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EFFECTS OF NOREPINEPHRINE AND THYROXINE ON THE TURNOVER RATE OF PLASMA FREE FATTY ACIDS

Kiyoshi MORIYA, Hiroshi MAEKUBO, Kenichi HONMA, and Shinji ITOH

Department of Physiology, Hokkaido University School of Medicine, Sapporo 060, Japan

Abstract Effects of norepinephrine infusion on rectal temperature, plasma free fatty acids (FFA) concentration and its turnover rate were studied in rats treated chronically with norepinephrine, thyroxine, or both. Chronic treatments with these hormones resulted in greater increases in rectal temperature and FFA turnover rate in response to norepinephrine as compared with the alterations in controls. Norepinephrine-induced elevation of FFA concentration was smaller in norepinephrine-treated and norepinephrine plus thyroxine-treated rats than in controls, and in thyroxine-treated rats the elevation was similar to that of controls. The regression coefficient of FFA concentration on its turnover rate was greater in all the treated groups than in controls although positive correlations were observed among both variables in the former and the latter. From the results it was inferred that the greater increase in removal of plasma FFA was produced by norepinephrine in parallel with the greater increase in rectal temperature by norepinephrine in rats treated with norepinephrine or thyroxine than in the controls.
The effects of norepinephrine infusion were also studied in surgically thyroidectomized rats. In thyroidectomized rats adapted to warm or cold, the smaller increases in rectal temperature and in the turnover rate of plasma FFA were induced by norepinephrine as compared with the changes in intact rats. The alterations in FFA concentration produced by norepinephrine were less in warm-adapted thyroidectomized rats and rather greater in cold-acclimated thyroidectomized rats than in respective controls.

In cold-acclimated rats it was observed in the previous report (MORIYA *et al.*, 1974) that rectal temperature and the turnover rate of plasma free fatty acid (FFA) during norepinephrine infusion attained higher levels than those in warm-

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森谷 潔，前久保博士，本間研一，伊藤真次

adapted control ones in spite of lower elevation of FFA concentration in the former than in the latter. The concentration and the turnover rate in plasma FFA at rest were on the same levels in both groups, respectively. Positive correlations were observed between the concentration and the turnover rate in plasma FFA, rectal temperature and logarithm concentration of plasma FFA and rectal temperature and logarithm turnover rate of plasma FFA in both groups when these valiables included everything before and during infusion of norepinephrine. The regression coefficients of FFA concentration on FFA turnover rate in plasma and of logarithm concentration in plasma FFA on rectal temperature were greater in cold-acclimated rats than in controls. The intersections of two correlation curves in each case were around resting levels of FFA concentration, the turnover rate of FFA and rectal temperature in both groups. While there was no difference in regression coefficient of logarithm turnover rate in plasma FFA on rectal temperature among both groups. From the results it was inferred that removal of circulating FFA which might be activated by norepinephrine was significantly greater in cold-acclimated rats than in controls since in the steady state conditions used in this study the turnover rate represented the rate of FFA release from adipose tissues and of its removal by the tissues. Furthermore, it was also inferred that an increase in FFA removal from the circulation might be induced by norepinephrine in parallel with an increase in rectal temperature, and that this phenomenon might be an important basis for nonshivering thermogenesis developed in cold acclimation.

LEBLANC *et al.* (1964, 1970, 1972) reported that animals treated chronically with either norepinephrine or thyroxine, or both showed a greater increases in oxygen consumption and rectal temperature in response to exogenous norepinephrine and a greater resistance to extreme cold of 25°C than controls. The increments found in these rats were similar to those observed in cold-acclimated ones. Thereafter, HSIEH and WANG (1971), NAGASAKA (1972) and AGISHI and ITOH (1975 a, b) ascertained the increase in norepinephrine-induced calorigenesis in animals treated with these hormones. According to these results it was indicated that norepinephrine and thyroxine are essential for the development of cold acclimation.

On the other hand, it was recognized that thyroid hormones have an effect on the metabolic rate and play an important role in the maintenance of body temperature, and that in thyroidectomized rats lesser calorigenesis was induced by norepinephrine than in intact rats (HONMA, 1975). According to PAUL and HOLMES (1973) in thyroidectomized conscious dogs the lower level of FFA turnover rate in plasma and the same concentration of plasma FFA were observed during rest and acute cold exposure, as compared with those in normal dogs.

The present work was designed to determine that the increase or decrease in the removal of plasma FFA was usually induced by norepinephrine in parallel with the rise or fall of calorigenesis by norepinephrine in animals treated chroni-

cally with norepinephrine or thyroxine and in thyroidectomized animals.

METHODS

Animals used in this study were male rats of the Wistar strain with an average body weight of 220 g at the beginning of treatment. They were provided with water and chow (Oriental MF) *ad libitum*. In the first series of experiments rats were divided into the following four groups. Group 1 was olive oil controls, injected s.c. daily with 0.15 ml of oil/100 g body weight. Group 2 was norepinephrine (NE)-treated rats, injected s.c. with norepinephrine bitartrate (Wako Chem. Co.) suspended in olive oil in a daily dose of 60μg/100 g. Groups 3 was thyroxine (T4)-treated rats, injected s.c. with 5 μg thyroxine natrium (Sigma Chem. Co.)/100 g. Group 4 was NE+T4-treated rats, injected s.c. daily with norepinephrine and thyroxine in doses equal to those in Groups 2 and 3. All treatments were given daily for 4 weeks in accordance with the period reported by AGISHI and ITOH (1975 a, b) until the day before the experiment. In the second series of experiments, the following four groups of rats were used. Group 1 was warm-adapted controls, kept at 22°C. Group 2 was thyroidectomized (TX) rats, kept at room temperature of 22°C. Group 3 was cold-acclimated control rats, exposed to environment of 5°C for 2 to 4 weeks. Group 4 was cold-acclimated TX rats which were thyroidectomized after exposure to cold for 2 to 4 weeks. Thyroidectomy was performed surgically and the animals were provided with 1% calcium lactate solution in place of tap water and used for experiments 2 weeks after the surgery.

After 18 hr fasting, rats were anaesthetized with urethane (150 mg/100 g body weight, s.c.). A continuous infusion of ^{14}C-palmitate (New England Nuclear Corp., specific activity: 500 Ci/mol)-albumin complex solution was given *via* the right femoral vein using a constant infusion pump which delivered 14 μl of solution (^{14}C: 5 mμCi) per min through an indwelling polyethylene catheter. Blood samples were taken from the jugular vein into heparinized syringes at intervals as indicated below. These samples were used for determinations of plasma FFA concentration and its specific activity (SA). The first sample was drawn 20 min after the infusion of ^{14}C-palmitate. Immediately after the sampling, norepinephrine infusion was started concurrently with ^{14}C-palmitate *via* another femoral vein at a constant rate of 2 μg per min. The second blood sample was drawn 20 min after the norepinephrine infusion. Under steady state conditions, infusion rate of ^{14}C-palmitate divided by SA of plasma FFA represents the turnover rate of plasma FFA (ARMSTRONG *et al.*, 1961; MORIYA *et al.*, 1974).

Plasma FFA concentration was determined by the method of DUNCOMBE (1963). Radioactivity in the FFA fractions which were isolated by the method of STEINBERG *et al.* (1964) was counted with a scintillation spectrometer.

Rectal temperature was measured at a site 5 cm from the anus in a room with

a temperature 25°C. It was measured by the use of thermister thermometer just before the start of norepinephrine infusion and 10, 20, and 30 min after the start of the infusion.

RESULTS

1. Changes in rectal temperature by norepinephrine infusion in NE-, T4-, and NE+T4-treated rats

Effect of intravenous infusion of norepinephrine at a rate of 2 μg/min on rectal temperature was observed in olive oil controls, NE-, T4-, and NE+T4-treated rats under urethane anaesthesia. Effect of saline infusion was also surveyed as control experiments.

Before the infusion rectal temperatures were on the same level in these four groups (Table 1). In the case of infusion of physiological saline a gradual lowering of the rectal temperature was observed in all groups, and the mean change of three rats for 20 min was −1.41°C for controls, −0.65°C for NE-treated ones, −0.83°C for T4-treated ones and −0.33°C for NE+T4-treated rats. An infusion of norepinephrine resulted in a significant elevation of rectal temperature in NE-, T4-, and NE+T4-treated groups, but not in controls. It is apparent that the greater increase was induced by norepinephrine in the treated groups than in the controls.

Table 1. Changes in rectal temperature during norepinephrine infusion (2 μg/min for 30 min) in rats treated with norepinephrine (NE) and thyroxine (T4) under urethane anaesthesia.

Group	No. of rats	Before (°C)	Δ10 min	Δ20 min	Δ30 min
Olive oil control	6	35.32±0.15	−0.27±0.03	−0.33±0.03	−0.43±0.03
P			<0.001	<0.001	<0.001
NE-treated	10	34.75±0.21	+0.39±0.07**	+0.99±0.13**	+1.36±0.18**
P			<0.001	<0.001	<0.001
T4-treated	9	35.79±0.23	+0.21±0.05**	+0.53±0.08**	+0.78±0.13**
P			<0.001	<0.001	<0.001
NE+T4-treated	10	35.97±0.14	+0.42±0.26*	+1.12±0.26**	+1.47±0.36*
P			<0.001	<0.01	<0.01

Mean±SEM * $P<0.01$, ** $P<0.001$ *vs.* olive oil control

Rectal temperature measured just before the start of norepinephrine infusion is given as before, and changes in rectal temperature during 10, 20, and 30 min period after the start of infusion are given as Δ10, Δ20, and Δ30.

2. Changes in the concentration and the turnover rate of plasma FFA induced by norepinephrine infusion in NE-, T4-, and NE+T4-treated rats

Effects of norepinephrine infusion on the concentration and the turnover rate of plasma FFA were examined in the four groups of rats. Although an aver-

Table 2. Changes in the concentration and the turnover rate of plasma FFA produced by norepinephrine infusion (2 μg/min for 20 min) in rats treated with norepinephrine (NE) and thyroxine (T4) under urethane anaesthesia.

Group	No. of rats	Body weight	FFA concentration (μEq/liter)			Turnover rate (μEq/min/100 g)		
			before	after	difference	before	after	difference
Olive oil control	6	365±17	689±72	1315±45	626±41	2.25±0.24	3.54±0.23	1.29±0.17
NE-treated	10	325±17	679±30	1109±48*	430±58*	2.81±0.18	4.33±0.19*	1.52±0.21
T4-treated	9	300±14	702±49	1416±64	693±42	3.15±0.28	5.41±0.33**	2.26±0.25**
NE+T4-treated	10	300±18	787±37	1168±51	381±47*	3.66±0.27*	5.32±0.36**	1.66±0.30

Mean±SEM * $P<0.05$, ** $P<0.01$ *vs.* olive oil control

age body weight of rats in each groups was 220 g at the beginning of treatments, an increase in body weight for 4 weeks seemed to be less in NE-, T4-, and NE+T4-treated rats than in controls, presumably, as a result of the lipolysis activated by norepinephrine or thyroxine (Table 2). Before the infusion plasma concentrations of FFA in the four groups were not different, and the turnover rate of plasma FFA was the highest in NE+T4-treated rats, the lowest in controls and intermediate in NE- and T4-treated rats (Table 2). As seen in this table, norepinephrine infusion caused significant increases in the concentration and the turnover rate of FFA of each group, respectively. It should be noted that in NE- and NE+T4-treated rats norepinephrine-induced elevation of FFA concentration was less in spite of higher increase in FFA turnover rate, as compared with the changes in controls. In T4-treated rats the elevation of plasma FFA concentration was similar to that of controls, but the turnover rate of FFA showed a marked increase. After saline infusion, no significant changes were observed in both concentration and turnover rate of FFA in all groups.

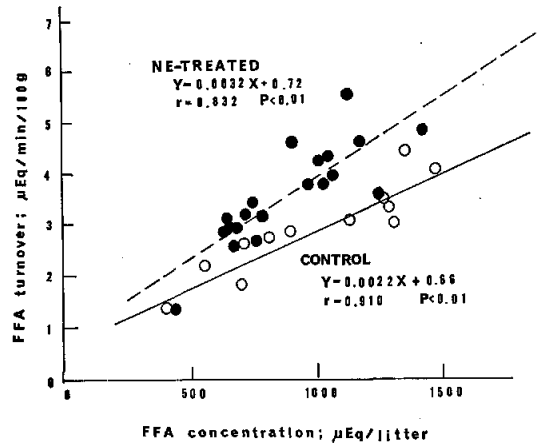

Fig. 1. Relationship between FFA concentration and its turnover rate of plasma in NE-treated rats and control ones.

When the relationship between FFA concentration (X) and its turnover rate (Y) was examined, significant positive correlations were obtained in these four groups (Fig. 1 and Table 3). The regression equations were calculated by the method of least squares and included the values before and during norepinephrine infusion. Each regression coefficient for the treated rats was significantly greater than that for controls (Table 3).

3. Elevation of rectal temperature in relation to the concentration and the turnover rate of plasma FFA in NE-, T4- and NE+T4-treated rats

In order to investigate the possibility that the elevation of rectal temperature

Table 3. Relationship between the concentration (X) and the turnover rate (Y) in plasma FFA in rats treated with norepinephrine (NE) and thyroxine (T4).

Group	n	$\bar{X}$	$\bar{Y}$	Regression equation	Regression coefficient	Correlation coefficient	P*
Olive oil control	12	1002	2.90	$Y=0.0022X+0.66$	0.0022	0.910	<0.01
NE-treated	20	894	3.52	$Y=0.0032X+0.72$	0.0032	0.832	<0.01
P vs. control					<0.01		
T4-treated	18	1059	4.28	$Y=0.0033X+0.72$	0.0033	0.874	<0.01
P vs. control					<0.01		
NE+T4-treated	20	978	4.49	$Y=0.0039X+0.68$	0.0039	0.720	<0.01
P vs. control					<0.01		

n; the number of variables.
$\bar{X}$ and $\bar{Y}$; the respective average of X and Y.
* for correlation coefficient.

induced by norepinephrine is related to the increase in FFA concentration or FFA turnover rate, rectal temperature was analysed for logarithm concentration of FFA and logarithm turnover rate of FFA (Tables 4 and 5). In this case the correctional value was added to each rectal temperature measured 20 min following norepinephrine infusion since rectal temperature of rats was gradually lowered by urethane anaesthesia. The value was an average change in rectal temperature during infusion of physiological saline for 20 min and was detailed in part 1 of results. As shown in Table 4, positive correlations were obtained between rectal temperature (Y) and logarithm concentration of FFA (X) in all groups. The regression coefficients for NE- and NE+T4-treated groups were greater than that

Table 4. Relationship between logarithm concentration of plasma FFA (X) and rectal temperature (Y) in rats treated with norepinephrine (NE) and thyroxine (T4).

Group	n	$\bar{X}$	$\bar{Y}$	Regression equation	Regression coefficient	Correlation coefficient	P*
Olive oil control	12	2.974	35.86	$Y=2.28\log X+29.08$	2.28	0.600	<0.05
NE-treated	20	2.934	35.56	$Y=6.51\log X+16.47$	6.51	0.803	<0.01
P vs. control					<0.01		
T4-treated	18	2.992	36.47	$Y=2.65\log X+28.55$	2.65	0.498	<0.05
P vs. control					NS		
NE+T4-treated	20	2.977	36.69	$Y=6.85\log X+16.31$	6.85	0.676	<0.01
P vs. control					<0.05		

n; the number of variables.
$\bar{X}$ and $\bar{Y}$; the respective average of X and Y.
* for correlation coefficient.

Table 5. Relationship between the logarithm turnover rate of plasma FFA (X) and rectal temperature (Y) in rats treated with norepinephrine (NE) and thyroxine (T4).

Group	n	$\bar{X}$	$\bar{Y}$	Regression equation	Regression coefficient	Correlation coefficient	P*
Olive oil control	12	0.440	35.86	$Y=2.19 \log X+34.89$	2.19	0.471	NS
NE-treated	20	0.534	35.56	$Y=4.58 \log X+33.12$	4.58	0.604	<0.01
P vs. control					NS		
T4-treated	18	0.605	36.47	$Y=3.17 \log X+34.56$	3.17	0.529	<0.05
P vs. control					NS		
NE+T4-treated	20	0.635	36.69	$Y=5.08 \log X+33.47$	5.08	0.594	<0.01
P vs. control					NS		

n; the number of variables.
$\bar{X}$ and $\bar{Y}$; the respective average of X and Y.
* for correlation coefficient.

for controls. Positive correlations were also obtained between rectal temperature (Y) and logarithm turnover rate of FFA (X) although in control rats the significance of correlation among both values was lower than the lowest significant level, and no difference in these regression coefficients was observed between groups treated with norepinephrine, thyroxine, or both and controls (Table 5).

4. Changes in rectal temperature by norepinephrine infusion in thyroidectomized rats

Effect of norepinephrine infusion at a rate of 2 μg and 0.5 μg per min on rectal temperature was observed in TX rats adapted warm and cold environments.

Table 6. Changes in rectal temperature during norepinephrine infusion in thyroidectomized (TX) rats under urethane anaesthesia.

Group	No. of rats	Before (°C)	Δ10 min	Δ20 min
Norepinephrine infusion rate; 2 μg/min				
Room control	15	36.18±0.25	−0.33±0.11	−0.51±0.19
TX	7	32.76±0.32**	−0.84±0.07*	−1.39±0.06*
Cold-acclimated control	15	36.49±0.41	+0.52±0.13	+1.11±0.26
TX	7	33.92±0.53*	−0.33±0.10**	−0.24±0.12*
Norepinephrine infusion rate; 0.5 μg/min				
Cold-acclimated control	4	35.60±0.20	−0.16±0.16	−0.04±0.29
TX	5	31.93±0.59*	−0.42±0.10	−0.75±0.19

Mean±SEM * $P<0.01$, ** $P<0.001$ *vs.* respective control

Rectal temperature measured just before the start of norepinephrine infusion is given as before, and changes in rectal temperature during 10 and 20 min period after the start of the infusion are given as Δ10 and Δ20.

Table 7. Changes in the concentration and the turnover rate of plasma FFA produced by norepinephrine infusion for 20 min in thyroidectomized (TX) rats under urethane anaesthesia.

Group	No. of rats	Body weight	FFA concentration (μEq/liter)			Turnover rate (μEq/min/100 g)		
			before	after	difference	before	after	difference
Norepinephrine infusion rate; 2 μg/min								
Room control	14	310	580±22	1185±46	605±42	1.99±0.13	3.39±0.19	1.39±0.14
TX	7	335	426±42	907±57**	481±27	1.11±0.15**	1.87±0.14***	0.75±0.06**
Cold-acclimated control	16	350	626±30	992±38	366±29	2.29±0.21	4.21±0.31	1.92±0.22
TX	7	285	347±24**	1109±71	763±87***	0.91±0.11***	3.04±0.27*	2.14±0.18
Norepinephrine infusion rate; 0.5 μg/min								
Cold-acclimated control	4	200	672±68	866±88	194±28	3.26±0.31	4.03±0.34	0.82±0.15
TX	5	205	466±66	851±73	384±56*	1.33±0.19**	2.47±0.24*	1.14±0.19

Mean±SEM * $P<0.05$, ** $P<0.01$, *** $P<0.001$ *vs.* respective control

Before the infusion rectal temperature of these TX rats were lower than that of respective controls (Table 6). During the infusion at a rate of 2 μg per min rectal temperature fell gradually in warm-adapted controls, warm-TX and cold-TX rats, while it rose in cold-acclimated rats. Infusion of norepinephrine at a rate of 0.5 μg per min was ineffective to induce elevation of rectal temperature in every group, but the extent of the decrease was much less in cold-acclimated controls as compared with that in cold-TX rats. Thus, it was shown that thyroidectomy suppresses the calorigenic action of norepinephrine.

5. Changes in the concentration and the turnover rate of plasma FFA in thyroidectomized rats

Before the norepinephrine infusion, plasma concentration of FFA and the turnover rate of plasma FFA of warm-TX and cold-TX rats were lower than those of the respective controls (Table 7). Norepinephrine infusion caused significant increases in the both variables of each group. In warm-adapted rats the elevation of FFA concentration and turnover rate was smaller in TX rats than in the controls. While in cold-acclimated rats, FFA concentration of TX rats increased to a similar level as the controls in spite of a lower level of turnover rate in plasma FFA.

In these groups positive correlations were obtained between FFA concentration (X) and its turnover rate (Y) in plasma when both variables included everything before and during infusion of norepinephrine at a rate of 2 μg per min (Table 8). The regression coefficients of FFA concentration on FFA turnover rate for TX rats in the both different ambient temperatures were significantly smaller than that for respective controls (Table 8).

Table 8. Relationship between the concentration (X) and the turnover rate (Y) in plasma FFA in thyroidectomized (TX) rats.

Group	n	$\bar{X}$	$\bar{Y}$	Regression equation	Regression coefficient	Correlation coefficient	P*
Room control	28	883	2.69	$Y=0.0024X+0.57$	0.0024	0.809	<0.01
TX	14	606	1.49	$Y=0.0018X+0.32$	0.0018	0.917	<0.01
P *vs.* control					<0.01		
Cold-acclimated control	32	809	3.25	$Y=0.0047X+0.58$	0.0047	0.753	<0.01
TX	14	728	1.98	$Y=0.0028X+0.04$	0.0028	0.944	<0.01
P *vs.* control					<0.01		

n; the number of variables.
$\bar{X}$ and $\bar{Y}$; the respective average of X and Y.
* for correlation coefficient.

DISCUSSION

In this study chronic treatments of rats either with norepinephrine or thyro-

xine, or both caused greater responses to infused norepinephrine in elevating rectal temperature and the turnover rate of plasma FFA in comparison with the responses in controls. On the other hand, the effect of norepinephrine on increasing FFA concentration was less in NE- and NE+T4-treated rats than in controls, but in T4-treated rats it was similar to that in controls (Tables 1 and 2). Although positive correlation was observed between FFA concentration and its turnover rate in each group, the regression coefficient was greater in NE-, T4-, and NE+T4-treated rats than in controls (Table 3). These results are consistent with the general concept that the turnover (=uptake) of plasma FFA changes in parallel with the concentration of plasma FFA by mass action effect, although the ratio of FFA turnover rate to its concentration was greater in NE-, T4- and NE+T4-treated rats than in controls. Thyroid hormone potenciates the effect of norepinephrine on lipolysis in adipose tissue (DEYKIN and VAUGHAN, 1963; CALDWELL and FAIN, 1971), therefore norepinephrine-induced lipolysis may be enhanced more in T4-treated rats than in controls, resulting in higher mobilization of FFA to the circulation. Thus, it can be deduced that FFA uptake by the tissues may be stimulated more strongly by norepinephrine in T4-treated rats than in controls from the fact that the regression coefficient of FFA concentration on its turnover rate was greater in the former than in the latter. It is also inferred that in NE+T4-treated rats as well as T4-treated rats FFA uptake from the circulation may be activated more strongly by norepinephrine than in controls.

Since the near changes were induced by norepinephrine in rectal temperature and in oxygen consumption of rats under our experimental conditions (unpublished data) and others (LEBLANC and PAULIOT, 1964), calorigenesis by norepinephrine was inferred from the elevation of rectal temperature. The regression coefficient of logarithm concentration in plasma FFA on rectal temperature was greater in NE- and NE+T4-treated rats than in controls although positive correlation among the both variables was observed in each group (Table 4). While in the formers the regression coefficient of logarithm turnover rate in plasma FFA on rectal temperature was not different from that of the latter (Table 5). Therefore, at the same FFA concentration, rectal temperature was higher in NE- and NE+T4-treated rats than in the controls, and rectal temperature was the same in both groups when compared at the same level of the turnover rate of FFA. These results seem to suggest that an equal percentage of FFA uptaked by the tissues might be utilized for calorigenesis in the tissues of NE- and NE+T4-treated rats and of controls, and that the turnover (=uptake) rate of FFA might be stimulated more strongly by norepinephrine in the formers than in the latter. Between T4-treated rats and controls, no differences in regression coefficients of logarithm concentration of FFA on rectal temperature and of logarithm turnover rate of FFA on rectal temperature were observed. These results may be consistent with the fact that the greater regression coefficient of FFA concentration on its turnover rate was induced by norepinephrine in T4-treated rats as well as NE- and NE+T4-treated

ones than in controls, since FFA concentration in plasma represents the equilibrium of release from adipose tissues and uptake by the tissues. Thus, it may be concluded that FFA uptake by the tissues augmented by norepinephrine contributes in common to the greater elevation of rectal temperature induced by norepinephrine in NE-, T4-, and NE+T4-treated rats than in the controls. In this study, the effect of intracellular level of chronical treatments with norepinephrine and thyroxine remained to be solved.

It is known that thyroid hormone affects catecholamine metabolism in normal and cold-exposed rats (SELLERS *et al.*, 1971). At 4°C mild hypothyroid rats excreted more norepinephrine and hyperthyroid rats excreted less norepinephrine than euthyroid rats, although in each case the excretion rate was higher than that at room temperature, indicating the presence of partial compensatory secretion among these two hormones (SELLERS *et al.*, 1951, 1974). From these observations it is assumed that the effects of two hormones on the development of nonshivering thermogenesis are possibly mediated through the common processes although the mechanisms of actions of two hormones are obscure.

In the present study plasma thyroxine and triiodothyronine concentrations were determined by radioimmunoassay (by courtesy of Dr. Norimichi Konno). Plasma thyroxine was found to be 12 μg/dl$\pm$0.8 (SEM) and triiodothyronine was 97 ng/dl$\pm$7.5 in T4-treated rats and 2.7$\pm$0.3 and 28$\pm$4.9 in olive oil controls, respectively. These values obtained in T4-treated rats are compatible to those in hyperthyroid animals proposed by AZIZI *et al.* (1974). According to SWANSON (1957), thyroxine secretion rate was 5 μg/100 g/day in rats exposed to cold, and so the dose used in this study seems within the realm of physiological dose.

It is apparent that the lower rectal temperature was induced by thyroidectomy (Table 6). At rest the concentration and the turnover rate of plasma FFA seemed to be lower levels in TX rats than in intact rats, and to be more pronounced in the turnover rate than in the concentration of FFA. During norepinephrine infusion smaller increases in both variables were induced in warm-adapted TX rats, while in cold-acclimated TX rats lower level of FFA turnover rate was produced in spite of the same level of FFA concentration, as compared with those in respective controls (Table 7). Although positive correlation was observed between plasma FFA concentration and its turnover rate in each group, the regression coefficient was smaller in TX rats than in intact rats (Table 8). Deficiency of thyroid hormones has reported to cause a decrease in FFA mobilization from adipose tissues (DEBONS and SCHWARTZ, 1961). However, from the present results, it was suggested that absence of thyroid hormones might result in a greater suppression on uptake of FFA than on its mobilization in parallel with a fall of calorigenesis.

Dose of norepinephrine used in this study may be also within physiological range since LEDUC (1961) suggested that the maximum secretion rate into the blood in cold-exposed rats was about 60 μg/100 g/day. In this study increases in rectal temperature induced by norepinephrine were greater in NE- and NE+T4-treated

rats than in controls, therefore, the role of norepinephrine for the development of nonshivering thermogenesis was confirmed in accordance with other investigators. The increase or decrease in uptake of plasma FFA was produced by norepinephrine in parallel with the rise or fall of calorigenesis by norepinephrine in this study. Accordingly, it seems that exploration of intracellular process of plasma FFA turnover rate will contribute to clarifying basic mechanisms underlying nonshivering thermogenesis.

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