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ACUTE EFFECT OF THYROTROPIN ON PHOSPHATIDYLINOSITOL DEGRADATION AND TRANSIENT ACCUMULATION OF DIACYLGLYCEROL IN ISOLATED THYROID FOLLICLES

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SUMMARY Pig thyroid follicles isolated by collagenase treatment were incubated with or without thyrotropin. 1) Within 30 seconds the intracellular diacylglycerol content was doubled but then returned to the initial level after a further 30 sec incubation. 2) In the follicles prelabeled with [^{32}P]-phosphate or [^{14}C]-arachidonate, thyrotropin-dependent degradation was observed in phosphatidylinositol. 3) Diacylglycerol containing [^{14}C]-arachidonate was increased in parallel with the accumulation of total diacylglycerol mentioned in 1). The physiological role of the rapid thyrotropin action was discussed in relation to phosphatidylinositol turnover and possible production of free arachidonate which was known to be a precursor of prostaglandin.

INTRODUCTION

It has been known that TSH stimulates [^{32}P]-phosphate incorporation into phospholipids, especially into PI[1/] (1 - 3). This is considered to be an example of the 'phosphoinositide effect' which is induced by various neurotransmitters as well as peptide hormones. Although Michell suggested that the primary reaction was activation by the stimulant of PI-specific phospholipase C (E.C. 3,1,4,10) (4), the physiological significance of this effect is not yet known. On the other hand, Burke found TSH-induced synthesis of thyroidal PGE (5) and Haye et al. (6) suggested that such a PGE synthesis was caused by TSH-induced arachidonic acid production, which was derived from PI by hypothetical phospholipase A_2 specific to PI. The TSH-induced PGE synthesis as a long term phenomenon, however, was not confirmed by other investigators (7 - 8). Instead, cAMP, a second messenger of TSH, was found to inhibit PGE synthesis.

Recently, Toccafondi et al. reported that PGE release from thyroid was stimulated by TSH for only short period after the addition of the hormone and suggested that cAMP increased by TSH abolished the PGE release in a later period (9). As is generally known, the arachidonic acid content in PI is much higher than in other phospholipids and phospholipase C of animal tissues including thyroid

1/ Abbreviations used are: PI, phosphatidylinositol; DG, diacylglycerol; MG, monoacylglycerol; KRB, Krebs-Ringer-Bicarbonate; PGE, prostaglandin E; cAMP, cyclic adenosine 3'5'-monophosphate; TSH, thyrotropin.

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TABLE 1

Effects of TSH on the [^{32}P] incorporation into individual phospholipids of pig thyroid follicles

Phospholipid type*	Incorporation into phospholipid (dpm/mgP**)		Increase***
	-TSH	TSH	
phosphatidylcholine	38140	66826	175
phosphatidylethanolamine	30711	58960	192
phosphatidylinositol	69906	263427	377
sphingomyelin	8913	18116	203
others	7367	9373	127

Two tenth g of packed thyroid follicles were incubated in 2 ml of KRB-buffer containing 50 μCi of [^{32}P]-phosphate with or without 160 mU of TSH. The specific radioactivity of labeled phosphate in the reaction medium was 21 μCi/μmole. Values are means of duplicates.

* Phosphatidylserine was neglected because of its very low content in pig thyroid as reported by Macchia and Pastan (14)

** P content was determined according to Ames et al. (13)

*** % increase in labeled phospholipid responding to TSH

is PI specific (10). These facts led us to examine whether TSH-dependent PI turnover is initiated by TSH-dependent PI degradation that can supply arachidonic acid for acute and transient PGE synthesis. Recent findings of thrombin-induced PI degradation in platelets (11, 12) and temporary accumulation of DG as an intermediate for PGE suggested that a similar mechanism may work in thyroid, as a primary action of TSH on thyroidal plasma membrane. In the present paper, we describe TSH induction of PI degradation and transient accumulation of DG in thyroid follicles.

MATERIALS AND METHODS

1) Isolation of thyroid follicles Pig thyroid glands were obtained from a local slaughterhouse. The isolated follicles were prepared by a method originally contrived by Ui and Sho (unpublished data). The whole lobe of the gland was perfused with cold KRB-buffer containing 10 mM glucose and then with 0.1 % collagenase dissolved in the buffer. The perfused glands were incubated in the collagenase solution at 37 C for 1 h. The follicles were liberated by gentle sqeezing of the collagenase-treated glands immersed in KRB-buffer, collected by centrifugation at 44 X g for 5 min and washed several times with KRB-buffer to remove blood cells and single cells. The follicle-preparation responded to TSH, resulting in a "phosphoinositide effect", as shown in Table 1. The results indicated that the preparation was as active as the slices, in terms of the regulatory mechanism of phospholipid metabolism. In addition, the use of the preparation avoided the interference in glyceride analyses by the coexistence of fat-rich extraneous tissues.

2) Determination of total diacylglycerol increase About 0.2 g of packed follicles were preincubated in 1.9 ml of KRB containing 10 mM glucose at 37 C for 30 min. At the end of the preincubation, 0.1 ml of TSH solution (1.6 U/ml of 1 % BSA) was added. One tenth ml of 1 % BSA replaced the TSH solution in the control vessels. At the time indicated, the reaction was stopped by the addition of 20 volumes of chloroform-methanol (2 : 1) and vigourous shaking. After washing with 0.2 M KCl the lower phase was collected and applied to a silicate column for neutral lipids. The lipid fraction eluted with chloroform-methanol (95 : 5) was dried and subjected to a thin layer chromatography according to Skipski et al. (15). The DG fraction on the TLC plate was scraped off and analyzed as acyl esters by Shapiro's method with a modification for microscale-determination (16).

3) Radiotracer experiments About 0.4 g of packed follicles was suspended in a final volume of 2.0 ml of KRB-buffer containing 10 mM glucose and preincubated for 30 min. Into the suspensions, 10 μCi/ml each of [^{32}P] phosphate or 0.33 μCi/ml each of [^{14}C]-arachidonic acid (17) was added and incubation was continued for 2 to 3 h. The labeled follicles were spun down at 380 X g for 5 min, washed with cold KRB-buffer 3 times and finally suspended in the original volume of KRB-glucose. Incubation with TSH and DG isolation from the incubation mixture were performed as described in the previous section. Phospholipids were separated by thin layer chromatography using chloroform-methanol-acetic acid-H_2O (50 : 30 : 8 : 4).

Phospholipids separated on the plate were visualized with iodine vapor, scraped, suspended in toluene scintilator : Triton X-100 (2 : 1), and counted in a Beckman LS 7500.

4) Chemicals The TSH prepared from whale pituitary (18) was kindly supplied by Drs. Takahashi and Ui, Institute of Endocrinology, Gunma University. The biological activity was 1.6 U/mg. Collagenase type IV was purchased from Worthington, BSA from Sigma. Other chemicals were reagent grade.

RESULTS

1) Transient increase in total diacylglycerol content Fig. 1 shows the time course of the change in total DG content of follicles incubated with or without TSH (80 mU/ml). The DG content of follicles increased immediately after the addition of TSH, reached the maximum within 30 sec and returned to the control level (74 nmoles/g tissue) within 1 min from the beginning. The control level of DG was not as low as that of platelets reported by Rittenhouse-Simmons (9), possibly because of the complex structure of thyroid follicles.

2) [^{32}P]-phosphatidylinositol degradation To find the source of DG produced under the influence of TSH, metabolic changes in [^{32}P]- or [^{14}C] arachidonate-labeled phospholipids were examined. Table 2 summarizes the results of such experiments in which thyroid follicles prelabeled with [^{32}P]-phosphate (A) or with [^{14}C]-arachidonic acid (B) were incubated for 5 min in the presence or absence of TSH. In the absence of TSH, no more than 5 % of labeled phospholipids were degraded during incubation while, in the presence of TSH, almost 1/3 of [^{32}P]-labeled or 1/6 of [^{14}C]-arachidonate labeled PI disappeared. There were no other phospholipids which decreased appreciably, except [^{32}P]-phosphatidylcholine. Therefore, if the degradation of PI was catalyzed by PI-specific phospholipase C, the PI could be the main source of DG shown in Fig. 2.

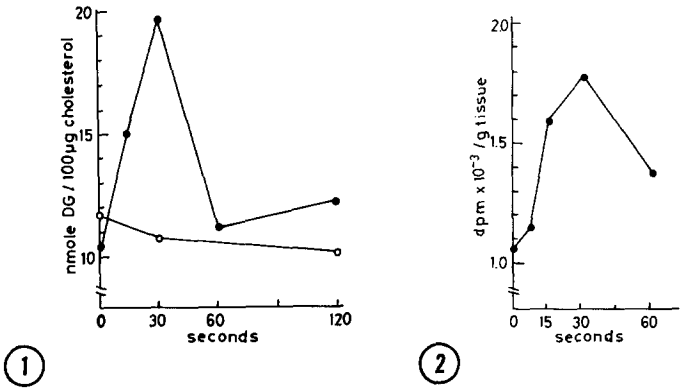

Fig. 1. Early effect of TSH on total DG content in isolated thyroid follicles
Values are means of duplicates and expressed as total DG in nmoles per 100 µg of cholesterol which has been known to be uninfluenced by TSH. The cholesterol content was 0.7 mg/g tissue. Isolated follicles were preincubated in KRB-glucose for 30 min and, after the TSH addition, the reaction was stopped by the addition of a chloroform-methanol mixture. DG was isolated by thin layer chromatography, and determined as acylesters. Similar results were obtained in an identical experiment. (o–o) - TSH, (●–●) + TSH.

Fig. 2. Time course of labeled DG production of [^{14}C]-arachidonic acid in the presence of TSH
Values are means of duplicates. Follicles were preincubated with [^{14}C]-arachidonic acid for 2 h and washed. The prelabeled follicles were treated by the same procedure as described in the legend for Fig. 1, except that the labeled DG isolated was counted.

3) Transient production of [^{14}C]-arachidonate labeled diacylglycerol If the newly produced DG was derived from PI, the DG should be arachidonic acid rich. To prove this, thyroid follicles prelabeled with [^{14}C]-arachidonate were incubated with or without TSH for 30 sec and changes in labeled diacylglycerol as well as phospholipids were determined. As shown in Table 3, even such short incubation caused appreciable degradation of PI responding to TSH, while no change was found in the other phospholipids. About 40 % of this decrease in [^{14}C]-arachidonate labeled PI was compensated by the increase in [^{14}C]-arachidonate labeled DG.

This early increase in [^{14}C]-arachidonate labeled DG was confirmed by a time course study. As shown in Fig. 2, the labeled DG increased, reached its maximum at 30 sec after the onset of TSH addition and decreased thereafter. The pattern was similar to that of the change in total DG shown in Fig. 1.

DISCUSSION

The present study disclosed that TSH-induced degradation of PI and concomitant production of DG containing arachidonic acid. However, the higher level of DG did not continue longer than a minute and the increase in the amount of DG was always

TABLE 2

TSH dependent changes in [^{32}P]-phosphate and [^{14}C]-arachidonate labeled phospholipids

Phospholipid type	Specific activity (dpm/g tissue)* after 5 min incubation -TSH	TSH	Decrease**
[A]prelabeled with [^{32}P]-phosphate			
phosphatidylcholine	7045 ± 125	6460 ± 115	8
phosphatidylethanolamine	973 ± 53	982 ± 70	–***
phosphatidylinositol	2293 ± 103	1458 ± 180	36
sphingomyelin	263 ± 45	240 ± 23	–
others	280 ± 38	495 ± 88	–
[B]prelabeled with [^{14}C]-arachidonate			
phosphatidylcholine	3105 ± 284	3049 ± 198	–
phosphatidylethanolamine	1053 ± 102	1181 ± 102	–
phosphatidylinositol	6372 ± 267	5445 ± 234	15
sphingomyelin	99 ± 13	132 ± 20	–
others	132 ± 13	106 ± 13	–

Thyroid follicles were preincubated for 3 h with [^{32}P]-phosphate [A] or for 2 h with [^{14}C]-arachidonate [B]. The labeled follicles were washed and incubated for 5 min with or without TSH.
* Values are means of quadraplicates with SE
** % decrease in labeled phospholipid responding to TSH
*** not significant

TABLE 3

Effect of TSH on the degradation of [^{14}C]-arachidonic acid labeled PI and formation of DG

Lipid type	Specific activity (dpm/g tissue)* Incorporation during preincubation	after 30 sec incubation -TSH	TSH
phosphatidylcholine	19455 ± 395	19140 ± 70	19300 ± 285**
phosphatidylethanolamine	5300 ± 45	5565 ± 310	5340 ± 25**
phosphatidylinositol	15645 ± 190	15155 ± 405	14025 ± 270***
1,2-diacylglycerol	1445 ± 130	1590 ± 230	2070 ± 70***

* Values are means of triplicates with SE
** not significant
*** P<0.01 vs. incorporation during preincubation.

less than the decrease in the amount of PI. This may be caused by further degradation and/or utilization of DG for resynthesis of PI through phosphatidic acid. In fact, TSH stimulated [^{32}P] incorporation into PI in isolated follicles as well as thyroid slices, and the stimulation continued for 2 - 3 h, suggesting that PI turned over via DG and then phosphatidic acid. Therefore at least a part of the acute metabolic changes in PI and DG is supposed to be the initial step in PI turnover in the long term experiments, although we have no quantitative evidence to support this theory. Concerning DG degradation, it is noted that PI metabolism in platelets has been discussed with reference to the production of prostaglandin precursor or free arachidonic acid by further degradation of DG. As the basis of such a discussion, DG lipase was found in plasma membranes of platelets in addition to erythrocyte (19) and brain (20). We have actually found DG lipase and suggested that MG lipase also is present in thyroid plasma membrane (21). Since TSH-induced cAMP accumulation was reported (22) to occur a few minutes after the addition of TSH, it is still possible for transient prostaglandin synthesis to occur during the first few minutes of TSH action, even if the accumulated cAMP inhibited the synthesis. Therefore, at least a part of the DG may participate as a source of prostaglandin.

Although the physiological significance of the present finding is not completely clear at this stage of the study, we assume a new mechanism of hormone action in which the conversion of a considerable portion of PI to DG changes the nature of the plasma membrane, and possible prostaglandin synthesis and release (9) participate in the thyroidal information system. In addition, it is noticed that DG containing an unsaturated fatty acid residue like arachidonate has been reported to have the ability to stimulate cAMP-independent and Ca^{++} dependent protein kinase (23, 24).

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REFERENCES

1. Freinkel, N. (1957) Endocrinology 61, 448-460
2. Scott, T. W., Jay, S. M. and Freinkel, N. (1966) Endocrinology 79, 591-600
3. Scott, T. W., Mills, S. C. and Freinkel, N. (1968) Biochem. J. 109, 325-332
4. Michell, R. H. (1975) Biochem. Biophys. Acta 415, 81-147
5. Burke, G., Chang, L-L., and Szabo, M. (1973) Science 180, 872-875
6. Haye, B., Champion, S. and Jacquemin, C. (1973) FEBS Lett. 30, 253-260
7. Margotat, A., Rolland, P-H., Charrier, B. and Mauchamp, J. (1978) FEBS Lett. 95, 347-351
8. Boeynaems, J. M., Waelbroeck, M. and Dumont, J. E. (1979) Endocrinology 105, 988-995
9. Toccafondi, R. S., Patrono, C., Pinca, E., Aterini, S., Rotella, C. M. and Tanini, A. (1980) Abstracts of VIII International Thyroid Congress, pp 104

10. Jangalwala, F. B., Freinkel, N. and Dawson, R. M. C. (1971) Biochem. J. 123, 19-33
11. Rittenhouse-Simmons, S. (1979) J. Clin. Invest. 63, 580-587
12. Bell, R. L. and Majerus, P. W. (1980) J. Biol. Chem. 255, 1790-1792
13. Ames, B. N., Dubin, D. T. (1960) J. Biol. Chem. 235, 769-775
14. Macchia, V., Tamburrini, D. and Pastan, I. (1969) Endocrinology 86, 787-792
15. Skipski, V. P., Smolone, A. F. and Sullivan, R. C. (1965) Biochem. Biophys. Acta 106, 386-396
16. Shapiro, B. (1953) Biochem. J. 53, 663-666
17. Rittenhouse-Simmons, E., Russell, F. A. and Deykin, D. (1976) Biochem. Biophys. Res. Commun. 70, 295-301
18. Takahashi, H. and Ui, N. (1972) J. Biochem. 71, 531-541
19. Michell, R. H., Coleman, R. and Finean, J. B. (1973) Biochem. Biophys. Acta 318, 306-312
20. Cabat, M. C. and Gatt, S. (1977) Biochemistry 16, 2330-2334
21. Igarashi, Y. and Kondo, Y. Submitted to Biochem. Biophys. Res. Commun.
22. Povey, P. M., Rees Smith, B., Davies, T. F. and Hall, R. (1976) FEBS Lett. 72, 251-255
23. Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. and Nishizuka, Y. (1979) Biochem. Biophys. Res. Commun. 91, 1218-1224
24. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. and Nishizuka, Y. (1980) J. Biol. Chem. 255, 2273-2276