# The Role of Calcium Ion in Methoxamine-Induced Amylase Release from Parotid Glands of Euthyroid and Hypothyroid Rats

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**Abstract**—The change in responsiveness to $\alpha$-adrenergic stimulation and to calcium ions in amylase release from parotid glands of hypothyroid rats was compared with that in euthyroid rats. Calcium depletion and addition of verapamil or W-7 in the medium caused a decrease in methoxamine-induced amylase release in both eu- and hypothyroid rats. Moreover, addition of calcium ions to $Ca^{2+}$-free medium markedly increased the methoxamine-induced amylase release in proportion to its concentration in hypothyroid rats. These results suggest that calcium ions play an important role in methoxamine-induced amylase release from parotid glands of hypothyroid rats as well as those of the euthyroid ones.

It is well known that amylase release from the parotid glands is provoked mainly via activation of $\beta$-adrenoceptors (1–3). On the other hand, $\alpha$-adrenergic stimulation is less effective for this enzyme secretion (3, 4). The previous investigations in this laboratory have shown that amylase was released from parotid slices in higher ratio in hypothyroid rats than in euthyroid rats after $\alpha$- or $\beta$-adrenergic stimulation (4). The increase in response to $\beta$-adrenergic stimulation in hypothyroid rats may account, at least in part, for the phosphorylation of specific proteins contained in the microsomal fraction by cyclic AMP-dependent protein kinase (5). In contrast, processes mediated through $\alpha$-adrenergic stimulation are thought to involve stimulation of phospholipid metabolism and calcium mobilization (6–8). The present study was designed to examine the relationship between an increased responsiveness of parotid glands from hypothyroid rats to $\alpha$-adrenergic stimulation and calcium ions.

All studies were carried out on male Wistar rats weighing 170–250 g. Rats were made hypothyroid by feeding on a 0.15% propylthiouracil diet for more than 12 weeks and fasted for 16–20 hr prior to use. Parotid glands were quickly removed, trimmed and placed in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose and 5 mM $\beta$-hydroxybutyrate. Parotid slices were prepared by cutting the glands into small pieces; approx. 14–17 mg of the slices were weighed and preincubated for 20 min in 5 ml medium at 37°C under $O_2$ : $CO_2$ (95% : 5%) as the gas phase. After preincubation, the medium was removed with a pipette, the slices were rinsed once with fresh buffer, and then 5 ml of fresh, prewarmed, gassed incubation buffer was added to the tissue. Methoxamine (MTX, Nippon Shinyaku Co.) was added to each vial and the reaction was started. The slices were further incubated for 15 min. When the effect of verapamil (Knoll, Eisai Pharmaceutical Co.) on MTX-induced amylase release was examined, verapamil was added 10 min prior to the addition of MTX. During incubation, the non-stimulated amylase release from the glands was approx. 2% of the total in euthyroid rats and approx. 1% of that in the hypothyroid ones. Total amylase was

determined by summation of the amount in the medium and that remaining in the tissue at the end of the incubation. The percentage of total amylase released into the medium was then calculated. Statistical significance between euthyroid and hypothyroid rats was determined by Student's *t*-test.

As shown in Fig. 1A, in hypothyroid rats, amylase release induced by $10^{-4}$ M MTX was considerably reduced in the medium containing a lower concentration of calcium ion ($Ca^{2+}$) as compared with that in the medium containing a physiological concentration of $Ca^{2+}$. Hypothyroid rats showed a marked increase in MTX-induced amylase release in proportion to $Ca^{2+}$ concentration in the medium, whereas the increase in amylase release was minimal in euthyroid rats. This was clear at 2.5 mM $Ca^{2+}$, and amylase release was approx. 3-fold higher in the hypothyroid rats than the euthyroid ones.

In order to clarify the difference of the effect of calcium ions on $\alpha$-adrenergic stimulation between euthyroid and hypothyroid rats, the following experiments were performed. Islet-activating protein (IAP) is a new protein isolated from the culture medium of *Bordetella pertussis* (9), and its remarkable action is a reversal of the $\alpha$-adrenergic effect on insulin secretion (10). Thus, the effect of this protein on MTX-induced amylase release was investigated. Rats were injected intravenously with 1 $\mu$g of IAP and used in the experiments after 1 week. As shown in Fig. 1A, it elevated the ratio of amylase release in all cases, but there was no difference between euthyroid and hypothyroid rats. Then, the effect of verapamil on amylase release was examined. As shown in Fig. 1B, it dose-dependently suppressed amylase release induced by MTX. This phenomenon was compatible with the result of $Ca^{2+}$-dependent amylase release shown in Fig. 1A.

Table 1 shows effects of N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide

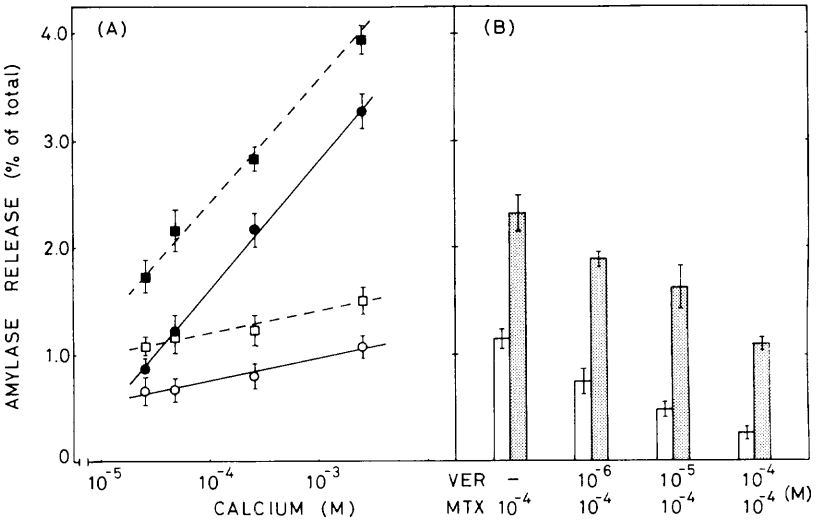


**Fig. 1.** (A) Effect of $Ca^{2+}$ concentration on methoxamine-induced amylase release from parotid glands of euthyroid, hypothyroid and islet-activating protein (IAP)-treated rats. The tissue slices were preincubated for 20 min at 37°C and transferred to new medium. After addition of methoxamine ($10^{-4}$ M), incubations were continued for 15 min. Net amylase release was defined as the difference between total amount released and basal release. Values are the means±S.E. of 5–6 experiments for each point. ○——○ euthyroid, □------□ IAP-treated euthyroid, ●——● hypothyroid, ■------■ IAP-treated hypothyroid. (B) effect of verapamil concentration on MTX-induced amylase release from euthyroid and hypothyroid rats. After 20 min preincubation, the slices were incubated with verapamil (VER) for 10 min. Then, methoxamine was added into the medium, and incubations were continued for 15 min. Values were the means±S.E. of 4 experiments. ▭ euthyroid, ▩ hypothyroid.

Table 1. Effect of W-7 and $Ca^{2+}$ depletion on MTX-induced amylase release from parotid glands of euthyroid and hypothyroid rats

| | Amylase release (%) | |
|---|---|---|
| | Euthyroid | Hypothyroid |
| Control (MTX alone, $10^{-4}$ M) | 1.31±0.18 | 2.59±0.16* |
| MTX ($10^{-4}$ M) +W-7 ($5\times10^{-5}$ M) | 0.30±0.08** | 2.02±0.23*** |
| MTX ($10^{-4}$ M) +$Ca^{2+}$-free +EGTA ($10^{-3}$ M) | 0.26±0.06** | 1.20±0.18** |

MTX=methoxamine, EGTA=ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, W-7= N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide. Values are the means±S.E. of 5 experiments. *$P<0.05$, compared to control euthyroid, **$P<0.01$, compared to its respective control, ***$P<0.05$, compared to its respective control.

(W-7) and $Ca^{2+}$ depletion in the medium on MTX-induced amylase release. Amylase release was markedly reduced by addition of $5\times10^{-5}$ M W-7, a calcium antagonist, or by incubation in $Ca^{2+}$-free medium with $10^{-3}$ M EGTA in euthyroid rats. On the contrary, effects of $Ca^{2+}$ depletion and W-7 on MTX-induced amylase release were less effective in hypothyroid rats than in the euthyroid ones. These observations suggested that α-adrenergic stimulated amylase release from rat parotid glands was more or less dependent on extracellular $Ca^{2+}$. These results were very consistent with those reported by Petersen et al. (11). Moreover, it was shown that calmodulin antagonists failed to demonstrate the involvement of calmodulin in the cyclic AMP-induced amylase release in rat parotid glands (12). However, W-7 markedly reduced MTX-induced amylase release in euthyroid rats in the present experiment. This difference may be due to the different agonists used in these experiments. In the former study, dibutyryl cyclic AMP was used for amylase release induction, but MTX was used in the present experiment. Therefore, the results presented here suggest that α-adrenergic agonists-induced amylase release from parotid glands of euthyroid rats was more intensely suppressed by addition of W-7 or $Ca^{2+}$ depletion than in hypothyroid rats. However, addition of $Ca^{2+}$ into the $Ca^{2+}$-free medium markedly increased the amylase release up to the physiological concentration in hypothyroid rats. Although the mechanism of action in these phenomena still remains to be clarified, it was shown in the present study that extracellular $Ca^{2+}$ plays an important role on α-adrenergic agonist-induced amylase release in the hypothyroid status as well as in the euthyroid one.

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