EXISTENCE OF TWO POOLS OF PROSTAGLANDINS DURING STIMULATION OF THE THYROID BY TSH

Bernard HAYE, Serge CHAMPION and Claude JACQUEMIN*
Laboratoire de Biochimie, Faculté des Sciences, B.P. No. 347, 51062 Reims-Cedex, France

Received 13 February 1974

1. Introduction

In a previous work [1] we have shown that Kuehl's hypothesis [2,3], taken over by Burke [4,5], according to which the prostaglandins would represent an obligatory intermediate in the hormonal stimulation of adenyl cyclase, could not be applied to the stimulation of the thyroid by TSH. Our results have been simultaneously confirmed by those of Wolff [6]. Finally, Burke himself was puzzled by the observation [7] that dibutyryl adenosine monophosphate cyclic (DBC) was able to increase the level of prostaglandins in rat or mouse thyroid.

We decided to study the relations which could exist between the process of prostaglandin biosynthesis that we have described, where TSH stimulates a phospholipase A_2 which preferentially liberates arachidonate from phosphatidyl-inositol, and the effect of DBC.

The results that we have obtained show the independence of the two pathways. DBC or cAMP have no effect on phospholipase A_2, but they activate a lipase which liberates arachidonate from neutral lipids. It is therefore possible to distinguish two pools of prostaglandins, one which is pre-cAMP and not compulsory, the other which is post-cAMP and is a consequence of the activation of adenyl-cyclase.

* With the technical collaboration of Mrs O. Legue.

** We thank the slaughter-house of the City of Reims for providing us with the glands.

2. Materials and methods

2.1. *Preparation of labeled lipids using ^{14}C-acetate*

Pig thyroid glands were obtained from the slaughter-house** prepared and incubated as previously described [8] in the presence of 250 μCi of ^{14}C-acetate (^{14}C-acetate C-U 91,3 mCi/mM, C.E.N. France). After extraction, the total phospholipids are chromatographed according to Marinetti [9] and eluted from a preparative chromatographic run. Measurements of phosphorus, according to Shibuya [10], and of radioactivity allow a determination of specific radioactivity.

Neutral lipids are analyzed by thin-layer chromatography according to Mangold [11] and eluted. Measurements of glycerol and of radioactivity allow a determination of specific radioactivity.

2.2. *Preparation of triglycerides labeled with ^{14}C-arachidonate*

For analytical purposes, 200 mg of pig thyroid are incubated in 2 ml of Krebs–Ringer–Tris, pH 7.4, for 20 min in the presence of 0.05 μCi of arachidonic acid (Arachidonic acid, the Radiochemical Centre, Amersham, 54 mCi/mM). At the end of the incubation time, the total lipid extract is analyzed on one hand by thin-layer chromatography according to Lands and Samuelsson [12] which allows a separation of phospholipids, prostaglandins E_2 and $F_2\alpha$, arachidonic acid and neutral lipids, and on the other hand in the above-mentioned system of Mangold which allows an analysis of neutral lipids.

Lipids are visualized by spraying a solution of iodine (1%) in methanol. The spots are scraped and transferred into scintillation vials.

For preparative purposes, 2 g of pig thyroid are incubated in 10 ml of Krebs–Ringer–Tris, pH 7.4, for 20 min in the presence of 5 μCi of arachidonic acid.

2.3. *Biosynthesis of prostaglandins in vitro*

After incubation the slices are homogenized in the incubation mixture. The medium is acidified to pH 3 with 2 M citric acid or 0.03 N HCl and the total lipid extract is chromatographed as previously described.

Prostaglandins E are determined by the spectrophotometric technique method of Wallach [13] after treatment with KOH–methanol.

2.4. *Measurement of phospholipase A_2 activity*

The phospholipase A_2 activity in a thyroid homogenate is determined as previously described [1].

2.5. *Measurement of lipase activity*

The lipase activity in a thyroid homogenate is determined by measuring the amounts of radioactive fatty acids liberated from ^{14}C-triglycerides. Experimental details are given in the legend of fig. 3. At the end of the incubation time the free fatty acids are extracted by the method of Dole [14]. An aliquot is counted to determine the radioactivity. ^{14}C-Arachidonic acid is determined as previously described [1].

Comparisons between the tests and the controls were done using the 't' test of Student [15].

3. Results

We have checked that, in our experimental conditions, DBC as well as TSH increased the level of type E prostaglandins in slices of pig thyroid. PGE were determined after transformation into PGB. It can be seen in fig. 1 that TSH (50 mU/ml) causes after 15 min a 2-fold increase and after 45 min a 3-fold increase in the level of PGE. The effect of DBC (50 μg/ml) is in the same direction, but is less important, and a 2-fold increase is only obtained after 45 min.

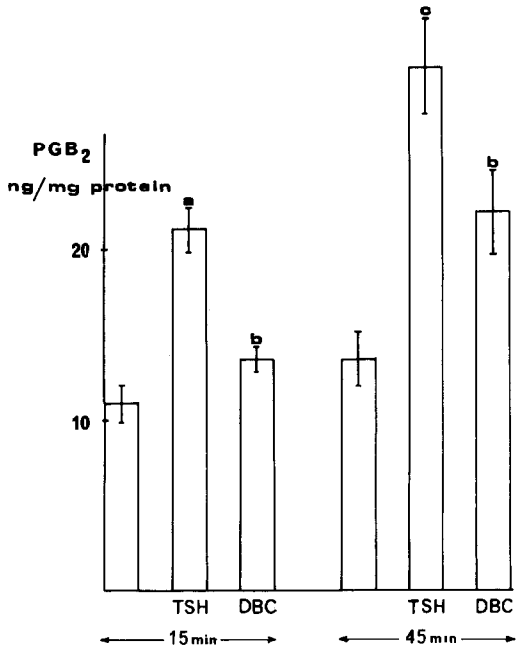


Fig. 1. Biosynthesis of prostaglandins in slices of pig thyroid. After a 30 min preincubation in Krebs–Ringer–Tris containing glucose, the slices (0.5 g) are incubated in Krebs–Ringer–Tris containing 2 mM glutathione, in the presence or absence of TSH 50 mU/ml and of DBC 50 μg/ml. At the end of the incubation, the slices are homogenized in the medium. After acidification at pH 3 with 2 M citric acid or 0.03 N HCl, the total lipids are extracted. A treatment with KOH–methanol [13] allows a spectrophotometric determination of prostaglandins as PGB_2 at 278 mμ. (a) $p < 0.01$; (b) $p < 0.05$; (c) $p < 0.001$.

As the doses used, for both effectors, allow maximal responses, it was possible that the effect of TSH is not entirely mediated by cAMP. We have therefore studied the effect of DBC and of cAMP on phospholipase A_2, whose stimulation by TSH liberated from phosphatidyl-inositol arachidonate which is a substrate of prostaglandin synthetase. For all the doses used, we obtained no significant response with cyclic nucleotides (table 1). Two hypotheses could then be proposed concerning the mode of action of cAMP in prostaglandins synthesis. The cAMP (or the DBC) could either activate the prostaglandin synthetase, and this hypothesis had been suggested by Burke [7] or could increase the concentration of free arachidonate from a precursor which is different from the phospholipids, for instance the triglycerides.

Table 1
Activity of phospholipase A_2 in a thyroid homogenate

	^{14}C-Arachidonic-acid released from ^{14}C-Phosphatidyl-inositol (cpm)	
Control	220 ∓ 40	
TSH 50 mU/ml	880 ∓ 35	(a)
DBC 50 μg/ml	205 ∓ 25	(b)
Theophylline 2 mM	230 ∓ 30	(b)
cAMP 50 μg/ml + theophylline 2 mM	225 ∓ 50	(b)

The reaction mixture (500 μl) contains the emulsified ^{14}C-phospholipid (phosphatidyl-inositol), Tris maleate buffer pH 7.4 20 mM, thyroid homogenate (900 μg of protein as measured by the method of Lowry [16], indomethacin (10 μg/ml) and the effectors TSH, DBC and cAMP. The values represent the radioactivity found as arachidonic acid after 30 min incubation. (a) $p < 0.01$; (b) N.S.: versus control.

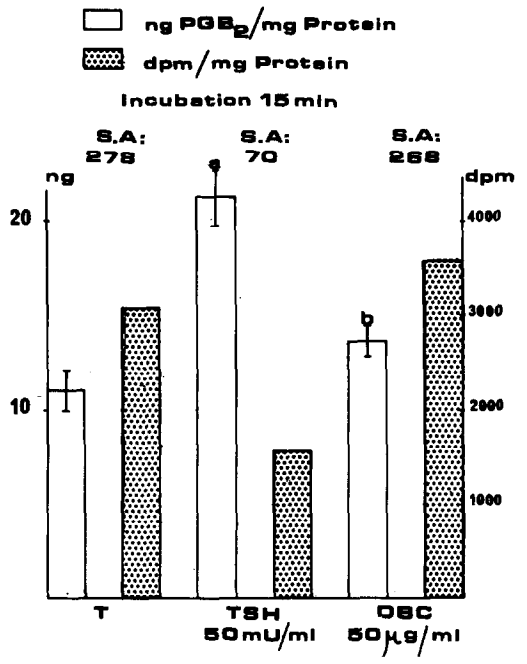


Fig. 2. Specific radioactivity of thyroid prostaglandins after incubation of slices in the presence of ^{14}C-arachidonic acid. The experimental procedure was given with fig. 1. Counting spot PGE_2 after thin-layer chromatography [12] allows the determination of specific activity (S.A.) in dpm/ng of PGB_2. (a) $p < 0.01$; (b) $p < 0.05$.

In order to test the first hypothesis, we have incubated for 15 min slices of pig thyroid with ^{14}C-arachidonate of high specific radioactivity (54 mCi/mM) in the presence of TSH (50 mU/ml) or of DBC (50 μg/ml). Prostaglandins E were purified, their concentration was determined as PGB, and their ^{14}C content was measured.

The results show that both effectors increase the prostaglandins' concentration, but TSH decreases the conversion of ^{14}C-arachidonate, whereas DBC increases this conversion (fig. 2). The specific radioactivity is four times lower after action of TSH, but is not really modified by DBC. This result is compatible, in the case of DBC, with an activation of the prostaglandin synthetase; in the case of TSH this effect, which must exist, is masked by the isotopic dilution of arachidonate following the stimulation of phospholipase A_2. This is confirmed by the effect of EDTA (20 mM) which inhibits phospholipase A_2. In the presence of EDTA, TSH and DBC increase the radioactivity present in the prostaglandins (100% in 30 min).

An action, direct or not, or cAMP on the activity of prostaglandin synthetase did not exclude however another action on the liberation of arachidonate from triglycerides.

In a preliminary work (unpublished results), we have shown that slices of pig thyroid take up labeled arachidonic acid and incorporate it into neutral lipids and phospholipids. After 45 min of incubation, 45% of the radioactivity is found within the slices and is distributed the following way: 60% as free arachidonic, acid, 23% incorporated into neutral lipids and 9% into phospholipids. The spot of triglycerides represents about 50% of the radioactivity found in neutral lipids.

We have then, using this technique, prepared triglycerides labeled with arachidonic acid (specific radioactivity: 0.25 Ci/mole of glycerol).

Triglycerides containing ^{14}C-fatty acids or ^{14}C-arachidonate are incubated in the presence of albumin with a pig thyroid homogenate; the lipolysis is measured (without albumin there is no lipolytic activity). It can be seen that DBC, and to a lesser

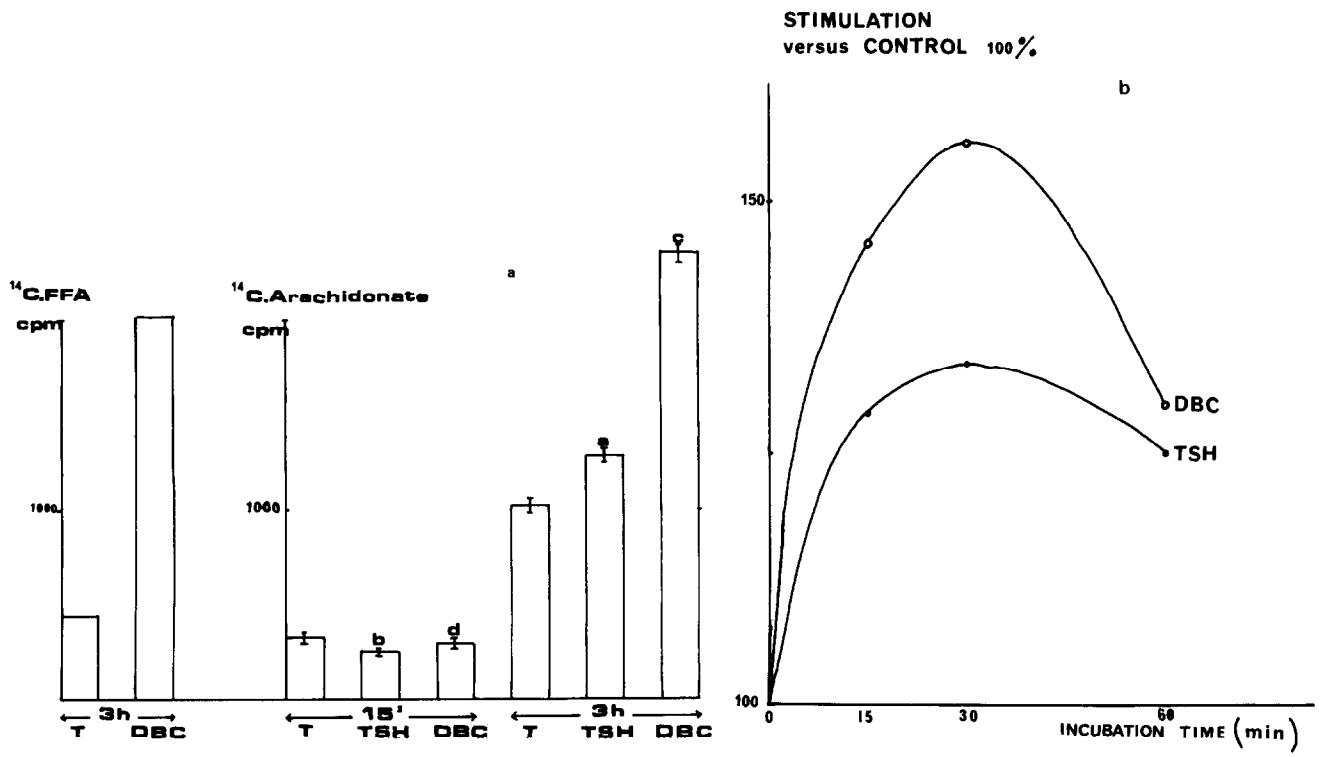


Fig. 3. Lipase radioactivity of a thyroid homogenate. (3a) Without ATP generating system: The incubation mixture (500 μl) contains the emulsified ^{14}C-triglycerides, phosphate buffer pH 7.4 50 mM with a final concentration of 3% serum albumin free of fatty acids; theophylline 2 mM, ATP 1 mM, $MgCl_2$ 10 mM, EDTA 1 mM, indomethacin 5 μg which is added to prevent transformation of the liberated arachidonic acid into prostaglandins, and the effectors TSH (50 mU/ml) and DBC (50 μg/ml). (a) $p < 0.01$; (b) $p < 0.05$; (c) $p < 0.001$; (d) N.S. Versus control. (3b) With an ATP generating system: In addition the incubation mixture contains an ATP generating system: phosphoenolpyruvic acid 8 mM and pyruvate kinase 4 I.U. •–•–• TSH 50 mU/ml; ○–○–○ DBC 50 μg/ml. All experiments significantly different from control $p < 0.01$.

extent TSH, have after 3 hr of incubation a lipolytic effect in the thyroid (fig. 3). This lipolysis can play a role in the biosynthesis of prostaglandins by cutting off from the triglycerides the arachidonate that they contain.

The experimental conditions that we have used to measure this lipolysis do not appear satisfactory however, as a significant effect is only obtained after 2 or 3 hr of incubation. The stimulation of the lipolysis in the adipose tissue is due to the activation of the lipase by phosphorylation under the action of cAMP dependent-protein kinase. Protein kinases have been described in the thyroid [17], so that such a scheme was not improbable. We have therefore repeated our incubations using a homogenate supplemented with an ATP-generating system.

It can be seen that the introduction of PEP (phosphoenolpyruvic acid) + pyruvate kinase stimulates lipolysis in the absence of TSH or DBC. However these effectors significantly increase the liberation of ^{14}C-arachidonate after 15 min (fig. 3b). We think that the increase of lipolysis over the background is due to protein kinases which are not cAMP dependent. This uncontrolled involvement in lipolysis action is perhaps without biological significance and is perhaps an artefact resulting from tissue homogenization.

4. Discussion

The increase in intracellular concentration of prostaglandins in the thyroid during stimulation by

TSH was first described by Burke et al. [18]. We have confirmed this result and shown that the increase in prostaglandins concentration is due to an increase of their synthesis [1]. But the action of TSH is complex and takes place through different mechanisms.

It seems, but this result is preliminary and is based on a determination of specific radioactivity, that TSH, via cAMP, stimulates the activity of prostaglandin synthetase.

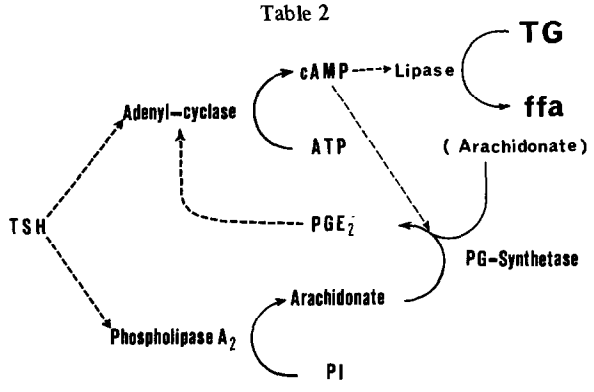
The other way, by which TSH stimulates prostaglandins synthesis, is the increase in the intracellular concentration of free arachidonate, a limiting substrate of prostaglandin synthetase.

The liberation of arachidonate occurs at the expense of two distinct pools of precursors, the phospholipids and particularly, phosphatidyl-inositol, and the triglycerides by two different and independent mechanisms.

From the phospholipids, arachidonate is liberated by action of a phospholipase A_2 stimulated by TSH through a process in which cAMP is not involved. The synthesis of prostaglandins (PGE_2) which follows is however not essential to adenyl-cyclase stimulation [1].

From the triglycerides, arachidonate is liberated by a c-AMP dependent lipase. By analogy with the reactions that have been described in the adipose tissue, the following sequence of reactions can be proposed : stimulation of adenyl-cyclase by TSH, the c-AMP generated activates a protein kinase which phosphorylates the lipase and causes its activation. The existence of c-AMP dependent-protein kinases in the thyroid, whose substrates have not yet been determined [15] is in favor of this hypothesis.

Table 2



The results we have just reported are not in opposition to those we have obtained earlier, but rather are complementing them and they are summarized in table 2.

The significance of the two pools of prostaglandins from the point of view of the regulation of the thyroid metabolism will be discussed elsewhere.

Acknowledgements

This work was supported by grants from the CNRS (ATP no. 401). We warmly thank Dr. J. E. Pike (Upjohn Company, Kalamazoo, Michigan) who provided us with the prostaglandins, the NIH (NIH-TSH B_6 2.54 U/mg) and Endopancrine Laboratories who provided us with the TSH.

References

[1] Haye, B., Champion, S. and Jacquemin, C. (1973) FEBS Letters 30, 253.
[2] Kuehl, Jr., F. A., Humes, J. L., Tarnoff, J., Cirillo, V. J. and Ham, E. A. (1970) Science 169, 883.
[3] Kuehl Jr., F. A., Humes, J. L., Mandel, L. R., Cirillo, V. J., Zanetti, M. E., and Ham, E. A. (1971) Biochem. Biophys. Res. Commun. 44, 1464.
[4] Burke, G. (1970) Am. J. Physiol. 218, 1445.
[5] Sato, S., Szabo, M., Kowalski, K. and Burke, G. (1972) Endocrinology 90, 343.
[6] Wolff, J. and Moore, W. V. (1973) Biochem. Biophys. Res. Commun. 51, 34.
[7] Burke, G. (1973) Prostaglandins 3, 291.
[8] Jacquemin, C. and Haye, B. (1970) Bull. Soc. Chim. Biol. 52, 153.
[9] Marinetti, G. V. (1962) J. Lipid Research. 3, 1.
[10] Shibuya, I., Honda, B. and Marvo, B. (1967) Agr. Biol. Chem. 31, 111.
[11] Mangold, H. K. (1961) J. Am. Oil. Chemists. Soc. 38, 708.
[12] Lands, W. E. M. and Samuelsson, B. (1968) Biochim. Biophys. Acta. 164, 430.
[13] Wallach, D. P. and Daniels, E. G. (1971) Biochim. Biophys. Acta. 231, 445.
[14] Dole, V. P. (1956) J. Clin. Invest. 35, 150.
[15] Lison, L. (1958) Statistique appliquée à la biologie expérimentale. Gauthier-Villars, Paris.
[16] Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[17] Rappaport, L., Leterrier, J. F. and Nunez, J. (1971) Biochimie 53, 721.
[18] Yu, S. C., Chang, L. and Burke, G. (1972) J. Clin. Invest. 51, 1038.