THYROXINE MODULATION OF EPINEPHRINE STIMULATED SECRETION OF RAT PAROTID α-AMYLASE

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SUMMARY

Thyroxine has been shown to modulate cyclic AMP dependent secretion of parotid α-amylase by enhancing the sensitivity of membrane bound adenyl cyclase to epinephrine. Thyroxine by itself is without effect.

Experiments in our laboratory and in collaboration with others suggest that thyroxine may have its primary site of action at the surface of the cell membrane (1-4). These studies led us to propose a general mechanism of action for thyroxine (T_4) that involves interaction of T_4 with a component of the cell membrane which results in a modulation of the ability of other hormones to stimulate the adenyl cyclase system.

This relationship has been clearly established in two different cell types. In adipose tissue thyroxine acts as a positive modulator of sensitivity to epinephrine (4-7) and in the thyrotrophs of the anterior pituitary as a negative modulator of sensitivity to thyrotropin releasing factor (TRF) (3,8,9).

As a test of our hypothesis we have carried out studies on parotid α-amylase secretion (1). Catecholamines induce a rapid secretion of α-amylase in the rat parotid gland and cyclic AMP has been shown to be an intermediate in this process (10,11). It was, therefore, of interest to see whether thyroxine could act as a modulator of epinephrine induced amylase secretion in this tissue. It has been known for many years that the parotid gland can accumulate norepinephrine (12). This tissue also contain an enzyme system which catalyzes the synthesis of organically bound iodide (13)

The physiological significance of these observations has remained obscure.

MATERIALS AND METHODS

Slices of rat parotid gland were prepared essentially as has previously been described (14). Animals used were male rats weighing 100-150 g (Sprague-Dawley derived Holtzman). Epinephrine bitartrate and thyroxine were obtained from Sigma. Tissue slices were given a preincubation wash according to the procedure of Schramm (10). Krebs-Ringer bicarbonate buffer which contained 5 mM sodium β-hydroxybutyrate, but not containing glucose, and unless otherwise specified 6 mM KCl was used after thorough gassing with 95% O_2:5% CO_2. Approximately 100 mg of slices were added to 3 ml of buffer in each incubation tube which was then arranged in a manner similar to that previously described (14) and continuously gassed at 37° with 95% O_2:5% CO_2. Enzyme secretion was determined by removal of 10 μliter aliquots of the medium at the time intervals shown and assayed for α-amylase using the Nelson-Somogyi procedure for reducing sugars (15) as recommended by Robyt and Whelan using glucose as a standard (16). Protein content of tissues was determined in a manner similar to that described by Grand and Gross (14). All values represent the average of replicates of at least four incubations.

RESULTS

Previous studies with this system have utilized high levels of KCl in the medium in order to facilitate enzyme release (10). Our method of determining amylase was highly sensitive to very low concentrations of the enzyme, since the measurement of the action of the enzyme by allowing it to produce reducing equivalents resulted in an amplification of several orders of magnitude. It was thus considered possible that we might be able to carry out these experiments using more physiological levels of potassium ion. This was of importance because of the known ability of high potassium ion concentrations to mimic hormonal effects

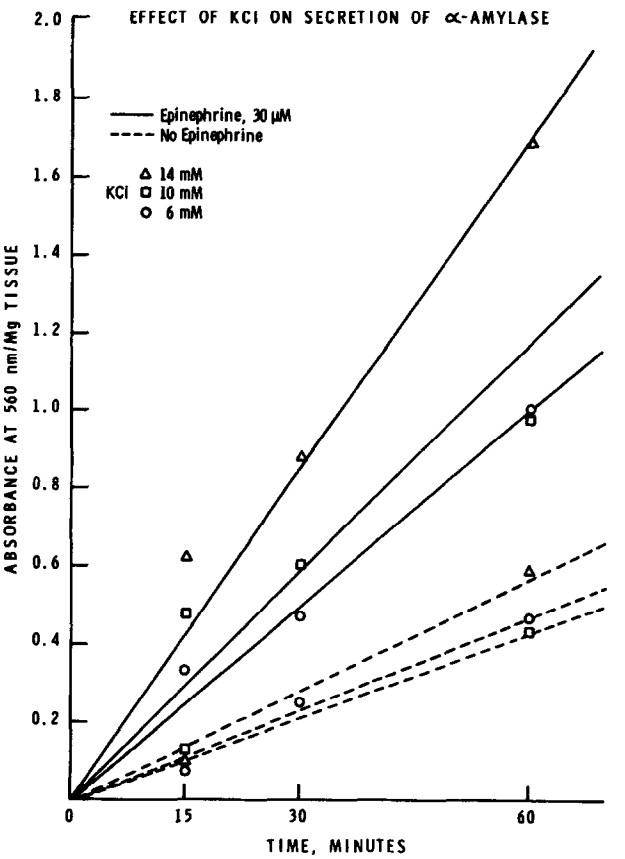

FIG. 1 - Secretion of α-amylase in the presence or absence of a maximal stimulatory concentration of epinephrine with varying concentrations of KCl.

(17). The significant induction of α-amylase secretion by maximal levels of epinephrine at these low KCl concentrations is shown in Fig. 1.

The ability of thyroxine to increase the sensitivity of this tissue to the epinephrine stimulated secretory process is illustrated in Figs. 2-4 It can be seen from Fig. 3 that thyroxine by itself is without effect. The rapid nature of this effect is shown in Fig. 4. In additional experiments we have observed that at high concentrations of epinephrine (30 μM) no additional enzyme secretion was induced in the presence of thyroxine even when the thyroxine concentration was raised to 10^{-5} M.

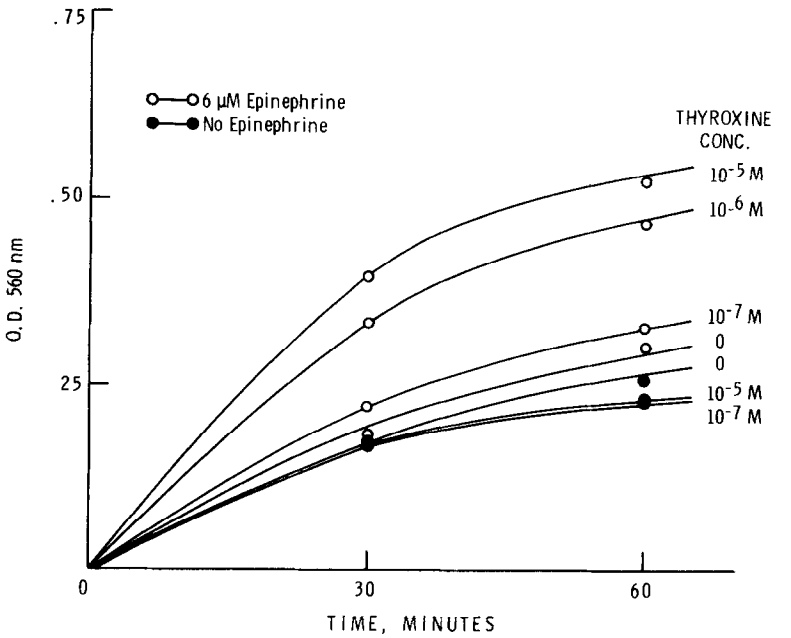

FIG. 2 - Effect of thyroxine concentration on secretion of α-amylase in the presence and absence of 6 μM epinephrine. Absorbance values are calculated per 100 μg of tissue protein.

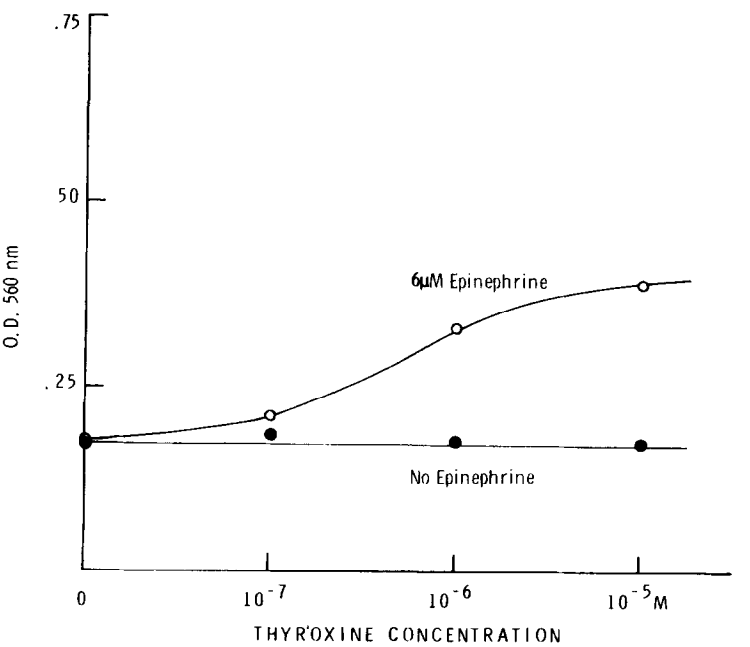

FIG. 3 - Data taken from the experiment as shown in Fig. 2 at 30 min and replotted as a function of thyroxine concentration.

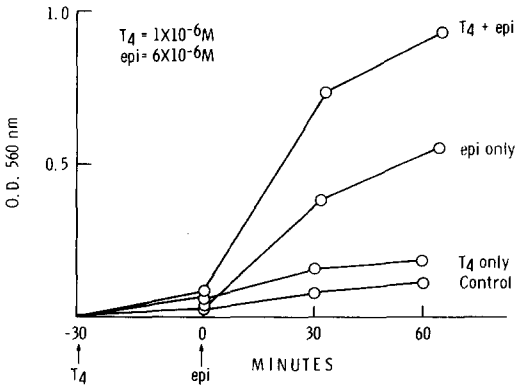

FIG. 4 - Time course of secretion of α-amylase after 30 min preincubation with 1 x 10^{-6} M thyroxine (T_4) and in the presence or absence of epinephrine (epi). Times of addition of the hormones are indicated on the abscissa. Absorbance values are calculated per 100 μg of tissue protein.

Thus, thyroxine alone does not directly provoke the adenyl cyclase mediated response; enhanced secretion of the enzyme occurs through the ability of thyroxine to modulate the effect of catecholamine stimulation. These results lend further support to the concept of thyroxine interaction with a membrane component of the catecholamine receptor site or of the adenyl cyclase complex. These experiments also suggest that the parotid secretory process may prove to be a useful model system for studying in more detail the mechanism of action of thyroxine.

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