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STIMULATION OF ARACHIDONIC ACID RELEASE FROM THYROID PHOSPHOLIPIDS

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The present study demonstrates that exposure of pig thyroid slices to thyrotropin stimulates the arachidonate release from endogenous phospholipids. The experiments with ^{14}C-arachidonic acid show that the release of arachidonic acid is mostly from phosphatidylcholine, phosphatidylinositol and neutral lipids. The liberation of arachidonate from thyroid phospholipids is Ca^{2+} dependent. The addition of calcium ionophore A23187 to medium augments the release of arachidonate. Addition of ionophore and egzogenous Ca^{2+} markedly stimulates the arachidonate release, what suggests that thyroid phospholipase A_2 can be regulated by the extend of saturation of the enzyme with Ca^{2+}. The arachidonate release from phospholipids caused by thyrotropin is potentiated by adenosine. This effect shows that adenosine modulates TSH action and supports the idea that adenosine takes part in the physiological regulation of thyroid cells.

Key words: *thyroid gland, phospholipids, phospholipase A_2, thyrotropin, adenosine, Ca^{2+}.*

INTRODUCTION

It is well documented that phospholipid metabolism in the thyroid is affected by thyrotropin (1—4). Thyrotropin stimulates both adenyl cyclase and inositol signalling system in the thyroid (5, 6). Changes in cellular lipid metabolism initiated by receptor-mediated activation of various phospholipases including phospholipase A_2, phospholipase C and phospholipase D play an important role in normal and pathological thyroid glands. Phospholipases A_2 are the class of enzymes which catalyse the hydrolysis of arachidonic acid and other unsaturated fatty acids from the sn-2 position of phospholipids (7). Arachidonic acid is converted *via* cyclo-oxygenase and lipooxygenase pathway to prostaglandins and other eicosanoides. Thus, phospholipase A_2 could be a potential intracellular regulator of prostaglandin synthesis. The presence of phospholipase A_1, phospholipase A_2 and lysophospholipase activities have been reported in bovine thyroid using exogenous phosphatidylcholine and phosphatidylethanolamine as

substrates (8). Haye et al. (9) have shown the existence of phospholipase A_2 which preferentially liberates arachidonate from phosphatidylinositol and which is stimulated by thyrotropin. The phospholipase A_2 activity could be regulated by various effectors in the cell (10). Among the effectors of phospholipase A_2, Ca^{2+}-ions appear a major regulator of enzyme activity (10). Recent studies indicate, that adenosine or an unhydrolysable adenosine derivate inhibits TSH-induced cAMP accumulation but enhances TSH-induced activation of phospholipase C and the subsequent Ca^{2+} signal transduction system (11, 12).

The present experiments were undertaken to examine the effect of TSH, adenosine and Ca^{2+}-ions on arachidonic acid release from endogenous phospholipids in pig thyroid glands.

MATERIAL AND METHODS

All reagents were of the highest analytical purity. The solvents were pured by redestillation. ^{14}C-Arachidonic acid was obtained from Amersham Buchler (specific radioactivity 7,03 MBq/mg), aethylene glycol-bis (β-aminoethyl ether) -N,N,N′,N′-tetraacetic acid (EGTA) from Ferak Berlin, calcium ionophore A 23187, thyrotropic hormone (bovine, 2 U/mg), adenosine and the reference compounds: L-α-phosphatidylethanolamine, L-α-phosphatidylserine, L-α-phosphatidylinositol, L-α-phosphatidylcholine, L-α-phosphatidic acid, sphingomyelin were obtained from Sigma Chemical Co.

The thyroid glands were obtained from slaughterhouse immediately after killing the animals.

Assay of arachidonic acid incorporation

Thyroids were first freed of connective tissue and fat and then sliced. The slices were incubated for 30 min at 37°C in 10 mM of Tris-HCl buffer, ph 7.0 (1 ml/g of slices) containing 1 mM KH_2PO_4, 0.5 mM $CaCl_2$, 0.9 mM $MgSO_4$, 110 mM KCl, 10 mM NaCl, 2 mM EGTA and ^{14}C-arachidonic acid (0.25 μCi/1 g of slices). During incubation the slices were shaken constantly. After incubation slices were washed three times in the same medium without labelled arachidonic acid. Next, the slices were divided into 1 g portions.

Assay of arachidonic acid release

The slices labelled with ^{14}C-arachidonic acid were incubated in the same buffer as above (1 g slices/1 ml of buffer) at 37°C for 1 h in the presence or absence of various efectors: TSH (100 mU/ml), adenosine (1 mM), Ca^{2+}(2 mM) or calcium ionophore 23187 (5 μM) depending of experimental conditions. The reaction was terminated by addition 3 ml of 1 M HCl. The content of each tube test was homogenized. Then chloroform-methanol (1:2 by vol) was added and the phospholipids and neutral lipids were extracted as previously described (13). Extract of phospholipids and neutral lipids was applied to silica gel TLC plates, which were developed in the two solvent systems: chloroform-methanol-NH_4OH-water (98:80:12.5:9 by vol.) in the first direction and chloroform-methanol-acetic acid-water (98:13:50:10 by vol.) in the second direction. Spots were coloured with iodine vapours. Phospholipids were identified on TLC plates by comparison with standards. Spots of phospholipids were scraped from plates into the

scintillation vials, 0.5 ml of chloroform-methanol-water (1:2.2:1 by vol) and 5 ml of Bray's scintillator liquid were added and counted for radioactivity by a liquid scintillator spectrometer LKB Wallac 1209 Rac Beta.

Statistically significant differences were calculated according to Student's t-test.

RESULTS

Thyroid slices prelabeled with ^{14}C arachidonic acid were incubated with thyrotropin or Ca^{2+} for 1 h, and changes in labeled phospholipids were determined. As shown in *Table 1* thyrotropin causes appreciable liberation of arachidonate from phosphatidylcholine, phosphatidylinositol and neutral lipids. The stimulation of arachidonic acid release by TSH suggests the activation of phospholipase A_2. The results of present investigation show that the arachidonic acid release from thyroid phospholipids is Ca^{2+} dependent. The addition of external Ca^{2+} augmented arachidonate release. The arachidonate liberation from labeled phospholipids was also stimulated by calcium ionophore A23187 in the presence or in the absence of external Ca^{2+} (*Table 2*). In the latter case, the Ca^{2+} is released from intracellular stores. The addition of ionophore A23187 and Ca^{2+} ions markedly increased arachidonic acid release from phospholipids. These results show that thyroid phospholipase A_2 can be regulated by the extend of the enzyme saturation with Ca^{2+} ions. The addition of adenosine to incubation medium causes only insignificant changes in labeled phospholipids. However, the addition of both agonists, adenosine and TSH, induces marked increase of arachidonic acid liberation from phospholipids. (*Table 3*). These results indicate that there is synergic action of TSH and adenosine in arachidonic acid release from thyroid phospholipids.

Table 1. Effect of thyrotropin and Ca^{2+}-ions on arachidonic acid release from thyroid phospholipids

Phospholipid	[^{14}C]-Arachidonate content (cpm/100 mg tissue)		
	CONTROL	TSH	Ca^{2+}
Phosphatidylcholine (PC)	3848 ± 265	3250 ± 258^{x}	3574 ± 240
Phosphatidylethanolamine (PE)	848 ± 70	790 ± 62	703 ± 55^{x}
Phosphatidylinositol (PI)	2445 ± 187	1768 ± 168^{xx}	1530 ± 160^{xx}
Phosphatidylserine (PS)	526 ± 57	545 ± 65	390 ± 47^{x}
Phosphatidic acid (PA)	139 ± 38	129 ± 35	138 ± 36
Sphingomyelin (SPH)	283 ± 45	308 ± 51	224 ± 40
Neutral lipids (NL)	13263 ± 1450	10085 ± 960^{x}	12508 ± 1510

Thyroid slices were preincubated for 30 min with [^{14}C]archidonic acid and were washed and incubated for 60 min with TSH (100 mU/ml) or $CaCl_2$ (2 mM) or without effectors (control). Phospholipids were extracted and determined by twodimensional chromatography. The data are expressed as the mean ± SD from four separate experiments. Statistical significance: $^{x}p < 0.05$, $^{xx}p < 0.01$ vs. control

Table 2. Effect of calcium ionophore A23187 and Ca^{2+} on arachidonic acid release from thyroid phospholipids

Phospholipid	[^{14}C]-Arachidonate content (cpm/100 mg tissue)		
	CONTROL	CALCIUM IONOPHORE	CALCIUM IONOPHORE plus Ca^{2+}
Phosphatidylcholine	1703 ± 142	1605 ± 128	1223 ± 105^{xx}
Phosphatidylethanolamine	396 ± 45	322 ± 42	204 ± 32^{xxx}
Phosphatidylinositol	1198 ± 80	1046 ± 76	667 ± 55^{xxx}
Phosphatidylserine	236 ± 42	155 ± 35	123 ± 16^{x}
Phosphatidic acid	56 ± 9	38 ± 11	45 ± 10
Sphingomyelin	275 ± 39	223 ± 32	164 ± 24^{x}
Neutral lipids	7634 ± 320	7986 ± 455	7504 ± 480

The labeled slices with [^{14}C]-arachidonic acid were incubated in the presence ionophore A23187 (5 μM) or ionophore plus Ca^{2+} (5 mM) and in absence of effectors (control). Phospholipids were extracted and determined as described in Methods. The data are expressed as the mean ± SD from three separate experiments. Statistical significance: $^{x}p < 0.05$, $^{xx}p < 0.01$, $^{xxx}p < 0.001$ vs. control

Table 3. Effect of thyrotropin and adenosine on arachidonic acid release from thyroid phospholipids

Phospholipid	[^{14}C]-Arachidonate content (cpm/100 mg tissue)			
	CONTROL	TSH	ADENOSINE	ADENOSINE + TSH
Phosphatidylcholine	2372 ± 298	1915 ± 351^{x}	2268 ± 257	1834 ± 214^{xx}
Phosphatidylethanolamine	601 ± 45	540 ± 58	556 ± 67	479 ± 61^{xx}
Phosphatidylinositol	1885 ± 259	1281 ± 329^{xx}	1695 ± 244	1104 ± 305^{xxx}
Phosphatidylserine	156 ± 45	160 ± 44	162 ± 39	125 ± 31
Phosphatidic acid	109 ± 34	98 ± 31	84 ± 32	114 ± 36
Sphingomyelin	141 ± 35	150 ± 42	143 ± 32	136 ± 30
Neutral lipids	12402 ± 877	8396 ± 613^{xxx}	11562 ± 823	11055 ± 723^{xx}

The thyroid slices were prelabeled by incubation with [^{14}C]-arachidonic acid and then were stimulated with TSH (100 mU/ml) or adenosine (1 mM) or TSH plus adenosine for 1 h. Phospholipid extraction and measurements were processed as described in Methods. All results are presented as mean ± SD from six experiments. Statistical significance: $^{x}p < 0.05$, $^{xx}p < 0.01$, $^{xxx}p < 0.001$ vs. control

DISCUSSION

Two enzymatic pathways may be involved in the release of aracidonic acid from lipids: phospholipase A_2 and phospholipase C with sequential action digliceride lipase. Phospholipase A_2 action leads to the direct release of

arachidonic acid with accumulation of lysophospholipid. Arachidonic acid can also be liberated indirectly in the phosphoinoditide cycle. In the present study it was shown that thyrotropin stimulates the release of arachidonic acid from prelabeled thyroid slices. Arachidonic acid was released mostly from phosphatidylcholine, phosphatidylinositol and neutral phospholipids. The stimulation of arachidonic acid release from phosphatidylcholine indicates the activation of phospholipase A_2. In our previous report (4) we have demonstrated the decrease of phosphatidylcholine and the increase of lysophosphytidylcholine in pig thyroid membranes in the presence of thyrotropin. The concomitant production of lysophosphatidylcholine suggested the involvement of phopholipase A_2. Shimegi et al. (12) also have found that in FRTL-5 thyroid cells arachidonic acid release was associated with lysophosphatidylcholine production and conclude that arachidonic acid is produced by phospholipase A_2. Because phosphatidylcholine is the principal phospholipid in the thyroid (about 50% of the pool of phospholipids), its hydrolysis by phospholipase A_2 may be the important source of arachidonic acid.

We observed that thyrotropin caused also a significant decrease of radioactivity in phosphatidylinositol. Haye et al. (9) have described a phosphatidylinositol specific phospholipase A_2, which is stimulated by thyrotropin, and have suggested the role for this enzyme in the release of arachidonic acid for prostaglandin synthesis. They also have suggested the presence of two distinct sources of prostaglandin in the thyroid: phospholipids and triglicerides. From the phospholipids, arachidonate is liberated by action of phospholipase A_2 stimulated by TSH through the process in which cAMP is not involved. From the triglicerides, arachidonate is liberated by cAMP dependent lipase (14). Both enzymes can potentially release arachidonate and thus contribute to the thyrotropin stimulated prostaglandin biosynthesis. It was shown that in neutral lipids the arachidonate amount is much higher in diacylglicerols than in monoacylglicerols or triacylglicerols. However, the most important pool of esterified arachidonate are triacylglicerols (15). In the present investigation, the labeled arachidonate content in neutral thyroid lipids was much higher than in phospholipids, what is consistent with findings of Haye and Jaquemin (15). Neutral lipids besides phospholipids may be an important source of arachidonic acid in the thyroid.

The present results show that the release of arachidonic acid in the thyroid is Ca^{2+}-dependent. The addition of calcium ionophore A23187 increases the liberation of labeled arachidonic acid from thyroid slices probably by the mechanism of releasing Ca^{2+} from internal stores and hence increasing phospholipase A_2 activity. The addition of ionophore A23187 and Ca^{2+} augmented arachidonic acid release from phospholipids. This observation suggests, that thyroid phospholipase A_2 can be regulated by the extend of

saturation with Ca^{2+}. Ca^{2+} ions, thus appearing a major regulator of arachidonic acid release in the thyroid. Intracellular Ca^{2+} mobilization can be induced in phosphoinosidide cycle (16). In this regulator system, the receptor activated phospholipase C hydrolyses phosphatidylinositol-(4,5)-bisphophate into diacylglicerol and inositol-(1,4,5)-trisphosphate which releases Ca^{2+} from intracellular stores. In addition, activation of the Ca^{2+}-phosphatidylinositol cascade is often associated with increased Ca^{2+} entry from extracellular medium. TSH-induced activation of phospholipase C, followed by Ca^{2+} mobilization and phospholipase A_2 activation, stimulates the arachidonic acid release. Tahara et al. (17) have showed that in FRTL-5 thyroid cells TSH regulates all three steps involved in prostaglandin synthesis i.e. arachidonic acid release from membrane phospholipids, cyclooxygenase action and individual prostaglandin formation. The arachidonic acid release induced by thyrotropin involves a pertussis toxin-sensitive G protein and is not cyclic AMP mediated (12, 17). In FRTL-5 thyroid cells arachidonic acid could be converted to prostaglandins E_2(PGE_2), prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), prostaglandin D_2(PGD_2) and other metabolites. This prostaglandin synthesis is under multihormonal control. It was demonstrated that cyclooxygenase and PGE_2 and PGD_2 isomerase like activities are also regulated by TSH but this regulation also involves insulin/insulin-like growth factor-I and one or more components in the serum (17). The ability of TSH to increase prostaglandin synthesis is consistent with results in most other thyroid studies (9, 15, 19). Thyrotropin has proposed to be a physiological inducer of the phosphoinositide-Ca^{2+} response. However, this view has not been widely accepted, since in all tested thyroid systems, the thyrotropin concentration required for the activation of phosphoinositide-Ca^{2+} responses was usually far greater than necessary to induce cAMP response. Recent studies indicate that adenosine (or its unhydrolysable derivates), probably *via* A_1 type of P_1-receptors, inhibits TSH-induced cAMP formation but sensitizes TSH-induced phospholipase C and the subsequent Ca^{2+} signal transduction system, such that TSH activates phospholipase C at much lower, near physiological, concentrations (11, 12). The results of this study confirm the cooperation of both agonists, adenosine and TSH, in arachidonic acid release from thyroid phospholipids. Adenosine augments TSH-induced arachidonic acid release probably by increasing Ca^{2+} which, in turn, activates Ca^{2+}-sensitive phospholipase A_2. It also has been shown, that thyrotropin-induced H_2O_2 production, an essential process for iodide organification and thyroid hormone synthesis, is mediated by Ca^{2+} signalling followed by phospholipase A_2 activation and potentiated by an adenosine derivate (18). Prostaglandins synthesized from arachidonates stimulate many different parameters of thyroid gland metabolism, suggesting an important role in regulating thyroid function (19). It was found that an increased release of PGE_2 could promote a local vasodilatation and increase thyroid blood flow. Adenosine is known as

vasodilator and its role in reactive hyperemia is generally accepted (20). In our experiments we showed that adenosine ougments TSH-induced arachidonic acid release. It could be suggested that prostaglandins are involved in vasodilation effect of adenosine. It may be one of the adenosine regulation mechanisms on the thyroid gland.

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