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# LACK OF THYROID HORMONE EFFECT ON ACTIVATION ENERGY OF NaK–ATPase

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In order to differentiate whether activation of NaK–ATPase in thyroid thermogenesis is due to increased numbers of active 'sodium pump' units or due to a change in the kinetics of the enzyme, the effect of $T_3$ on activation energy ($E_a$) of NaK–ATPase was determined in rat liver, kidney and brain. Injection of $T_3$ produced significant increases in the specific activity of NaK–ATPase in liver and kidney but not in brain homogenates. $T_3$ injections produced no significant change in the $E_a$ of NaK-ATPase in any of the three tissues.

The data are compatible with the hypothesis that thyroid stimulation of the sodium pump is brought about by an increase in the number of active pump units.

*Keywords:* NaK–ATPase; thyroid hormone; thyroid thermogenesis

Thyroid thermogenesis is mediated by stimulation of energy expenditure for transmembrane active sodium transport (Ismail-Beigi and Edelman, 1970, 1971; Asano et al., 1976). There is extensive evidence showing that $Na^+$-plus-$K^+$-$Mg^{2+}$-dependent ATPase (NaK–ATPase) is the enzymatic expression of the sodium pump (Skou, 1965; Glynn and Karlish, 1975). The tyroid-induced stimulation of the sodium pump is brought about by an increase in the specific activity of NaK–ATPase in target tissues (Ismail-Beigi and Edelman, 1971; Israel et al., 1973; Asano et al., 1976).

As a means of determining the mechanism of thyroid-induced stimulation of NaK–ATPase, the effect of triiodothyronine ($T_3$) on the activation energy ($E_a$) of the enzyme was determined in two thermogenically responsive tissues (rat liver and kidney) and compared to that of a control tissue (brain). Furthermore, the effect of $T_3$ on the respiration of liver slices was also determined at various temperatures.

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## MATERIALS AND METHODS

Male Sprague–Dawley rats (200–250 g body weight) were given three subcutaneous injections of either 50 $\mu$g $T_3$ per 100 g body weight or diluent on alternate days and were studied 24–48 h after the third injection. Each ATPase assay, with and without $10^{-3}$ M ouabain was performed in quadruplicate on crude homogenates of liver, kidney and brain as described previously (Ismail-Beigi and Edelman, 1971). The NaK–ATPase activity was calculated as total ATPase activity minus the ATPase activity in the presence of ouabain. Ten diluent and ten $T_3$-injected rats were used for assay of ATPase activity of each tissue. In order to determine the $E_a$ of the enzymes, assays were performed at 31 and 36°C and $E_a$ was calculated by the use of Arrhenius equation. These two temperatures were chosen for the study since, in preliminary experiments, the enzymes were found to be unstable at 40°C, and at the lower temperatures of 25–26°C, the rates were too low to get accurate results. Oxygen consumption ($QO_2$) was determined in liver slices in a Warburg respirometer (Ismail-Beigi and Edelman, 1970, 1971) at 31, 37 and 40°C. Sodium transport-dependent and -independent respiration were estimated by the use of ouabain.

The Mg–ATPase and NaK–ATPase activity of each tissue in the various thyroid states were calculated as mean ± S.E.M. Unpaired Student's *t*-test was used for statistical analysis (Snedecor and Cochran, 1967) taking *P* values of greater than 0.05 as insignificant.

## RESULTS

The effect of $T_3$ on $QO_2$ of liver slices at 31, 37 and 40°C is shown in table 1. The thermogenic effect of the hormone is present in the temperature range examined. Sodium transport-dependent respiration ($QO_2(t)$) showed a larger increase with elevation of temperature as compared to $Na^+$ transport-independent respiration ($QO_2'$). Thus at lower temperatures, a smaller fraction of the $T_3$-induced increase in $QO_2$ is attributable to an increase in $QO_2(t)$.

The effect of $T_3$ on liver, kidney and cerebral tissue ATPases is summarized in table 2. In the liver, injection of $T_3$ produced a 38% increase in NaK–ATPase activity assayed at 31°C (statistically not significant) and a 48% increase in the enzyme's activity at 36°C which was significant. $T_3$ produced no significant change in Mg–ATPase activity at either assay temperature. In the kidney, $T_3$ produced no change in the activity of Mg–ATPase, while NaK–ATPase activity increased by 26% both at 31 and 36°C; the change at 36°C being statistically significant. The effect of $T_3$ on activities of NaK–ATPase in liver and kidney at 36°C are close to the results reported previously at 37°C (Ismail-Beigi and Edelman, 1971). Injection of $T_3$ produced no change in Mg–ATPase or NaK–ATPase activity of cerebral homogenates assayed at either temperature. The lack of effect of $T_3$ on NaK–ATP-

Table 1
Effect of temperature on $QO_2$ of liver slices ± $T_3$. Rats were injected with 50 μg $T_3$/100 g body weight or diluent on alternate days × 3. The values are expressed in μl$O_2$/h/mg dry weight, mean ± S.E.

| | $QO_2$ | | |
|---|---|---|---|
| *(a) Euthyroid* | | | |
| Ouabain | 31° (n = 10) | 37° (n = 14) | 40° (n = 12) |
| NONE | 6.2 ± 0.2 | 8.2 ± 0.3 | 9.6 ± 0.4 |
| $10^{-3}$ M | 4.1 ± 0.2 | 5.1 ± 0.2 | 5.8 ± 0.4 |
| $QO_2(t)$ | 2.1 ± 0.2 | 3.1 ± 0.2 | 3.8 ± 0.3 |
| *(b) Euthyroid + $T_3$* | | | |
| Ouabain | 31° (n = 8) | 37° (n = 14) | 40° (n = 8) |
| NONE | 8.6 ± 0.3 | 12.4 ± 0.4 | 13.8 ± 0.4 |
| $10^{-3}$ M | 5.1 ± 0.2 | 5.5 ± 0.3 | 6.4 ± 0.6 |
| $QO_2(t)$ | 3.5 ± 0.2 | 6.9 ± 0.3 | 7.4 ± 0.6 |

Table 2
ATPase activity of rat liver, kidney and cerebral homogenates at 31°C and 36°C from euthyroid rats ± $T_3$. Rats were injected with diluent or 50 mg $T_3$/100 g body weight × 3 on alternate days. ATPase activity expressed as μmol $P_i$/h/mg protein, mean ± S.E.M., $n = 20$. $P$ values are of the difference produced as a result of injection of $T_3$ as compared to the diluent-injected controls.

| Tissue | Thyroid status | 31°C | | 36°C | |
|---|---|---|---|---|---|
| | | Mg–ATPase | NaK–ATPase | Mg–ATPase | NaK–ATPase |
| Liver | Euthyroid | 3.14 ± 0.30 | 0.55 ± 0.10 | 4.00 ± 0.40 | 0.82 ± 0.10 |
| | +$T_3$ | 3.60 ± 0.50[a] | 0.76 ± 0.10[a] | 4.20 ± 0.50[a] | 1.22 ± 0.13[c] |
| Kidney | Euthyroid | 13.5 ± 0.5 | 2.05 ± 0.20 | 16.0 ± 0.8 | 3.10 ± 0.20 |
| | +$T_3$ | 13.9 ± 0.7[a] | 2.60 ± 0.40[a] | 15.9 ± 1.0[a] | 3.90 ± 0.30[b] |
| Cerebrum | Euthyroid | 7.71 ± 0.75 | 3.26 ± 0.27 | 9.45 ± 1.2 | 5.03 ± 0.60 |
| | +$T_3$ | 8.00 ± 0.90[a] | 3.40 ± 0.30[a] | 10.20 ± 1.1[a] | 4.97 ± 0.50[a] |

[a] Not statistically significant; [b] $P < 0.05$; [c] $P < 0.025$.

Table 3
Activation energy of ATPase enzymes in homogenates from euthyroid rats ± $T_3$. The data are derived from table 2. Activation energy is expressed in calories. None of the changes recorded for the various tissues as a result of $T_3$ injection are statistically significant.

| Tissue | Thyroid status | Mg–ATPase | NaK–ATPase |
|---|---|---|---|
| Liver | Euthyroid | 8800 ± 1300 | 14,700 ± 1500 |
| | Euthyroid + $T_3$ | 6000 ± 1200 | 17,000 ± 2000 |
| Kidney | Euthyroid | 6400 ± 1200 | 15,200 ± 1500 |
| | Euthyroid + $T_3$ | 5100 ± 1000 | 14,900 ± 1300 |
| Cerebrum | Euthyroid | 7600 ± 1200 | 15,900 ± 1500 |
| | Euthyroid + $T_3$ | 9000 ± 1200 | 14,000 ± 2000 |

ase activity confirms previous data (Ismail-Beigi and Edelman, 1971) and is in accord with the fact that mammalian adult brain does not thermogenically respond to thyroid hormones (Barker and Klitgaard, 1952).

As a means of differentiating a $T_3$-induced $V_{max}$ versus a $K_m$ effect on NaK–ATPase, the $E_a$ of the ATPases were calculated based on the data of table 2 and are summarized in table 3. In thermogenically responsive as well as unresponsive tissues, $T_3$ produced no significant change in the magnitude of $E_a$ of Mg–ATPase or NaK–ATPase enzyme. It should be noted, however, that the variance in the $E_a$ was rather large and small changes in the $E_a$ of NaK–ATPase would go unnoticed. In the various tissues, $E_a$ of Mg–ATPase was between 5100 and 9000 calories and that of NaK–ATPase was between 14,000 and 17,000 calories. Both these values are close to the figures reported previously for brain microsomal preparations (Robinson, 1969).

## DISCUSSION

Thyroid-induced increase in specific activity of NaK–ATPase (table 2) can be brought about by (a) $V_{max}$ effect, i.e., an increase in the number of active sodium pump units (synthesis of new sodium pumps or removal of inhibitor), or (b) a decrease in $K_m$ of the enzyme for intracellular ATP or sodium or for extracellular potassium. Since the magnitude of $E_a$ of an enzyme remains constant unless the kinetic behaviour (or catalytic mechanism) of the enzyme is altered, the effect of $T_3$ on $E_a$ of NaK–ATPase was determined as an indirect way of differentiating between the above two possibilities. The finding that $T_3$ has no demonstrable effect on $E_a$ of NaK–ATPase (table 3) while it increases the specific activity of the enzymes is compatible with the $V_{max}$ mechanism. In a recent report, Asano et al. (1976) did not find any change in $K_m$ for ATP of rat muscle membrane NaK–ATPase as a result of thyroid hormone injection, while the specific activity of the enzyme had increased. This information also supports the $V_{max}$ mechanism. Never-

Table 4
Apparent respiratory 'activation energy' of liver slices from euthyroid rats ± $T_3$. The data are derived from table 1. Activation energy is expressed in calories. $QO_2$ denotes the total respiratory rate; $QO_2'$ denotes sodium transport-independent respiration; $QO_2(t)$ denotes sodium transport-dependent respiration.

| | $QO_2$ | $QO_2'$ | $QO_2(t)$ |
|---|---|---|---|
| Euthyroid | 8900 ± 900 | 6700 ± 800 | 14,500 ± 1200 |
| Euthyroid + $T_3$ | 8800 ± 1000 | 5500 ± 900 | 16,000 ± 1100 |
| Δ | −100 | −1200 | +1500 |
| *P* | N.S. | N.S. | N.S. |

theless, further data on possible thyroid effects on the $K_m$ of NaK–ATPase for ATP, sodium, and potassium would be of importance.

Since some degree of fever may be seen in thyrotoxic states (Bard, 1961), it is noteworthy that the $T_3$-induced increase in $QO_2(t)$ persists at temperatures other than 37°C (table 1). The larger magnitude of $E_a$ of NaK–ATPase as compared to Mg–ATPase (table 3) is compatible with the greater temperature dependence of $QO_2(t)$ as compared to $QO_2'$. The respiratory rates summarized in table 1 are the net result of complex cellular processes and, therefore, interpretation of their temperature dependence is difficult. Nevertheless, it is interesting that the calculated apparent respiratory 'activation energy' of $QO_2(t)$ and $QO_2'$ (table 4) are numerically close to the $E_a$ values of NaK–ATPase and Mg–ATPase of table 3.

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