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INVOLVEMENT OF α_1-ADRENERGIC RECEPTORS IN STIMULATION OF PHOSPHATIDYLINOSITOL METABOLISM BY CATECHOLAMINES IN MOUSE THYROIDS

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Abstract—The effects of α-adrenergic agonists and thyroid stimulating hormone on the incorporation of radioactive phosphate into phosphatidylinositol were investigated in mouse thyroids *in vitro*. The incorporation of ^{32}P orthophosphate into phosphatidylinositol was stimulated by thyroid stimulating hormone, norepinephrine (a mixed α_1- and α_2-adrenergic agonist), methoxamine and phenylephrine (α_1-agonists) and slightly by clonidine and oxymetazoline (α_2-agonists) but not by isoproterenol (β-agonist). Prazosin (α_1-antagonist) inhibited the stimulation by norepinephrine of ^{32}P incorporation into phosphatidylinositol, but yohimbine (α_2-antagonist) was less effective. Although norepinephrine inhibits the thyroid stimulating hormone-induced release by activating α-, especially α_1-adrenoceptors in mouse thyroids [M. L. Maayan *et al.*, *Metabolism* **26**, 473 (1977); M. L. Maayan *et al.*, *Endocrinology* **101**, 284 (1977); T. Muraki *et al.*, *Endocrinology* **110**, 51 (1982)] α_1-agonists did not decrease the stimulation of turnover elicited by thyroid stimulating hormone and did not have additive action with it. These results suggest that (1) the stimulation of phosphatidylinositol turnover of mouse thyroids elicited by adrenergic agonists is mediated by activation of α_1-adrenoceptors and (2) the inhibitory effect of norepinephrine on the thyroid stimulating hormone-induced release of thyroxine is not mediated by norepinephrine-inhibition of phosphatidylinositol-turnover stimulated by thyroid stimulating hormone.

It is known that neurotransmitters, hormones and drugs elicit an increased turnover of phosphatidylinositol (PI)† in various tissues [4, 5]. Although Michell [6] suggested that this effect is presumably initiated by activation of PI-specific phospholipase C, the physiological significance of this effect is not yet known.

TSH stimulates the incorporation of radioactive phosphate into phospholipids, especially into PI in the thyroid gland [7, 8]. It has been reported that the turnover of PI was also stimulated by cholinergic agonists in the thyroids from mice and dogs [9, 10], however, the effect of adrenergic agonists has not been examined extensively.

It is also known that catecholamines inhibit the release of thyroxine induced by TSH in the mouse thyroids *in vitro* by activating α-adrenoceptors [1, 2].

Recently, we demonstrated that this inhibitory effect of catecholamines was mediated through α_1-adrenergic receptors [3]. The elevation of thyroid cyclic AMP content induced by TSH was not inhibited by catecholamines [1, 3]. Therefore, the inhibitory effect of catecholamines is not mediated by inhibition of adenylate cyclase.

To study the possible role of PI turnover on inhibition by catecholamines, we have sought to determine whether catecholamines have an effect on the PI turnover in mouse thyroids. We have also examined how the TSH-induced stimulation of PI turnover is inhibited by activation of α_1-adrenoceptors like the thyroxine release elicited by TSH.

MATERIALS AND METHODS

Male, ddY strain mice, weighing 25–30 g, were stunned by a blow on the head and the thyroid gland was dissected out under magnification. The thyroid gland was weighed and was incubated in 0.4 ml of modified Krebs–Ringer bicarbonate buffer, pH 7.4, under 95% O_2 + 5% CO_2 at 37° for 3 hr unless otherwise stated. The Krebs–Ringer bicarbonate buffer consisted of NaCl 132.3 mM, KCl 5.2 mM, $MgCl_2$ 1.1 mM, $CaCl_2$ 2.0 mM, $NaHCO_3$ 15.0 mM, NaH_2PO_4 11.2 mM, containing 0.09% glucose, 0.005% ascorbic acid and 50 μCi/ml ^{32}P orthophosphate (NEX-053).

After incubation, phospholipids of the thyroid gland were extracted by homogenizing with 2 ml chloroform–methanol (2:1). After centrifugation of the homogenate (800 *g*, 5 min), the sediment was homogenized with another 2 ml chloroform–methanol and the supernatant was separated by centrifugation.

The combined supernatant was washed with 0.1 vol. of 2 M KCl solution and the organic phase evaporated to dryness under a stream of nitrogen. The residue was dissolved in a small volume of chloroform–methanol (2:1) and was spotted on thin

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† Abbreviations: phosphatidylinositol, PI; phosphatidylcholine, PC; phosphatidylethanolamine, PE; phosphatidic acid, PA; thyroid stimulating hormone, TSH; norepinephrine, NE; dibutyryl cyclic 3′:5′-adenosine monophosphate, dibutyryl cyclic AMP.

layer aluminium sheets precoated with silicagel 60 (Merck, Sharp & Dohme, West Point, PA) along with phospholipid standards such as phosphatidylcholine (PC), PI, phosphatidylethanolamine (PE) and phosphatidic acid (PA).

Major phospholipids were separated by one-dimensional TLC following the method of García-Sáinz and Fain [11]. The solvent system contained chloroform–methanol–28% NH_4OH–H_2O (130:70:5:5). Each phospholipid spot was visualized by iodine-vapour and cut out.

The ^{32}P incorporation into the phospholipids and the radioactivity of the medium were counted in a toluene scintillator (4g PPO, 0.1g POPOP/1 toluene) with a liquid scintillation spectrometer (Beckman LS-100).

Results are expressed as mean ± S.E. Significance of differences between means was assessed by using the *t*-test of Aspin and Welch [12].

These materials and methods differ little from our study [3] of thyroxine release in the mouse thyroid.

Chemicals. Norepinephrine bitartrate, dopamine HCl, TSH, dibutyryl cyclic AMP, PA, PI, PC and PE were purchased from Sigma Chemical Co. (St. Louis, MO); clonidine HCl from Tokyo Kasei Co. (Tokyo, Japan); DL-isoprotrenol HCl and yohimbine HCl from Nakarai Co. (Nagoya, Japan); oxymetazoline HCl from Chugai Pharmaceutical Co. (Nagoya, Japan); phenylephrine HCl from Kowa Co. (Nagoya, Japan); methoxamine HCl from Nippon Shinyaku Co. (Kyoto, Japan); prazosin HCl from Taito–Pfizer Co. (Tokyo, Japan). ^{32}P Orthophosphate in water (NEX-053) was purchased from New England Nuclear (Boston, MA).

RESULTS

The incubation of the mouse thyroid gland in the modified Krebs–Ringer bicarbonate buffer containing 50 μCi/ml ^{32}P, 0.09% glucose and 0.005% ascorbic acid, for 3 hr resulted in the incorporation of the radioactive phosphate into the phospholipids (Table 1).

The ^{32}P incorporation into phospholipids was the largest for PC, followed by PI, PE and PA in decreasing order. Addition of TSH (10 mU/ml) on NE (10^{-5} M) to the medium increased the incorporation of radioactivity into PC, PI and PA, while PE radioactivity remained unchanged.

NE (10^{-5} M) was more effective than TSH (10 mU/ml) in the stimulatory effect of ^{32}P incorporation into PI. Among the phospholipids, stimulation by TSH or NE of ^{32}P incorporation was the most marked for PI, so we chiefly checked for the effect of drugs on the incorporation of radioactive phosphate into PI fraction in the experiments.

The effect of TSH and NE on the incorporation of ^{32}P into PI was examined as a function of time (Fig. 1). The radioactivity of PI increased linearly up to 4 hr of incubation in the absence of NE or TSH. TSH (10 mU/ml) stimulated the incorporation of the radioactivity into PI after 1 hr of lag time, while NE (10^{-5} M) increased the ^{32}P incorporation linearly for up to 4 hr. Therefore, we examined the effect of drugs on the ^{32}P incorporation into PI at 3 hr of incubation in our experiments.

Addition of NE, a mixed α_1- and α_2-adrenergic agonist, to the medium in the concentration of

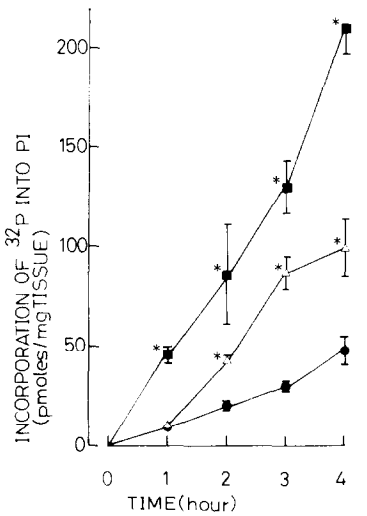

Fig. 1. Effect of TSH and NE on PI labeling as a function of time. Mice thyroids were incubated for the indicated time at 37° in 400 μl of Krebs–Ringer bicarbonate containing 50 μCi/ml ^{32}P, 0.005% ascorbic acid and 0.09% glucose under 95% O_2 + 5% CO_2. The concentrations of TSH and NE were 10 mU/ml and 10^{-5} M, respectively. (●), control; (△), TSH; (■), NE. Values and vertical bars represent means ± S.E. from 4 to 7 individual experiments. * $P < 0.05$ as compared to control at the indicated time.

Table 1. Effects of TSH and NE on the incorporation of radioactive phosphate into the phospholipids of mouse thyroids

	Incorporation of ^{32}P into phospholipids (pmoles/3 hr/mg tissue)		
	Control	NE(10^{-5} M)	TSH(10 mU/ml)
PA	6.3 ± 0.6	14.6 ± 1.5* (232)	9.8 ± 1.3* (156)
PI	29.8 ± 2.1	130.8 ± 12.2* (439)	87.0 ± 7.7* (292)
PC	120.9 ± 9.4	206.0 ± 12.2* (170)	175.8 ± 13.0* (145)
PE	25.5 ± 3.0	36.4 ± 8.0 (143)	21.6 ± 2.8 (85)

Mouse thyroids were incubated for 3 hr at 37° in Krebs–Ringer bicarbonate buffer containing 50 μCi/ml ^{32}P, 0.005% ascorbic acid, 0.09% glucose and hormones under 95% O_2 + 5% CO_2.

Each value represents mean ± S.E. of 7 to 10 experiments. Percentile stimulation from control value is shown in parentheses.

* $P < 0.05$ as compared to control.

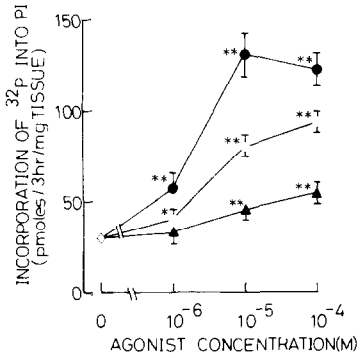

Fig. 2. Effect of α-agonists on the PI labeling. α-Agonists were added in the indicated concentrations. The thyroids were incubated 3 hr and other details are the same as in Fig. 1. Values and vertical bars represent means ± S.E. from 4 to 7 individual experiments. (●), NE; (○), methoxamine; (▲), clonidine. * $P < 0.05$ as compared to control; ** $P < 0.01$ as compared to control.

10^{-6} M ~ 10^{-4} M, resulted in a concentration-dependent increase in the incorporation of radioactivity into PI (Fig. 2). Methoxamine, an α_1-agonist, also increased the radioactivity of PI, while the effect of clonidine, an α_2-agonist, was much smaller.

To examine the relative potency of several adrenergic agonists to stimulate the PI labeling, we compared the effects of the adrenergic agonists at concentrations of 10^{-5} M (Table 2). NE (mixed α_1- and α_2-agonist) and pure α_1-agonists such as phenyleprine and methoxamine were potent in stimulatory effect of PI turnover; the α_2-agonists were less effective; and the pure β-agonist, isoproterenol, was not at all effective. The rank order of the stimulatory effect of adrenergic agonists was as follows: NE (mixed α_1- and α_2-agonist) > phenylephrine, methoxamine (α_1-agonists) > clonidine, oxymetazoline (α_2-agonists) > isoproterenol (β-agonist). In addition, dopamine and dibutyryl cyclic AMP had no effect.

To confirm the role of α_1-adrenoceptors, we examined whether the stimulation of PI turnover elicited by NE in the mouse thyroids was reversed by α-antagonists. Prazosin (α_1-antagonist) inhibited the PI labeling stimulated by NE (10^{-5} M) in a concentration-dependent manner, while yohimbine (α_2-antagonist) was less effective (Fig. 3): the IC_{50}'s of prazosin and yohimbine were 4.2×10^{-8} M and 4.8×10^{-6} M, respectively. Propranolol (β-antagonist) had no inhibitory effect on the action of NE (data not shown).

These results on adrenergic agonists and antagonists indicated the involvement of α_1-adrenoceptors rather than α_2- or β-adrenoceptors or dopamine receptors in the catecholamine stimulation of ^{32}P incorporation into PI of mouse thyroids.

Although α-adrenergic agonists are known to inhibit the thyroxine release elicited by TSH through α_1-adrenoceptors, the mediator of the inhibitory effect is not known. To examine the possible role of PI turnover in the inhibitory effect of α-agonists on the TSH-induced thyroxine release, we investigated the effect of α-agonists on the ^{32}P incorporation into PI stimulated with TSH (10 mU/ml) (Fig. 4). The stimulation of PI turnover induced by TSH was saturated at this concentration (data not shown).

NE (10^{-5} M), methoxamine (10^{-4} M) and clonidine (10^{-4} M) alone increased the PI turnover, and did not reduce the stimulation of PI turnover elicited

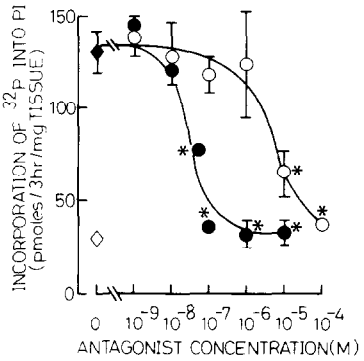

Fig. 3. Inhibition by α-antagonists of the NE-induced increase in PI turnover. The concentration of NE was 10^{-5} M. Values and vertical bars represent means ± S.E. from 4 to 7 individual experiments. (●), prazosin; (○), yohimbine; (◇), control; (◆), NE. * $P < 0.01$ as compared to NE.

Table 2. Effects of various drugs on the incorporation of ^{32}P into PI

Additions	No.	Incorporation of ^{32}P into PI (pmoles/3 hr/mg tissue)
None	10	29.8 ± 2.1 (100)
Norepinephrine	7	130.8 ± 12.1* (439)
Methoxamine	4	80.1 ± 5.5* (269)
Phenylephrine	4	91.0 ± 4.9* (305)
Clonidine	4	45.9 ± 6.1* (154)
Oxymetazoline	4	43.9 ± 3.9* (147)
Isoproterenol	4	26.8 ± 2.3 (90)
Dopamine	4	33.1 ± 3.2 (111)
Dibutyryl cyclic AMP	4	36.8 ± 8.2 (123)

The thyroids were incubated for 3 hr. The concentrations of drugs were 10^{-5} M except dibutyryl cyclic AMP (10^{-3} M).

Each value represents mean ± S.E. Percentile stimulation from control (no additions) is shown in parentheses.

* $P < 0.01$ as compared to control.

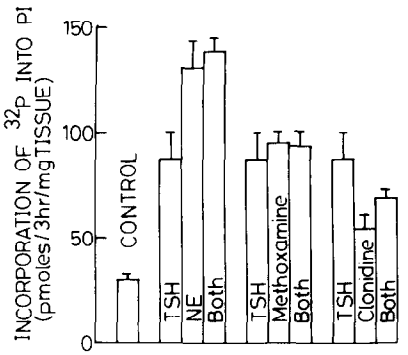

Fig. 4. Effect of α-agonists on the TSH-induced increase in PI turnover. The thyroids were incubated for 3 hr. Columns and bars represent means ± S.E. of 4–10 experiments. The concentrations of TSH, NE, methoxamine, clonidine are 10 mU/ml, 10^{-5} M, 10^{-4} M and 10^{-4} M, respectively. Other details are the same as in Fig. 1.

by TSH (10 mU/ml). However, the stimulatory effects of PI labeling of TSH and α-agonists were not additive (Fig. 4).

DISCUSSION

We observed that TSH increased the incorporation of radioactive phosphate into PI, PC and PA fraction in mouse thyroids. The largest stimulatory effect of TSH was seen on PI, confirming previous results obtained with the thyroids of other species [7, 8].

In addition, we found that NE stimulated the incorporation of radioactive phosphate into PI, PC and PA with the most pronounced stimulation seen again in PI.

It was previously reported that dibutyryl cyclic AMP did not mimic the effect of TSH on the stimulation of ^{32}P incorporation into phospholipids of the pig thyroid gland [13]. Similarly, we observed that dibutyryl cyclic AMP exerted no effect on PI labeling in mouse thyroids. This result agrees with the prior report that stimulation of PI turnover elicited by TSH is probably not mediated by cyclic AMP, although TSH stimulates the adenylate cyclase of the thyroid [14].

Since the stimulatory effect of NE was not antagonized by propranolol, and since isoproterenol had no effect on the PI metabolism, the effect of NE was not mediated through β-adrenoceptors.

The α-adrenoceptors are subdivided into α_1 and α_2 [15, 16]. It is suggested that stimulation of α_1-adrenoceptors activates PI turnover and elevates cytosolic Ca^{2+} ions, while stimulation of α_2-adrenoceptors induces nonspecific inhibition of adenylate cyclase [17], as observed with various tissues [18, 19].

We found that the stimulatory effect on PI turnover of α_1-agonists, phenylephrine and methoxamine, was stronger than that of the α_2-agonists, oxymetazoline and clonidine, and that the stimulatory effect of NE was more effectively antagonized by prazosin (α_1-antagonist) than by yohimbine (α_2-antagonist).

These results suggest that the involvement of α_1-adrenoceptors in catecholamine stimulation of PI turnover in the mouse thyroid.

Altman *et al.* [9] did not find any effect of epinephrine on the ^{32}P incorporation into phospholipids in the dog thyroid gland. The cause of this discrepancy is not clear. However, it should be noted that there is a species difference in the number of thyroidal sympathetic nerve terminals between mice and dogs [20].

It has been reported that inhibition by NE of TSH-induced thyroxine release from mouse thyroids was mediated by α-adrenoceptors [1, 2]. We have shown that this inhibitory effect of NE was mediated through activation of α_1-adrenoceptors [3].

It was claimed that this inhibitory effect of the catecholamine was not mediated by nonspecific inhibitory effect on thyroidal adenylate cyclase activity [2, 3].

We have shown that both TSH and α-agonists stimulated the PI turnover in mouse thyroids. The α-agonists, such as NE, methoxamine or clonidine, did not decrease the stimulation of PI turnover by TSH and did not show additive action with TSH. Therefore, it is not likely that α-agonists inhibit the thyroxine release elicited by TSH by diminishing the TSH-induced PI turnover, in spite of the involvement of α_1-adrenoceptors. Further studies are needed to clarify the inhibitory mechanism of NE on TSH-induced thyroxine release.

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