2 W/kg) was similar. It is likely that all rats attained the maximal rate of HP possible, but the high rates of DHL noted in the two groups with estrogen exceeded HP. Only in the ovariectomized rats not receiving estrogen did HP exceed

TABLE 2. *Pretest and posttest rectal temperature for −5°C tests in expt 1*

Thyroid Condition	Group		
	IN	OX + E_2	OX
Euthyroid, pre-PTU			
Pre	37.8 ± 0.3	37.6 ± 0.5	37.4 ± 0.8
Post	37.7 ± 0.5*	38.4 ± 0.7*	37.9 ± 0.4*
Hypothyroid, PTU-treated			
Pre	37.2 ± 0.4	36.9 ± 0.5	36.2 ± 0.8
Post	30.2 ± 3.5†	24.4 ± 2.3‡	36.1 ± 2.0†
Euthyroid, PTU-recovered			
Pre	37.5 ± 0.7	37.8 ± 0.6	37.5 ± 0.7
Post	38.2 ± 0.3†	38.4 ± 0.4†	38.4 ± 0.3†

Values are means ± SD in °C; n = 5. Groups: see Table 1 footnote for abbreviations. * Following 6-h exposure. † Following 3-h exposure. ‡ Following 3-h or less exposure.

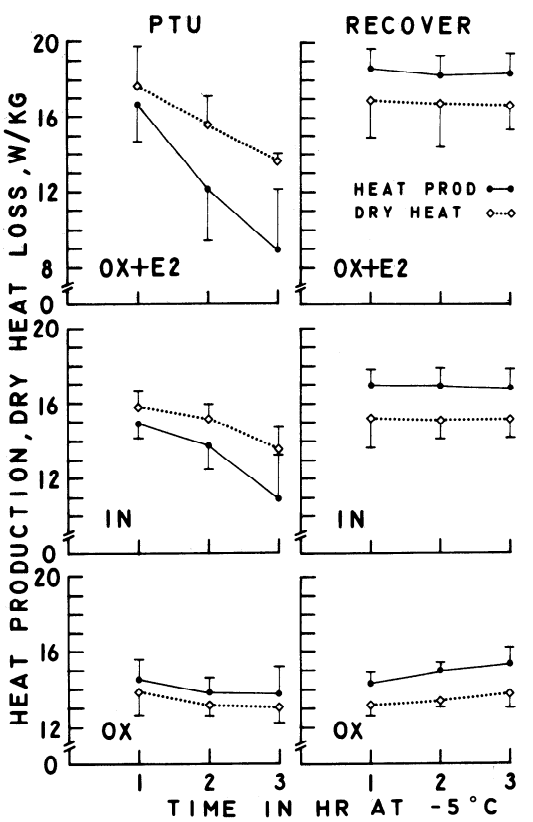

FIG. 3. Heat production (*filled symbols* connected by *solid lines*) and dry heat loss (*open symbols* connected by *broken lines*) as a function of time at −5°C for all groups during PTU treatment and following recovery from PTU. *Bracket* encloses 1 SD. Groups are indicated as in Fig. 1.

DHL (DHL/HP of 0.95 ± 0.07, 0.96 ± 0.05, and 0.96 ± 0.10 for the 1st, 2nd, and 3rd h, respectively). In contrast, DHL/HP of the estrogen-treated ovariectomized rats (1.12 ± 0.08, 1.34 ± 0.36, and 1.74 ± 0.74 for 1st, 2nd, and 3rd h, respectively) and the intact female rats (1.07 ± 0.08, 1.12 ± 0.08, and 1.30 ± 0.28 for 1st, 2nd, and 3rd h, respectively) always exceeded 1.0.

During the 1st h at −5°C, DHL for all groups was similar regardless of thyroid status. Thus the presence or absence of T_4 did not alter rates of DHL but did affect the ability of the rats to elevated HP above DHL. Thermal balance was restored after recovery from PTU, since HP of the intact female rats and the estrogen-treated ovariectomized rats rose above DHL (Fig. 3). As indicated in Table 2, T_{re} increased from pre- to posttest for all groups after recovery from PTU.

Body mass of the ovariectomized group not receiving estrogen replacement was greater than that of the estrogen-treated ovariectomized group and the intact group for all thyroid conditions ($P < 0.001$). Body masses are given in Table 3. Body mass changed little from the pre-PTU to the PTU condition, but following recovery from the PTU treatment, mass increased in all groups ($P < 0.001$).

Experiment 2. Thyroidectomized estrogen-treated ovariectomized rats were unable to maintain thermal balance at −5°C in a manner similar to PTU-treated estrogen-treated ovariectomized rats. Rates of DHL exceeded HP in the thyroidectomized estrogen-treated ovariectomized group during the entire test at −5°C, as shown in Fig. 4. In contrast, the thyroidectomized ovariectomized group not receiving estrogen was able to elevate HP above their relatively lower rates of DHL. However, unlike the PTU-treated rats, this group did not maintain this condition for the entire 3-h test, since DHL exceeded HP during the final hour (see Fig. 4). Pretest T_{rc} was the same for both the estrogen-treated (36.6 ± 0.8°C) and untreated (36.7 ± 0.3°C) thyroidectomized ovariectomized groups. At equivalent rates of HP during the 1st h at −5°C, HDL was highest for the estrogen-treated group ($P < 0.05$). As a consequence, T_{re} of the estrogen-treated group declined (−0.04°C/min) twice as fast as in the untreated group (0.02°C/min). Two rats in the estrogen-treated group were unable to complete the entire 3-h test (HP fell below 6 W/kg), whereas the entire untreated group completed the test at −5°C.

The less stressful test at 15°C did not uncover any differences between the two groups. Average HP for the estrogen-treated (12.98 ± 1.21 W/kg) and the untreated (12.92 ± 0.46 W/kg) ovariectomized thyroidectomized groups was similar. Average DHL was 11.72 ± 1.08 and 12.24 ± 0.90 W/kg for the estrogen-treated and untreated groups, respectively. No differences in pre- or posttest T_{re} were observed.

Average T_4 levels were 0.6 ± 0.4 and 0.5 ± 0.1 μg/100 ml, respectively, for the estrogen-treated and the untreated groups. Finally, there was no difference in body mass between the estrogen-treated (188 ± 17 g) and the untreated (193 ± 14 g) groups.

TABLE 3. *Body mass as a function of thyroid status and hormone condition in expt 1*

Thyroid Condition	Group		
	IN	OX + E_2	OX
Euthyroid, pre-PTU	213.2 ± 13.9	199.8 ± 14.9	260.6 ± 18.6
Hypothyroid, PTU-treated	213.6 ± 14.4	205.6 ± 28.4	279.4 ± 30.3
Euthyroid, PTU-recovered	246.0 ± 26.8	241.2 ± 44.6	320.2 ± 21.0

Values are means ± SD in grams; $n = 5$. Groups: see Table 1 footnote for abbreviations.

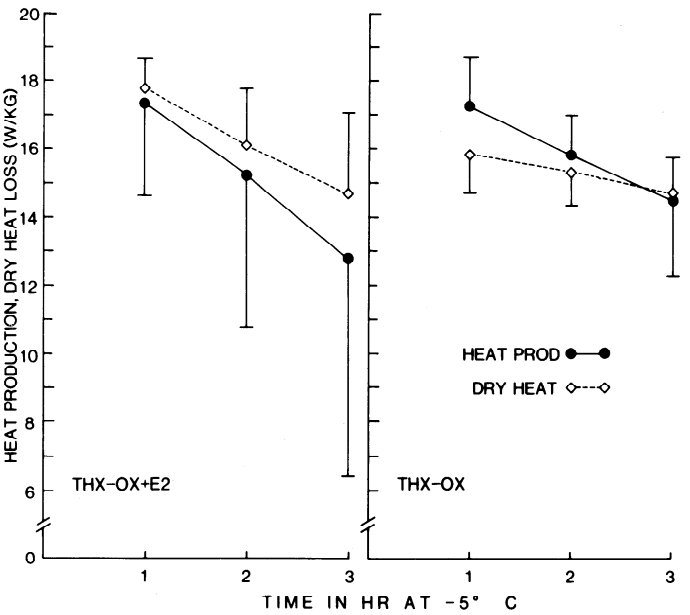

FIG. 4. Heat production (*filled symbols* connected by *solid lines*) and dry heat loss (*open symbols* connected by *broken lines*) as a function of time at −5°C for thyroidectomized rats. Thyroidectomized ovariectomized animals not receiving estrogen are indicated by THX-OX. Estrogen-treated animals are indicated by THX-OX+E_2. *Bracket* encloses 1 SD.

DISCUSSION

The present results suggest that one thermoregulatory effect of estrogen is to increase DHL. Surgical thyroidectomy or PTU treatment reduced the capacity of the estrogen-treated ovariectomized rats to oppose this higher rate of DHL with increased HP at −5°C. In contrast, the PTU-treated ovariectomized rats not receiving estrogen replacement maintained HP above DHL and remained in thermal equilibrium. The thyroidectomized ovariectomized rats not receiving estrogen had difficulty in elevating HP above DHL during the final hour of the 3-h test at −5°C. This indicates a difference between PTU treatment and thyroidectomy with regard to the animal's capacity to elevate HP during short-term cold exposure. However, DHL was highest in the estrogen-treated rats regardless of thyroid status. In the euthyroid condition, regardless of T_a, estrogen-treated rats were always able to maintain HP above their higher rate of DHL. This observation suggests that the levels of estrogen produced by the Silastic capsules in the present study neither depressed thyroid function nor prevented the rats from raising their metabolic rates. It seems likely that the increased rate of behavioral heat intake noted

previously (21) in cold-exposed estrogen-treated ovariectomized rats was not a response to depressed metabolism but rather to accelerated DHL.

Accelerated heat loss in intact female rats given pharmacologic supplements of ethynyl estradiol (EE) has been reported (8). Compared with rats in the present study treated with estradiol, the rats treated with EE showed a serious deficit in their ability to balance heat loss. For example, during relatively short exposures (60 min) to mild environmental cooling (T_a decreased from 28 to 19°C in 20 min), T_{re} of restrained EE-treated rats fell as much as 6°C. Furthermore, for any T_{re}, heat production of the EE-treated rats was lower than rats not receiving EE. This is in contrast to the present study in which euthyroid estrogen-treated ovariectomized rats were apparently able to compensate for their high rates of DHL by raising their HP proportionately. Perhaps the higher levels of EE produced by large capsules inhibited thyroid activity (5). As a consequence, the EE-treated rats might have been unable to elevate heat production adequately to compensate for their increased heat loss. This would be analogous to the thermal imbalance of the PTU-treated or the surgically thyroidectomized rats tested at −5°C in the present study. Differential effects of estradiol and ethynyl estradiol on hepatic lipase activity have been noted previously (20), and thus the type of estrogen administered could also be critical.

It is important to note that high rates of heat loss were associated with endogenous estrogen inasmuch as the intact female rats had higher rates of heat loss relative to the ovariectomized rats not receiving estrogen. The extent to which the dry heat loss of the euthyroid estrogen-treated ovariectomized rats exceeded the euthyroid intact female rats (see Fig. 2) may be related to differences in serum estrogen levels. The levels of estrogen produced by the Silastic capsule implants (30 pg/ml) did not exceed peak endogenous levels (50 pg/ml) noted by others for intact cycling rats (11). However, rats receiving estrogen via Silastic capsules are subjected to relatively invariant hormone levels because of the constant release of estrogen from the capsules, whereas the estrogen levels of intact rats vary with the estrous cycle. The relationship of the relatively low body temperature noted in intact cycling rats at proestrus (24) and early estrus (16) to rising estrogen levels (11) is unclear. It might be suggested that increased heat loss associated with rising estrogen levels is a contributing factor. Declining progesterone levels may also be involved, since progesterone administration is associated with increased thermogenesis in rats (6). Interestingly, the observation of increased heat production at proestrus (14) would imply that the normally cycling rat may be attempting to compensate for this change in energy balance. However, pilot observations (Tobet, Laudenslager, and Carlisle, unpublished) failed to confirm that rates of HP and DHL at proestrus (11.03 ± 0.47 and 9.39 ± 0.14) were different from those at diestrus I (11.16 ± 0.41 and 9.82 ± 0.31) during tests at 15°C.

Complex interactions exist among the ovarian, adrenal, and thyroid systems. Thus definitive statements regarding the unitary role of a single hormone are tenuous. It seems likely that adrenal function may have been important in the present study. For example, thyroid status affects circadian rhythms of coricosterone in the rat (18). Ovariectomy alone does not significantly affect plasma corticosterone-binding activity, but this activity is directly proportional to plasma T_4 levels (3). Furthermore, the combination of low T_4 levels and ovariectomy is additive and thus lowers corticosterone-binding activity maximally. The role of the adrenals in the increased HP and DHL associated with estrogen treatment has not been tested.

The mechanism by which estrogen accelerates heat loss is unknown. Body mass was reduced in the estrogen-treated rats, and reduced body mass is associated with increased heat loss per unit of mass (12). However, previous studies (8, 13) have discounted reduced body mass as a primary contributor to the increased heat loss noted in estrogen-treated rats. Because of the many effects of estrogen on the cardiovascular system (19), it may be that estrogen alters the peripheral vasculature so as to increase blood flow to the periphery or to interfere with cold-induced vasoconstriction. This could lead to increased heat loss from poorly insulated regions such as the tail, an effective heat exchanger in the rat (15). Estrogens may also influence thermoregulatory control mechanisms at the level of the hypothalamus, resulting in an altered thermoregulatory set point or gain (10). In summary, the thyroid does not appear to be necessary for the observation of increased heat loss associated with estrogen treatment, and it is possible that heat production is elevated as a compensatory response to increased heat loss in estrogen-treated rats.

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Present address of M. L. Laudenslager: Dept. of Psychology, Denver University, Denver, CO 80208.

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