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Reduced Norepinephrine Turnover in Interscapular Brown Adipose Tissue of Obese Rats After Ovariectomy

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Norepinephrine (NE) turnover, which is a reliable indicator of sympathetic nervous system (SNS) activity, was measured in the interscapular brown adipose tissue (IBAT), heart, and pancreas of ovariectomized (OVX), sham-operated rats receiving injections of estradiol benzoate (EB). Ovariectomized rats (OVX rats) ate much more than controls and became obese, whereas the administration of EB to obese OVX rats decreased their food intake to the level below that of sham-operated animals and body weight to the level of sham controls. The results from studies using the inhibition of NE biosynthesis with α-methyl-p-tyrosine or radiolabeled NE to measure NE turnover significantly demonstrated reductions in SNS activity in IBAT of OVX rats than in sham controls, whereas the injections of EB to OVX rats significantly restored the decrease of NE turnover in IBAT. NE turnover in heart and pancreas were similar in these three groups. It is suggested that reduced NE turnover in IBAT may be a major factor in the development of obesity after ovariectomy (OVX).

IT IS WELL-KNOWN that ovariectomy (OVX) increases food intake and body weight[1,2] and these changes can be inhibited or reversed by treatment with estradiol.[3,4] As to the cause, attention has been focused on the ventromedial hypothalamus (VMH), because of the following: (1) It contains many estradiol concentrating cells[5,6]; (2) implants of estradiol in the VMH of ovariectomized rats (OVX rats) suppress weight[3,7-9]; (3) lesions of VMH in OVX rats attenuate the response to estradiol.[2] However, because VMH is not only a center of satiety, but also a center of the sympathetic nervous system (SNS),[10] it is possible that the reduced SNS activity in the interscapular brown adipose tissue (IBAT) may be one of the important factors in the development of obesity in OVX rats. We have examined this by measuring norepinephrine (NE) turnover,[11-15] which is a reliable indicator of SNS activity in peripheral tissues, in IBAT, heart and pancreas of OVX rats, OVX rats treated with injections of estradiol benzoate (EB), and sham-operated rats.

MATERIALS AND METHODS

The 144 female Sprague-Dawley rats (approximately 200 g, 7 weeks old) used in these experiments were purchased from Charles-River Japan (Osaka, Japan).

Experiment 1: The Preliminary Study

An OVX was performed on 12 animals and 12 rats were sham operated. After surgery, the animals were housed in individual cages under conditions of controlled temperature (22 ± 2 °C) and artificial light from 7:00 am to 7:00 pm each day. Commercial powdered chow (Charles River Japan, Kanagawa, Japan) and tap water were available ad libitum. Four weeks after surgery, the animals were weighed and the study of NE turnover began between 9:00 am and 10:00 am. NE turnover was measured by determining the concentration of NE in IBAT, heart and pancreas at zero, three and six hours following the intraperitoneal injection of the methyl ester of α-methyl-p-tyrosine (80 mg/kg, Sigma Chemical, St Louis). This drug blocks tyrosine hydroxylase and prevents synthesis of NE.[11,14] The IBAT, heart, and pancreas were rapidly removed and dissected from connective tissue. Specimens were then frozen on dry ice and stored at −70 °C for later determination of NE. At the time of the assay (within 1 week), the frozen tissues were weighed and homogenized in ice-cold 0.1N perchloric acid containing 0.1 mmol/L reduced glutathione in a polytron (Kinematica, Luzern, Switzerland) and centrifuged at 0 °C. Aliquots of the supernatant were analyzed radioenzymatically for NE using a minor modification[15] of the method of Peuler and Johnson.[16]

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Table 1. NE Turnover Using the NE Biosynthesis Inhibition Technique in IBAT, Heart, and Pancreas of OVX and Sham-Operated Rats (Experiment 1)

Tissue	Organ Weight (g)	Endogenous NE (ng/organ)	Fractional NE Turnover k (%/h)	NE Turnover Rate (ng/organ/h)
IBAT				
OVX	0.143 ± 0.019	246.4 ± 6.8	4.1 ± 0.7	10.1 ± 2.1
Sham	0.139 ± 0.015	234.0 ± 7.4	14.2 ± 1.7	33.2 ± 5.2
P	NS	NS	<.01	
Heart				
OVX	0.935 ± 0.039	765.3 ± 51.0	6.8 ± 3.2	52.0 ± 29.6
Sham	0.844 ± 0.041	598.0 ± 47.3	6.0 ± 2.3	35.9 ± 17.7
P	NS	NS	NS	
Pancreas				
OVX	0.637 ± 0.041	391.8 ± 11.2	7.4 ± 2.0	29.0 ± 8.9
Sham	0.555 ± 0.048	388.1 ± 18.7	12.6 ± 1.8	48.9 ± 9.7
P	NS	NS	NS	

The fractional NE turnover (k) is expressed as mean ± SEM. The NE turnover rate is expressed as the mean with 95% confidence limits. Four rats were used at each time point to obtain the turnover data.

Abbreviation: NS, not significant.

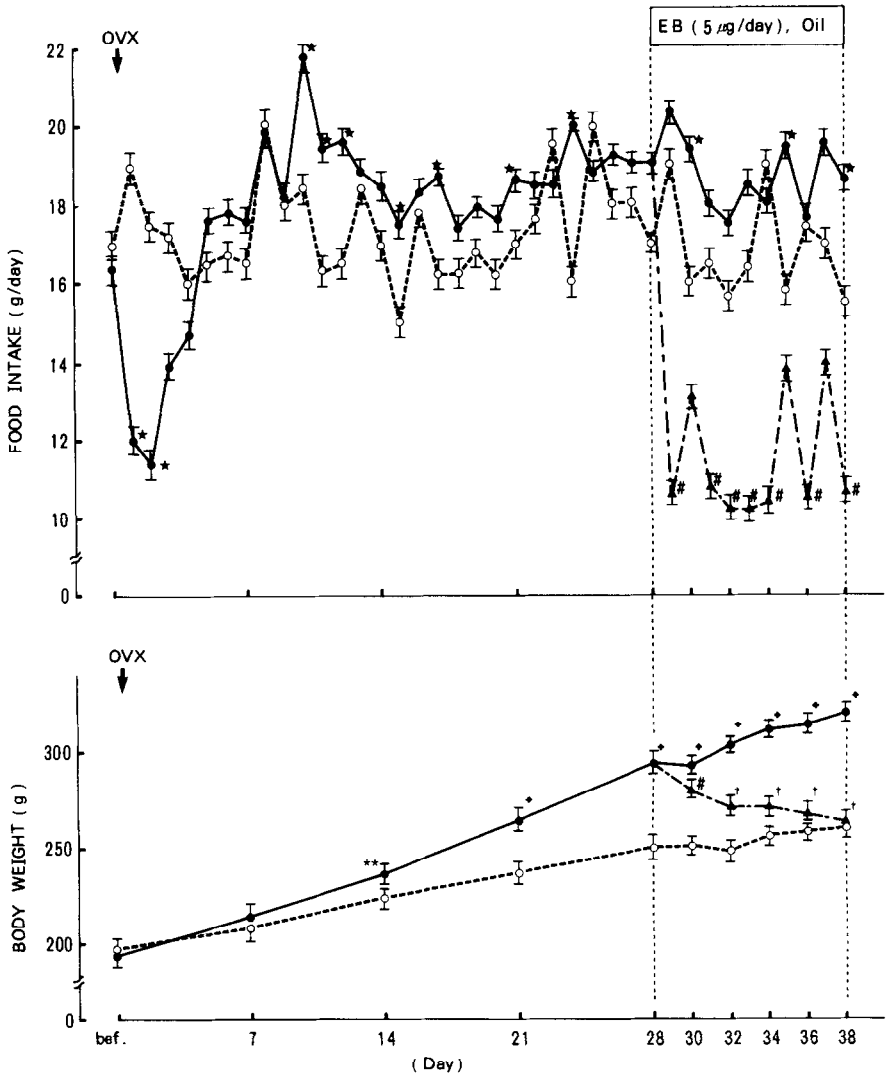

Fig 1. Changes of food intake and body weight in OVX + Oil (●), OVX + EB (▲), and sham-operated (○) rats.

Experiment 2: NE Turnover as Determined by the Inhibition Technique of NE Biosynthesis With α-Methyl-p-Tyrosine

Forty-eight rats were ovariectomized and 24 rats were sham operated. The rats were weighed and food intake was assessed by weighing the food administered and subtracting the amount remaining at the end of a 24 hour period, every day, in each group until the day before the study of NE turnover. Four weeks after surgery, 24 OVX rats were injected subcutaneously daily with 5 μg of estradiol benzoate (EB) in 0.1 mL sesame oil (OVX + EB) at the time of food measurement and the remaining 24 OVX rats were injected with sesame oil at the same dosage as the vehicle (OVX + Oil) for ten days. On the 11th morning following the administration of EB or oil, NE turnover was measured as in experiment 1.

Experiment 3: NE Turnover as Determined by the ^{3}H-NE Administration Technique

Forty-eight rats were housed in the same condition as in experiment 1. Thirty-two rats were ovariectomized and 16 rats were sham operated. By the same protocol as in experiment 2, OVX rats were divided into an OVX + EB group and an OVX + Oil group. On the 11th morning following the administration of EB or oil, NE turnover was determined by the ^{3}H-NE administration technique. Levo-[ring-2,5,6-^{3}H]-NE (sp act 44.4 Ci/mmol; New England Nuclear, Boston) was diluted to an appropriate concentration with isotonic saline and injected in a dose of 200 μCi/kg (0.76 μg · NE/kg) in a total volume of <0.5 mL intravenously (IV) into the tail vein of unanesthetized rats. One, three, five, and eight hours after the ^{3}H-NE administration, four animals from each experimental group were killed by cervical dislocation. The IBAT, heart, and pancreas were rapidly removed, frozen on dry ice and stored at −70 °C for later determination of NE (usually within 2 weeks). For NE analysis, the organ was weighed and homogenized with 3 mL/g of ice-cold 0.1N perchloric acid solution containing 0.1 mL of 0.1 mol EDTA, 0.1 mL of 1 mol $NaHSO_4$ and 45 μL of 0.14 mmol 3,4-dihydroxybenzylamine hydrobromide (DHBA, Sigma Chemical, St Louis) as the internal standard. NE was extracted by the method of Felice et al[17] and determined using high performance liquid chromatography (HPLC) with electrochemical detection (Bioanalytic System, USA) by the method of Riggin and Kissinger[18] using a Dowex AG ion exchange resin, 50–100 mesh (Muromac, MWC-1, Muromachi Chemical, Kyoto, Japan). Furthermore, a part of the aliquots of alumina eluate was counted for [^{3}H]-NE by scintillation spectrometry in a Packard TRI-CARB 460 liquid scintillation counter (Packard Instrument, Downers Grove, IL).

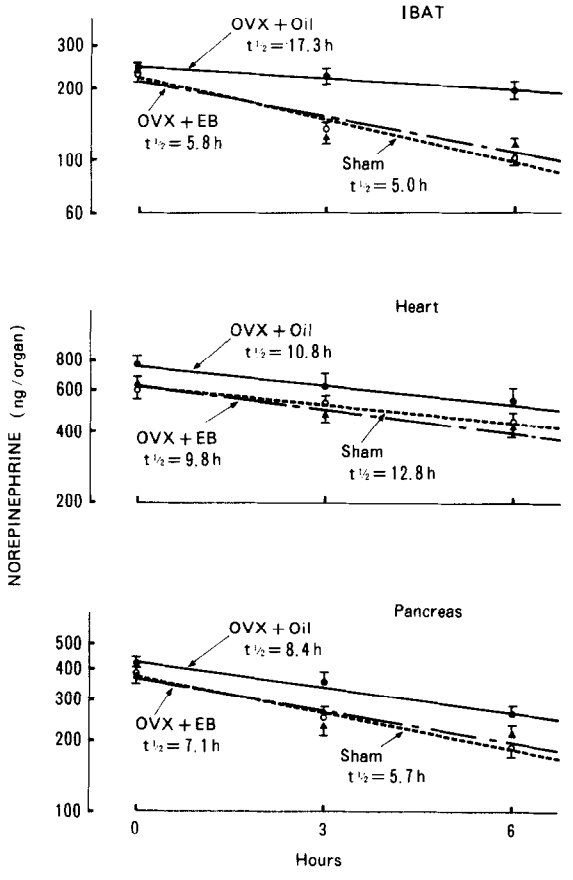

Fig 2. Norepinephrine turnover using the norepinephrine biosynthesis inhibition technique (experiment 2) in IBAT, heart, and pancreas of OVX + Oil (●), OVX + EB (▲), and sham-operated (○) rats. All data are plotted as the mean ± SEM for endogenous NE in tissues from eight animals in each group at zero, three, and six hours after the injection of α-methyl-p-tyrosine (80 mg/kg). The null hypothesis that all three regression lines could be represented by a common one was rejected for IBAT ($F_{2,66}$ = 13.2, P < .001), but not in heart ($F_{2,66}$ = 0.3, NS) and pancreas ($F_{2,66}$ = 2.0, NS).

Data are presented as mean ± SEM. Statistical analyses were performed using analysis of variance and of covariance.[19,20] In studies of NE turnover, the data were plotted semilogarithmically. The slope (fractional NE turnover rate, k) of the decline in endogenous NE after α-methyl-p-tyrosine injection or in NE specific activity over time after ^{3}H-NE administration was calculated by the method of least squares.[19,20] The statistical significance of each computed regression line was assessed by analysis of variance.[20] Comparison of fractional turnover rates was made by an analysis of covariance.[20] NE turnover rates were calculated as the product of the fractional turnover rate (k) times the endogenous NE content, which was the endogenous NE content at time zero in the experiments using α-methyl-p-tyrosine. The 95% confidence intervals were determined for the NE turnover rates as described previously.[21]

RESULTS

Experiment 1: The Preliminary Study

Four weeks after surgery, the body weight (292 ± 4 g) of OVX rats was significantly heavier than that (246 ± 6 g) of sham-operated animals (P < .05).

Table 1 shows the NE turnover data from IBAT, heart, and pancreas of OVX and sham controls. No significant differences in organ weights and basal NE contents of IBAT, heart, and pancreas were found in these groups. However, the fractional NE turnover rate (k) in IBAT was 4.1 ± 0.7%/h and 14.2 ± 1.7%/h (P < .01) for OVX and sham groups, respectively. The total NE turnover in IBAT was significantly decreased by OVX. In heart and pancreas, no significant differences in fractional NE turnover rate (k) and total NE turnover were observed.

Experiments 2 and 3

Figure 1 shows changes of body weight and food intake in OVX + Oil, sham and OVX + EB groups in experiment 2. The OVX group without treatment ate more than sham

Table 2. NE Turnover Using the NE Biosynthesis Inhibition Technique in IBAT, Heart, and Pancreas of OVX + Oil, Sham-Operated, and OVX + EB Rats (Experiment 2)

Tissue	Organ Weight (g)	Endogenous NE (ng/organ)	Fractional NE Turnover k (%/h)	NE Turnover Rate (ng/organ/h)
IBAT				
OVX + Oil	0.141 ± 0.013	243.4 ± 5.2	4.0 ± 1.2*	9.7 ± 3.2
Sham	0.135 ± 0.011	231.3 ± 5.5	13.9 ± 1.1	32.2 ± 3.3
OVX + EB	0.143 ± 0.014	242.7 ± 9.0	12.0 ± 1.6†	29.1 ± 5.1
Heart				
OVX + Oil	0.920 ± 0.031	768.9 ± 32.2‡	6.4 ± 1.9	49.2 ± 17.3
Sham	0.804 ± 0.032	597.0 ± 28.4	5.4 ± 1.5	33.8 ± 9.4
OVX + EB	0.845 ± 0.020	625.3 ± 17.9	7.1 ± 1.5	44.4 ± 10.9
Pancreas				
OVX + Oil	0.617 ± 0.028	421.5 ± 15.6	8.2 ± 1.8	34.6 ± 9.1
Sham	0.539 ± 0.032	386.1 ± 11.4	12.2 ± 1.4	47.1 ± 7.0
OVX + EB	0.538 ± 0.027	383.3 ± 12.9	9.8 ± 1.6	37.6 ± 7.6

The fractional NE turnover (k) is expressed as mean ± SEM. The NE turnover is expressed as the mean with 95% confidence limits. Eight rats were used at each time point to obtain the turnover data.

*Significantly different from Sham; $P < .001$.

†Significantly different from OVX + Oil; $P < .01$.

‡Significantly different from Sham; $P < .01$.

controls and the weight gain was significant, but the administration of EB to OVX rats decreased the food intake to levels below those of oil injected ovariectomized and sham operated animals, and body weights to level of sham controls ($P < .01$).

Figure 2 and Table 2 show the NE turnover data for the IBAT, heart, and pancreas of OVX + Oil, sham-operated and OVX + EB rats for experiment 2 obtained by the NE biosynthesis inhibition technique and Table 3 shows that for experiment 3 obtained by the ^{3}H-NE administration technique. In both experiment 2 and 3, the fractional NE turnover rate (k) and the total NE turnover rate in the IBAT of OVX + Oil group were significantly slower than those in the sham group and the administration of EB to OVX rats significantly restored the decrease of the NE turnover. In heart and pancreas, k and total NE turnover rate were similar in the three groups.

DISCUSSION

We have investigated the hypothesis that the reduced SNS activity in IBAT is one of the important factors in the development of obesity in OVX rats by measuring NE turnover in peripheral tissues of OVX rats. These results showed that OVX caused hyperphagia and led to a significant gain in body weight, and the administration of estradiol to obese rats after ovariectomy decreased their food intake and body weight, which was consistent with other reports.[1-4] Further, the NE turnover in IBAT of OVX rats was reduced significantly, compared to that of sham controls, and estra-

Table 3. NE Turnover Using ^{3}H-NE Administration Technique in IBAT, Heart, and Pancreas of OVX + Oil, Sham-Operated, and OVX + EB Rats (Experiment 3)

Tissue	Organ Weight (g)	Endogenous NE (ng/organ)	Fractional NE Turnover k (%/h)	NE Turnover Rate (ng/organ/h)
IBAT				
OVX + Oil	0.182 ± 0.017	266.8 ± 19.3	4.5 ± 1.6*	12.0 ± 5.5
Sham	0.169 ± 0.015	255.1 ± 9.4	12.6 ± 1.7	32.1 ± 5.7
OVX + EB	0.177 ± 0.013	261.3 ± 10.0	9.1 ± 2.0	23.8 ± 6.3
Heart				
OVX + Oil	0.836 ± 0.039*	697.2 ± 39.8†	6.7 ± 1.9	46.7 ± 16.7
Sham	0.720 ± 0.038	539.7 ± 27.9	10.1 ± 1.8	54.5 ± 13.0
OVX + EB	0.774 ± 0.036	545.9 ± 39.1	8.0 ± 1.7	43.7 ± 13.0
Pancreas				
OVX + Oil	0.575 ± 0.020	297.7 ± 23.5	11.5 ± 1.8	34.2 ± 8.5
Sham	0.566 ± 0.025	248.6 ± 15.6	13.9 ± 1.8	34.6 ± 6.4
OVX + EB	0.561 ± 0.019	299.0 ± 30.7	10.2 ± 2.0	30.5 ± 9.7

The fractional NE turnover (k) is expressed as mean ± SEM. The NE turnover is expressed as the mean with 95% confidence limits. Four rats were used at each time point to obtain the turnover data.

*Significantly different from Sham: $P < .05$.

†Significantly different from Sham: $P < .01$.

diol injections to OVX rats restored the decreased NE turnover in IBAT, although the NE turnover in heart and pancreas was similar in the OVX + Oil, sham-operated, and OVX + EB groups.

The NE turnover obtained by NE biosynthesis inhibition with α-methyl-p-tyrosine was consistent with that obtained by ^{3}H-NE administration. NE turnover has been shown to be a reliable indicator of the SNS activity.[11–15] Therefore, our results suggest that SNS activity is reduced in IBAT of OVX rats and the administration of estradiol to OVX rats restored this. We suggest that the decrease of estradiol following ovariectomy led to the reduction of estrogen binding to estrogen receptors in the VMH, which was less stimulated, leading to the decrease in NE turnover. To clarify this, we are now examining whether an antagonist of the estrogen receptor, Tamoxifen[22] (Nolvadex, ICI America, Wilmington, DE) can decrease NE turnover in IBAT and produce obesity. Mouridsen et al[23] reported that Tamoxifen treatment to advanced breast cancer caused marked body weight gain in 11 of 417 patients.

Our data on NE turnover after OVX shows that it differs in IBAT, compared with heart and pancreas. Although the cause of this discrepancy is unknown, we suggest that IBAT may be regulated more strongly by SNS than heart and pancreas[24] because in our previous study[25] using monosodium-L-glutamate (MSG) induced obese mice, we found that in the preobese stage, the NE turnover in IBAT of MSG mice was significantly decreased, which was not seen in heart. In the obese stage, the NE turnover was decreased both in IBAT and in heart of MSG mice.

It is now recognized[26] that BAT is a main effector of diet-induced thermogenesis (DIT) as well as nonshivering thermogenesis (NST), and that a defect in or an absence of BAT would predispose to obesity, as shown in genetically obese (ob/ob) mice,[27–29] VMH-lesioned weanling rats[30] and MSG-induced obese mice,[25,31] although the relevance of brown adipose tissue to thermogenesis per se, let alone the pathogenesis of obesity, is still now unknown in humans. In this study we have shown a reduced NE turnover of IBAT in obese rats after OVX and suggest that this may be a major factor in the development of obesity after OVX.

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