Metabolism of Glucose in Hyper- and Hypo-thyroid Rats *in vivo*

RELATION OF CATECHOLAMINE ACTIONS TO THYROID ACTIVITY IN CONTROLLING GLUCOSE TURNOVER

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1. In euthyroid rats, treatment with reserpine or 6-hydroxydopamine, which deprived neuronal terminals of catecholamines, resulted in increases in rates and rate coefficients for blood glucose turnover in the starved state as determined by decay of [U-¹⁴C,6-³H]-glucose. Conversely, the injection of adrenaline or noradrenaline into starved euthyroid rats caused a marked decrease in rate coefficients for glucose turnover. There was no change in the percentage glucose recycling under these conditions. 2. Adrenaline and noradrenaline caused more pronounced hyperglycaemia in hyperthyroid than in euthyroid rats owing to the greater activation of hepatic glucose production. 3. The increase in glucose turnover characteristic of hyperthyroidism was observed even after treatment with an α - or β -adrenergic antagonist, showing the insignificant role of the balance between α - and β -adrenergic receptors in the thyroid-dependent metabolic changes. 4. Rate coefficients for glucose turnover were not affected by reserpine treatment or catecholamine injections when rats had been rendered hyperthyroid. 5. Thus catecholamines are direct determinants of glucose-turnover rates in the starved state, and depend to some extent on the prevailing thyroid state.

Marked changes in glucose turnover rate dependent on thyroid status (Okajima & Ui, 1979*a*) were not mediated by endogenous insulin, which would be an important determinant of the feeding-induced increase in glucose consumption (Okajima & Ui, 1979*b*). Apart from insulin, endogenous catecholamines also could affect glucose turnover, since the administration of adrenaline to rats exerted profound influences on the hepatic production and the peripheral utilization of blood glucose (Yajima & Ui, 1974; Shikama & Ui, 1975*a,b*; Kusaka & Ui, 1977). The findings that many of the physiological responses characteristic of hyperthyroidism are similar to those induced by adrenergic stimulation (Harrison, 1964; Waldstein, 1966; Grossman *et al.*, 1971*a,b*) and that adrenergic receptor functions are dependent on thyroid status (Kunos *et al.*, 1974; Williams & Lefkowitz, 1977; Ciaraldi & Marinetti, 1977) appear to make it very likely that endogenous catecholamines are responsible for altered turnover rates of blood glucose in hyper- and hypo-thyroidism.

The purpose of the present paper is to describe results of experiments in which roles of endogenous and exogenous catecholamines in determining the rate of glucose turnover were studied in various thyroid states.

Experimental

Materials and methods were as in the preceding papers (Okajima & Ui, 1979*a,b*), except as follows. Food was withdrawn from the cage of rats 20h before the start of blood sampling. Reserpine was administered orally for 4 consecutive days at a dose of 0.5mg/100g body wt. on each day; experiments started 4h after the last administration. In some experiments, the reserpine treatment was prolonged up to 6 days. 6-Hydroxydopamine (2.5mg/100g) was injected intravenously once, 24h before the experiment.

The plasma concentration of cyclic AMP was determined by the sensitive method of Honma *et al.* (1977). In brief, 0.02ml of whole blood withdrawn from the tail vein was mixed with 0.2ml of 0.9% NaCl containing 10mM-EDTA. After centrifugation (700g, 10min), the supernatant (diluted plasma) was added with succinic anhydride dissolved in dioxan, and succinylated (3-carboxypropionylated) cyclic AMP was analysed radioimmunochemically with the use of rabbit anti-(cyclic AMP) antiserum and ¹²⁵I-labelled succinyl-cyclic AMP tyrosine methyl ester as a radioactive ligand. The radioactive ligand was synthesized by tyrosination of 2'-O-succinyl-cyclic AMP and subsequent iodination of the tyrosine

moiety (Steiner *et al.*, 1969). The smallest amount of cyclic AMP measurable by this method was 10fmol.

Reagents used for the radioimmunoassay of cyclic AMP were obtained as a Yamasa cyclic AMP assay kit, kindly given by Yamasa Shoyu Co., Choshi, Chiba, Japan. Sources of other reagents were: adrenaline, E. Merck Japan Ltd., Tokyo, Japan; noradrenaline and 6-hydroxydopamine, Sigma Chemical Co., St Louis, MO, U.S.A.; reserpine and phentolamine, Ciba-Geigy (Japan) Ltd., Tokyo, Japan; propranolol, Otsuka Pharmaceutical Co., Tokushima, Japan.

Results

Glucose turnover in rats treated with reserpine or 6-hydroxydopamine in various thyroid status

To decrease the amounts of endogenous catecholamines, rats had been treated with reserpine or 6-hydroxydopamine as described in the Experimental section. Reserpine is an agent that deprives sympathetic neurons and adrenal medullae of catecholamine vesicles, whereas 6-hydroxydopamine causes chemical sympathectomy by destroying neuronal terminals. On the basis of findings (Kunitada *et al.*, 1978) that the injection of tyramine into starved rats caused a sharp increase in plasma cyclic AMP as a

result of the β -adrenergic action of catecholamines released from neuronal terminals and adrenal medullae, a study was first made to confirm if the treated rats were actually deprived of endogenous catecholamines (Fig. 1).

In experiments shown in Fig. 1, theophylline was injected 30 min before tyramine to prevent generated cyclic AMP from being rapidly hydrolysed by phosphodiesterase. Plasma concentrations of cyclic AMP immediately before theophylline injection were 46 ± 2.7 , 52 ± 3.7 and 72 ± 10.6 pmol/ml of plasma for euthyroid, hyperthyroid and hypothyroid rats respectively. The injection of tyramine under these conditions caused striking increases in plasma cyclic AMP, though the increment was variable, depending on thyroid status. The prior treatment of rats with reserpine for 4 days completely abolished the cyclic AMP response of euthyroid and hyperthyroid rats to tyramine (Figs. 1a and 1b). In hypothyroid rats, however, tyramine caused a small increase in plasma cyclic AMP even after the 4-day regimen with reserpine; a 6-day regimen was required to abolish the cyclic AMP response (Fig. 1c). Likewise, there was no increase in plasma cyclic AMP after tyramine injection into 6-hydroxydopamine-treated euthyroid rats (Fig. 1a). Thus the treatment of rats with reserpine or 6-hydroxydopamine resulted in a significant decrease in endogenous catecholamine contents.

Fig. 1. Increases in the plasma concentration of cyclic AMP in response to tyramine under various conditions. Starved euthyroid (a), hyperthyroid (b) and hypothyroid (c) rats were injected subcutaneously with tyramine (1 mg/100 g body wt.) at zero time and with theophylline (2 mg/100 g) 30 min before the tyramine injection. ○, △, □, Without additional treatments; ●, ▲, ■, treated with reserpine for 4 days as described in the Experimental section; ▣, treated with reserpine for 6 days; ▼, treated with 6-hydroxydopamine as in the Experimental section. Mean values $\pm$ S.E.M. from four to eight rats are shown by symbols with vertical bars attached.

Pretreatment of euthyroid rats with reserpine was without effect on plasma concentrations of glucose and insulin, but significantly increased turnover rates of blood glucose determined with [U- ^{14}C ,6- ^3H]-glucose (Table 1). There was no change in the glucose recycling calculated from the difference in turnover rates between [^{14}C]- and [^3H]-glucose. The rate coefficient, which reflects the first-order kinetic constant, of glucose turnover also increased after reserpine treatment. The prior treatment of rats with hydroxydopamine caused effects similar to those caused by reserpine; not only rates but also rate coefficients of glucose turnover were higher in 6-hydroxydopamine-treated rats than in control euthyroid rats, without significant changes in the blood glucose concentration and recycling (Table 2). Thus depletion of endogenous catecholamines was associated with enhanced glucose turnover without changes in the circulating insulin concentration.

Glucose turned over more rapidly in hyperthyroid rats than in euthyroid rats, with gluconeogenesis being concurrently activated, as shown by enhanced glucose recycling (Table 1). The enhanced glucose turnover associated with slight hyperinsulinaemia confirms the results in our previous papers (Okajima & Ui, 1978, 1979*a,b*). In contrast with euthyroid rats, treatment of hyperthyroid rats with reserpine failed to increase rates and rate coefficients of glucose turnover (Table 1), despite deprivation of endogenous catecholamines, as evidenced by lack of the cyclic AMP response to tyramine (Fig. 1*b*). In other words, induction of hyperthyroidism was without effect on glucose-turnover rates if rats were deprived of endogenous catecholamines.

Glucose turned over more slowly in hypothyroid rats than in euthyroid rats, as reported in the preceding papers (Okajima & Ui, 1979*a,b*). Treatment of hypothyroid rats with reserpine for 4 days caused increases in both rates and rate coefficients of glucose

Table 1. Concentrations of blood glucose and plasma insulin, rates and rate coefficients for glucose turnover, and the percentage glucose recycling in starved rats as affected by reserpine treatment in various thyroid states

Blood samples (0.1 ml) were taken from the tail vein of starved rats injected with [U- ^{14}C ,6- ^3H]glucose (0.5 μCi of ^{14}C and 5 μCi of ^3H per 100 g body wt.) at 30, 45, 60, 75 and 90 min after the injection. Specific radioactivity of glucose in the sample was used for calculation of rates (R_e and $R_{6\text{H}}$) and rate coefficients (k'_e and $k'_{6\text{H}}$) for glucose turnover by the monoexponential method as described by Okajima & Ui (1979*a*). The glucose concentrations in the blood samples were averaged to obtain a representative value for each rat, whereas the plasma insulin concentration was determined at 90 min. The percentage glucose recycling was calculated based on the difference between R_e and $R_{6\text{H}}$ as described previously (Okajima & Ui, 1979*a*). Mean values $\pm$ S.E.M. are given with numbers of observations in parentheses. Effects of reserpine treatment are significant at: * $P < 0.05$ and ** $P < 0.01$. Abbreviation: n.d., not determined.

Treatment with reserpine	Blood glucose (mg/100 ml)	Turnover rate (mg/min per 100 g body wt.)		Rate coefficient (ml/min per 100 g body wt.)		Glucose recycling (%)	Plasma insulin (μ units/ml)
		$R_{6\text{H}}$	R_e	$k'_{6\text{H}}$	k'_e		
(a) Euthyroid							
– (9)	71 $\pm$ 3	0.52 $\pm$ 0.03	0.43 $\pm$ 0.02	0.73 $\pm$ 0.03	0.61 $\pm$ 0.02	17 $\pm$ 1.6	18 $\pm$ 1.8
+ (4)	80 $\pm$ 3	0.95 $\pm$ 0.11**	0.74 $\pm$ 0.08**	1.21 $\pm$ 0.16*	0.94 $\pm$ 0.12*	20 $\pm$ 0.8	20 $\pm$ 1.6
(b) Hyperthyroid							
– (6)	82 $\pm$ 3	0.80 $\pm$ 0.04	0.58 $\pm$ 0.03	0.97 $\pm$ 0.02	0.71 $\pm$ 0.01	27 $\pm$ 2.0	25 $\pm$ 1.2
+ (4)	90 $\pm$ 2	0.89 $\pm$ 0.06	0.70 $\pm$ 0.05	0.99 $\pm$ 0.07	0.78 $\pm$ 0.06	21 $\pm$ 2.3	32 $\pm$ 2.7
(c) Hypothyroid							
– (7)	67 $\pm$ 3	0.28 $\pm$ 0.01	0.24 $\pm$ 0.01	0.42 $\pm$ 0.03	0.37 $\pm$ 0.03	13 $\pm$ 1.5	14 $\pm$ 0.5
+ (4)	70 $\pm$ 3	0.42 $\pm$ 0.05*	0.35 $\pm$ 0.02**	0.60 $\pm$ 0.07*	0.50 $\pm$ 0.04*	16 $\pm$ 5.5	13 $\pm$ 1.3
+† (3)	83 $\pm$ 6	0.43 $\pm$ 0.04*	0.38 $\pm$ 0.04*	0.53 $\pm$ 0.09	0.47 $\pm$ 0.09	12 $\pm$ 2.8	n.d.

† Reserpine treatment was prolonged to 6 days.

Table 2. Effect of 6-hydroxydopamine on kinetic parameters for glucose turnover in euthyroid rats

Experimental conditions and expression of data are the same as in Table 1. *Effect of 6-hydroxydopamine is significant at $P < 0.05$.

Treatment with 6-hydroxydopamine	Blood glucose (mg/100 ml)	Turnover rate (mg/min per 100 g body wt.)		Rate coefficient (ml/min per 100 g body wt.)		Glucose recycling (%)
		$R_{6\text{H}}$	R_e	$k'_{6\text{H}}$	k'_e	
– (9)	74 $\pm$ 3	0.59 $\pm$ 0.03	0.48 $\pm$ 0.02	0.70 $\pm$ 0.03	0.65 $\pm$ 0.03	18 $\pm$ 1.2
+ (5)	71 $\pm$ 3	0.71 $\pm$ 0.04*	0.58 $\pm$ 0.03	1.02 $\pm$ 0.09*	0.83 $\pm$ 0.07*	19 $\pm$ 1.3

turnover. A similar degree of increases in turnover rates was induced when reserpine treatment was prolonged up to 6 days. The 6-day regimen caused a slight but significant hyperglycaemia, thereby decreasing the increments in rate coefficients as compared with the 4-day regimen. In any case, the increments in rates and rate coefficients of glucose turnover elicited by reserpine treatment were not larger in hypothyroid rats than in euthyroid rats. As a result, induction of hypothyroidism suppressed glucose turnover even in rats deprived of endogenous catecholamines.

Effects of adrenaline and noradrenaline on glucose-turnover rates in euthyroid and hyperthyroid rats

Adrenaline is a potent hyperglycaemic agent; a single subcutaneous injection into rats causes a progressive increase in the blood glucose concentration for the first 1.5–2 h, followed by a gradual return (see, e.g., Yajima & Ui, 1974; Shikama & Ui, 1975b). Such temporal changes in the blood glucose concentration demonstrate that the inflow and outflow rates of blood glucose are not equal to each other during this period. When euthyroid or hyperthyroid rats once injected with 10 μ g of adrenaline or noradrenaline were additionally injected with the same dose of the respective catecholamine 105 min later, however, hyperglycaemia was sustained at essentially a constant value from 15 to 105 min after the second

injection, reflecting steady-state kinetics of blood glucose (Fig. 2). Fig. 2 shows that adrenaline was more potent than noradrenaline as a hyperglycaemic agent, and that hyperthyroid rats were more responsive to these agents than were euthyroid rats.

[U- 14 C,6- 3 H]Glucose was injected concurrently with the second injection of the catecholamine (or of 0.9% NaCl in control rats) to estimate the steady-state rates and rate coefficients of glucose turnover; the results are shown in Table 3. After adrenaline injection, there were significant increases in the turnover rate as well as in the blood concentration of glucose. This increase was the only change accompanying the hyperglycaemia, since rate coefficients were conversely decreased. No change in glucose-turnover rates was induced by noradrenaline, despite significant hyperglycaemia. As a result, noradrenaline was as effective as adrenaline in decreasing the rate coefficients. Thus the capacity of euthyroid rats to take up glucose into peripheral tissue cells was markedly decreased by catecholamines such as adrenaline and noradrenaline. Glucose recycling was not changed by the catecholamines.

The injections of adrenaline or noradrenaline into hyperthyroid rats caused marked increases in turnover rates. Adrenaline caused hyperinsulinaemia, in accord with our previous results (Okajima & Ui, 1978). The percentage glucose recycling also increased on induction of hyperthyroidism (Okajima & Ui, 1979a), but it showed no change after the catechol-

Fig. 2. Blood concentration of glucose after the two consecutive injections of adrenaline or noradrenaline in euthyroid and hyperthyroid rats

Euthyroid (a) or hyperthyroid (b) rats were injected subcutaneously with adrenaline twice (●, ▲) or with noradrenaline twice (○, △) at a dose of 10 μ g/100 g body wt. at each time; 0.9% NaCl was injected instead of catecholamines as control (○, △). The first and the second injections of the catecholamine were 120 and 15 min respectively before zero time, at which time [U- 14 C,6- 3 H]glucose was injected to obtain the data shown in Table 3. Numbers of rats are shown in Table 3. Mean values $\pm$ S.E.M. are given by symbols with vertical bars attached.

amines. The catecholamine-induced increase in turnover rates in hyperthyroidism was explainable solely in terms of the mass-action effect of hyperglycaemia, as evidenced by unaltered rate coefficients. Hyperthyroidism was conceivably unresponsive to the inhibitory action of catecholamines on peripheral glucose utilization.

The injections of noradrenaline into reserpine-treated euthyroid rats caused a sustained mild hyperglycaemia associated with marked decrease in glucose-turnover rates (Table 4). As a result, there was no significant difference in glucose-turnover rates between reserpine-treated (Table 4) and untreated (Table 3) rats, if comparison was made after noradrenaline injection. It is likely therefore that the increase in glucose turnover induced by reserpine treatment (Table 1) was due to depletion of endogenous catecholamines that suppress glucose metabolism, rather than to resistance of the reserpine-treated rats to their suppressive action.

Effects of adrenergic antagonists on glucose turnover

Injection of either an α -adrenergic antagonist, phentolamine, or a β -adrenergic antagonist, pro-

pranolol, into rats did not cause significant changes in the blood glucose concentration (Table 5). Both phentolamine and propranolol caused slight increases in the glucose-turnover rate measured with $[U-^{14}C]$ -glucose or $[2-^3H]$ glucose. The injection of an α -adrenergic antagonist into rats causes hyperinsulinaemia (Kausal & Buse, 1967; Sumi & Ui, 1975). The increase in glucose turnover induced by phentolamine appeared to be due to the action of the insulin thus secreted, because there was no increase when the rats had been deprived of endogenous insulin by means of streptozotocin treatment (R_{2H} was 0.66 ± 0.03 for phentolamine-treated and 0.61 ± 0.04 for non-treated diabetic rats in the starved state). The increase in glucose turnover characteristic of hyperthyroidism was observed even after treatment with phentolamine or propranolol (Table 5), suggesting that the balance between α - and β -adrenergic receptors plays no important role in thyroid-dependent changes in glucose turnover.

Recovery from hypoglycaemia is known to depend on hyperglycaemic action of endogenous catecholamines as well as other hormones (Garber *et al.*, 1976). As shown in Fig. 3, insulin-induced hypoglycaemia was of shorter duration, with a more rapid

Table 3. *Effects of adrenaline and noradrenaline on kinetic parameters for glucose turnover and plasma insulin concentration in euthyroid and hyperthyroid rats*

Starved rats were injected with adrenaline or noradrenaline twice as in Fig. 2, and were then injected with $[U-^{14}C, 6-^3H]$ -glucose at 15 min after the second injection of the catecholamine. Other experimental conditions as well as expression of data are the same as in Table 1. Effects of catecholamines are significant at: * $P < 0.05$ and ** $P < 0.01$.

Treatment with catecholamines	Blood glucose (mg/100ml)	Turnover rate (mg/min per 100 g body wt.)		Rate coefficient (ml/min per 100 g body wt.)		Glucose recycling (%)	Plasma insulin (μ units/ml)
		R_{6H}	R_c	k'_{6H}	k'_c		
(a) Euthyroid							
None (11)	70 ± 2	0.56 ± 0.02	0.47 ± 0.02	0.81 ± 0.03	0.67 ± 0.03	18 ± 1.2	21 ± 1.0
Adrenaline (5)	$137 \pm 10^{**}$	$0.79 \pm 0.07^{**}$	$0.65 \pm 0.07^*$	$0.58 \pm 0.06^{**}$	$0.48 \pm 0.06^*$	19 ± 3.3	23 ± 3.5
Noradrenaline (4)	$99 \pm 2^{**}$	0.59 ± 0.03	0.48 ± 0.01	$0.60 \pm 0.02^{**}$	$0.49 \pm 0.02^{**}$	20 ± 2.5	26 ± 2.7
(b) Hyperthyroid							
None (11)	88 ± 2	0.90 ± 0.05	0.64 ± 0.04	1.01 ± 0.05	0.73 ± 0.04	28 ± 1.6	31 ± 3.6
Adrenaline (2)	179, 201	1.84, 2.02	1.26, 1.38	1.00, 1.04	0.63, 0.78	25, 37	51, 65
Noradrenaline (5)	$127 \pm 5^{**}$	$1.36 \pm 0.07^{**}$	$0.96 \pm 0.07^{**}$	1.07 ± 0.07	0.76 ± 0.06	29 ± 1.2	31 ± 2.7

Table 4. *Effect of noradrenaline on kinetic parameters for glucose turnover in reserpine-treated rats*

Reserpine-treated euthyroid rats were injected with noradrenaline and labelled glucose as described in Fig. 2 and Table 3. Other experimental conditions and expression of data are the same as in Table 1. Effects of noradrenaline are significant at: * $P < 0.05$ and ** $P < 0.01$.

Treatment with noradrenaline	Blood glucose (mg/100ml)	Turnover rate (mg/min per 100 g body wt.)		Rate coefficient (ml/min per 100 g body wt.)		Glucose recycling (%)
		R_{6H}	R_c	k'_{6H}	k'_c	
— (5)	78 ± 4	0.90 ± 0.06	0.73 ± 0.05	1.13 ± 0.12	0.91 ± 0.08	19 ± 2.2
+ (5)	$103 \pm 5^*$	$0.68 \pm 0.04^*$	$0.56 \pm 0.02^*$	$0.67 \pm 0.04^{**}$	$0.55 \pm 0.03^{**}$	17 ± 2.5

Table 5. Kinetic parameters for glucose turnover in euthyroid and hyperthyroid rats after treatment with an α - or a β -adrenergic antagonist

Phentolamine (0.5 mg/100 g body wt.), an α -adrenergic antagonist, or propranolol (1.5 mg/100 g), a β -adrenergic antagonist, was injected intraperitoneally into starved euthyroid or hyperthyroid rats 30 min before the injection of [U - ^{14}C , 2^3H]glucose. Other experimental conditions and expression of data are the same as in Table 1. Differences between euthyroid and hyperthyroid are significant at: * P < 0.05 and ** P < 0.01.

Thyroid status	Blood glucose (mg/100 ml)	Turnover rate (mg/min per 100 g body wt.)		Rate coefficient (ml/min per 100 g body wt.)	
		R_{2H}	R_e	k'_{2H}	k'_e
(a) Non-treated					
Euthyroid (10)	75 $\pm$ 3	0.71 $\pm$ 0.03	0.48 $\pm$ 0.03	0.96 $\pm$ 0.02	0.65 $\pm$ 0.04
Hyperthyroid (11)	90 $\pm$ 3**	1.24 $\pm$ 0.08**	0.75 $\pm$ 0.03**	1.40 $\pm$ 0.11**	0.86 $\pm$ 0.08*
(b) Phentolamine-treated					
Euthyroid (7)	82 $\pm$ 3	0.87 $\pm$ 0.04	0.57 $\pm$ 0.02	1.07 $\pm$ 0.04	0.69 $\pm$ 0.03
Hyperthyroid (8)	93 $\pm$ 4*	1.55 $\pm$ 0.06**	0.94 $\pm$ 0.04**	1.69 $\pm$ 0.08**	1.02 $\pm$ 0.06**
(c) Propranolol-treated					
Euthyroid (4)	77 $\pm$ 2	0.79 $\pm$ 0.06	0.54 $\pm$ 0.03	1.03 $\pm$ 0.06	0.70 $\pm$ 0.02
Hyperthyroid (4)	85 $\pm$ 7	1.40 $\pm$ 0.07**	0.89 $\pm$ 0.05**	1.67 $\pm$ 0.13**	1.07 $\pm$ 0.10*

Fig. 3. Changes in the blood glucose concentration in euthyroid and hyperthyroid rats after injection of insulin. Starved euthyroid ($\circ$, $\bullet$) or hyperthyroid (Δ , $\blacktriangle$) rats were injected subcutaneously with insulin (0.05 unit/100 g body wt.) at zero time. Propranolol ($\bullet$, $\blacktriangle$) or 0.9% NaCl ($\circ$, Δ) was injected as in Table 5 immediately before insulin. Mean values $\pm$ S.E.M. from four rats are shown by symbols with vertical bars attached.

return to the pre-insulin value, in hyperthyroid than in euthyroid rats. The recovery from the hypoglycaemia was interfered with efficiently by propranolol in euthyroid rats, but it was still observed after the β -blockade in hyperthyroid rats. Thus the ability of hyperthyroid rats to recover from insulin-induced hypoglycaemia appeared not to be solely related to β -adrenergic function.

Discussion

Catecholamines are known to exert acute effects on carbohydrate and lipid metabolism via stimulation of the membrane receptors. Consequently, there are rapid changes in plasma concentrations of metabolites, such as glucose, unesterified fatty acids etc., after a single injection with catecholamines. In the present study, however, it was found that the two consecutive injections of adrenaline or noradrenaline into starved rats at a 2 h interval caused a durable hyperglycaemia, with a constant blood glucose concentration maintained from 15 to 105 min after the second injection. The decay of labelled glucose was then measured during this period to obtain the steady-state rate of glucose turnover.

At the same dose of 20 μ g/100 g body wt., the hyperglycaemia was greater with adrenaline than with noradrenaline. In Table 3, adrenaline caused a roughly 96% increase in the concentration, and 38–41% increases in turnover rates, of blood glucose in the starved euthyroid rats. As a result, the rate coefficient for glucose turnover was significantly decreased by adrenaline. The increase in glucose turnover in the starved state may reflect increased hepatic output of glucose owing to activation of gluconeogenesis, whereas the decrease in rate coefficients indicates impaired capacity of peripheral tissues to utilize blood glucose. Thus the present results are in good agreement with previous conclusions (Yajima & Ui, 1975; Shikama & Ui, 1975a) that adrenaline-induced hyperglycaemia resulted from activation of hepatic gluconeogenesis associated with interference with removal of excess blood glucose by peripheral tissues.

The amount of glucose recycled via the Cori cycle (plus the glucose-alanine cycle), as calculated from

the difference in decay rate between [U- 14 C]- and [6- 3 H]-glucose, was 0.09 ± 0.01 mg/min per 100g body wt. in starved euthyroid rats; it increased to 0.14 ± 0.02 after adrenaline injections, in accord with the previous findings that adrenaline activates the Cori cycle (Kusaka & Ui, 1977), although the percentage contribution of the Cori cycle (plus glucose-alanine cycle) to the total turnover of blood glucose was not affected by adrenaline under the conditions used in the present study.

With noradrenaline, there was no increase in glucose-turnover rates despite slight hyperglycaemia. The noradrenaline-induced hyperglycaemia was therefore accounted for only by the inhibition of peripheral glucose utilization as revealed by the decrease in rate coefficients. Probably, the injection of noradrenaline did not activate hepatic gluconeogenesis *in vivo*. Thus adrenaline is a much stronger hyperglycaemic agent than noradrenaline by virtue of its capability of activating gluconeogenesis, whereas both catecholamines were equally effective in suppressing peripheral glucose utilization. Exton & Park (1968) reported that noradrenaline was as effective as adrenaline *in vitro* in enhancing gluconeogenesis in perfused rat liver. Since the minimum effective concentration of the catecholamines in the perfusate was much higher than their plasma concentrations in rats, it is conceivable that some unknown potentiator(s) may be involved *in vivo* in the action of adrenaline, but not in the action of noradrenaline, on hepatic gluconeogenesis.

The treatment of euthyroid rats with reserpine or 6-hydroxydopamine was without effect on the starvation concentration of blood glucose, but caused significant increases in turnover rates and rate coefficients calculated from the decay of [U- 14 C]- and [6- 3 H]-glucose. There were no detectable changes in the percentage recycling of glucose and the plasma concentration of insulin. The decrease in endogenous catecholamine contents after these treatments was revealed by failure of the treated rats to increase the plasma concentration of cyclic AMP in response to tyramine, a potent catecholamine-releasing agent (Kunitada *et al.*, 1978). Moreover, the injection of noradrenaline into reserpine-treated rats decreased rate coefficients to the values usually observed for non-treated normal rats (Table 4). It is concluded therefore that endogenous catecholamines, especially noradrenaline, function to suppress blood glucose turnover in the starved state, principally owing to an inhibition of peripheral glucose utilization. Probably the endogenous insulin plays an indispensable role in increasing glucose turnover in the fed state (Okajima & Ui, 1979b), whereas endogenous catecholamines take an important part in suppressing glucose metabolism in the starved state.

The above-mentioned metabolic actions of catecholamines were profoundly affected by induction of

hyperthyroidism. The hyperglycaemia induced by adrenaline or noradrenaline was greater in hyperthyroid rats than in euthyroid rats. This potentiation of catecholamine-induced hyperglycaemia was accounted for solely by enhanced hepatic glucose production, because the capacity of peripheral tissues to take up blood glucose, as reflected in rate coefficients, was not impaired by catecholamines in the hyperthyroid state. Conceivably, the gluconeogenic pathway in the liver became more responsive to activation by catecholamines in the hyperthyroid state. Similarly, metabolic and contractile responses of hearts (Hornbrook *et al.*, 1965; Young & McNeill, 1974; Roncari & Murphy, 1975; Fregly *et al.*, 1975) and lipolytic responses of adipocytes (Van Inwegen *et al.*, 1975; Malbon *et al.*, 1978) were reported to be enhanced by induction of hyperthyroidism, which also led to increases in the number of β -adrenergic receptors in heart (Williams & Lefkowitz, 1977; Ciaraldi & Marinetti, 1977) and cyclic AMP accumulation in fat-cells (Malbon *et al.*, 1978). Thus even noradrenaline, which was ineffective in the liver of euthyroid rats, came to activate gluconeogenesis in the liver of hyperthyroid rats.

Kunitada *et al.* (1978) have reported that insulin-induced hypoglycaemia caused release of adrenomedullary adrenaline and of pancreatic glucagon, which may facilitate the recovery from the hypoglycaemia by activating hepatic gluconeogenesis. The rapid recovery from the hypoglycaemia in hyperthyroid rats (Fig. 3) may reflect enhanced hepatic glucose production under these conditions. Participation of glucagon, as well as adrenaline, was suggested by the failure of a β -adrenergic antagonist to abolish the recovery.

Induction of hyperthyroidism resembled reserpine treatment in increasing rate coefficients for glucose turnover. Reserpine treatment did not further increase rate coefficients in hyperthyroid rats, suggesting that hyperthyroid rats might be depleted of endogenous catecholamines. An increase in the percentage recycling of glucose, however, was characteristic of hyperthyroidism and was not mimicked by reserpine treatment. Moreover, catecholamine injections failed to decrease rate coefficients in hyperthyroid rats, making the possibility of catecholamine deprivation unlikely. Thus it appears more likely that the capacity of hyperthyroid rats to take up glucose into their cells was unresponsive to endogenous and exogenous catecholamines, which did readily suppress this capacity of euthyroid rats. Hypothyroid rats could respond to endogenous catecholamines in this regard, as suggested by the reserpine-induced increase in rate coefficients. The fact that glucose turned over more rapidly in hyperthyroid than in euthyroid rats even after the treatment with α - and β -adrenergic antagonists confirms the conclusion that endogenous catecholamines play

only a minor role in increased glucose turnover characteristic of hyperthyroidism.

In conclusion, thyroid hormones and catecholamines play important roles, individually but in a mutually dependent manner, in determining glucose-turnover rates in the starved state. How catecholamines interact with thyroid in mammalian metabolic regulations remains for further investigation.

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