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BLOCKING OF DOG THYROID SECRETION *IN VITRO* BY INHIBITORS OF PROSTAGLANDIN SYNTHESIS*

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Abstract—Indomethacin, aspirin and paracetamol, three substances known to inhibit the synthesis of prostaglandins in various tissues, suppressed the TSH-induced release of butanol-extractable radioiodine by thyroid slices of dogs prelabelled *in vivo*. This parameter constitutes an index of the thyroid hormones secretion. The effect of indomethacin was dose-dependent and rapid in onset. The stimulatory effect of dibutyryl cyclic AMP (dB cAMP) on thyroid secretion was inhibited to the same degree as TSH effect. This inhibition was not caused by an energy deprivation, since lactate production and ATP concentrations were not altered. TSH-stimulated accumulation of cAMP was not modified by indomethacin. This suggests that some prostaglandin could play a role in thyroid hormone secretion at a step located after the generation of cAMP.

PGE_1 and other prostaglandins stimulate thyroid adenylate cyclase [1, 2], increase cAMP levels in thyroid gland [2, 3] and reproduce most of TSH effects on thyroid intermediary metabolism and specialized functions [2, 4, 5] in different animal species. On the other hand, TSH has been reported to enhance the synthesis [6] and the concentration [7] of various prostaglandins in the thyroid. However, the eventual role of prostaglandins in the thyroid remains to be defined. Burke has suggested that prostaglandins would mediate the stimulatory action of TSH on thyroid adenylate cyclase [8, 9] or conversely would be the messenger of cAMP in a negative feedback loop directed on thyroid adenylate cyclase [10], or would exert both effects simultaneously [10].

Jacquemin proposed a model in which TSH stimulates separately cAMP and prostaglandins synthesis [6] but cAMP and prostaglandins interact in a double positive feedback loop, cAMP enhancing prostaglandin synthesis and *vice versa* [11]. For Wolff, TSH could modulate cAMP metabolism by inducing a release of preformed membrane bound prostaglandins [13], rather than by increasing their synthesis [12].

The aim of the present work is to elucidate this controversial subject by studying the effect of various inhibitors of prostaglandin synthesis on several parameters of dog thyroid gland *in vitro* [14].

MATERIAL AND METHODS

Thyroid hormone secretion was measured as previously described [5]. Dogs were injected with 150 μCi ^{131}I and then given 300 mg thyroid extract daily, for 2 or 3 days. On the 4th day after ^{131}I injection, thyroid lobes were quickly removed, slices (0·25 mm thick) were prepared and incubated in Krebs–Ringer–phosphate buffer (pH 7·4) containing glucose (5·6 mM), $NaClO_4$ (1 mM) and methimazole (2 mM), at 37°, under air. Thyroid hormone secretion was estimated by the ratio between the butanol-extractable radioactivity in the medium at the end of the incubation and the total radioactivity of the slices before the incubation (% BEI). Lactate was assayed in the same incubation media by an enzymatic method [15].

Iodide incorporation into iodoproteins was measured as previously described [4]. Slices were incubated for 1 hr in Krebs–Ringer–phosphate buffer containing glucose (5·6 mM), KI (20 μM) and ^{131}I (0·5 μCi/ml), homogenized in a methimazole solution (10^{-3} M) and the proteins were precipitated with 5% trichloracetic acid (TCA). Results were expressed either as the ratio between TCA-precipitable and total radioactivities in the slices (cpm $PB^{131}I$/cpm ^{131}I) or as the ratio between TCA-precipitable radioactivity and the slice wet weight (cpm $PB^{131}I$/mg tissue).

Iodide uptake in the dog thyroid slices was measured by incubating them for 2 hr in a Krebs–Ringer–phosphate buffer containing glucose (5·6 mM), methimazole (2 mM), KI (2 μM) and ^{131}I (0·5 μCi/ml) and expressed as T/M where T and M are respectively the radioactivity per mg of tissue and the radioactivity per μl of medium.

For ATP and cAMP assay, slices were dropped in boiling water immediately after the test incubation, homogenized and centrifuged at 17,000 rev/min for 30 min. The supernatant was then assayed for ATP by the luciferine–luciferase method [16]. In other experiments, it was lyophilized, resolubilized in water and assayed for cAMP by the method of Gilman [17].

Paracetamol (4-acetamidophenol) and indomethacin were dissolved in dimethylsulfoxyde (DM SO) which was also added to the control flasks, at the final concentration of 1%. Aspirin was directly dissolved in the KRP medium, the pH of which was then adjusted to 7·4. In each type of experiment, slices were first preincubated with the tested agents (aspirin, paracetamol or indomethacin) or with DM SO alone

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for 1 hr and then transferred to fresh medium, containing the same concentrations of pharmacological agents for the test incubation. When used, TSH and dB cAMP were added at that moment. All incubations were carried out at 37°, under constant shaking (120 os./min). Results of representative experiments are expressed as means of triplicates ± standard deviation of the mean.

TSH (Thyrotropar®) was obtained from Armour, dB cAMP and the lactate assay kit from Boehringer-Mannheim. Aspirin was obtained from Merck–Darmstadt. Indomethacin and paracetamol were gifts from Merck, Sharp & Dohme and Union Chimique Belge respectively.

RESULTS

As shown in a preliminary report [14], TSH-induced secretion by dog thyroid slices, as measured by the release of butanol-extractable radioiodine [5], was inhibited by indomethacin (10 experiments). This inhibition was concentration-dependent, beginning at 1 μg/ml and was nearly complete at 50 μg/ml (Fig. 1). Graphical interpolation on the concentration–response curves gave an ID_{50}* of 20 μg/ml (56 μM) (mean of three experiments). This inhibitory effect was rapid in onset, being already detectable after a 40-min incubation (Fig. 2). It was more important when the slices were preincubated with the drug than without it (80 and 59 per cent respectively, mean of two experiments, 25 μg/ml indomethacin).

The release of non butanol-extractable radioiodine ($NBE^{131}I$), which is an index of follicle disruption [28], was not significantly increased by indomethacin: a 73 per cent inhibition of TSH-induced $BE^{131}I$ release and a 13 per cent increase of $NBE^{131}I$ release were observed in the presence of indomethacin, 25 μg/ml (mean of three experiments).

Indomethacin equally inhibited the secretory effect of dB cAMP and TSH (Table 1): 70 ± 5% inhibition for TSH (1 mU/ml, eight experiments) and 80 ± 5% inhibition for dB cAMP (100 or 200 μg/ml, four experiments) were observed in the presence of indomethacin, 25 μg/ml.

The inhibitory effect of indomethacin was mimicked by aspirin and paracetamol, both on TSH- and dB cAMP-induced thyroid secretion (Fig. 3). A

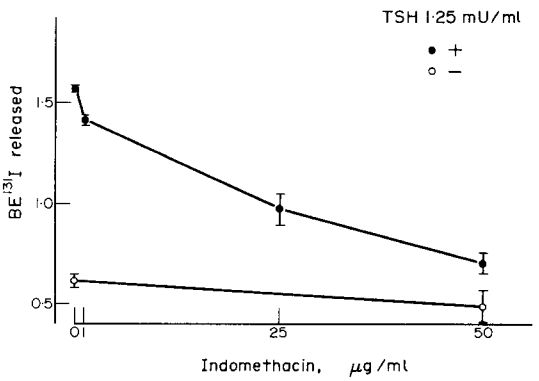

Fig. 1. Effect of various concentrations of indomethacin on basal and TSH-stimulated secretion by dog thyroid slices. Results are expressed as the percentage of the total ^{131}I radioactivity (slice + medium) present in the butanol extract of the incubation medium at the end of the test incubation. The drug was present during both the 1-hr preincubation and the 4-hr test incubation.

* ID_{50}: Drug concentration producing a 50 per cent inhibition.

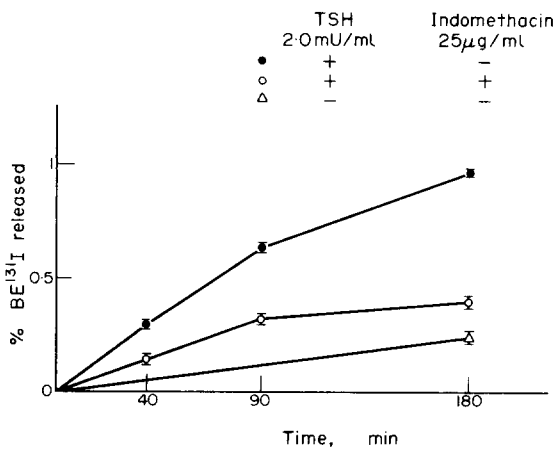

Fig. 2. Kinetics of the TSH-stimulated accumulation of butanol-extractable radioiodine in the slices incubation media, with and without indomethacin. The drug was present during the 1-hr preincubation and during the test incubation.

Table 1. Comparison of the effects of indomethacin on TSH and dB cAMP stimulations of thyroid secretion.

% $BE^{131}I$ released		Indomethacin 25 μg/ml
—	0·51 ± 0·06	0·50 ± 0·03
TSH 1·25 mU/ml	1·86 ± 0·07	0·97 ± 0·04
dB cAMP 100 μg/ml	1·44 ± 0·08	0·74 ± 0·10

The slices were first preincubated for 1 hr in the modified KRP medium containing indomethacin or DM SO 1% alone and then transferred to fresh media with the same composition, for a 4 hr-incubation, in the presence or absence of TSH and dB cAMP. Results are expressed as the percentage of the total ^{131}I radioactivity (slice + medium) present in the butanol extract of the incubation medium at the end of this incubation.

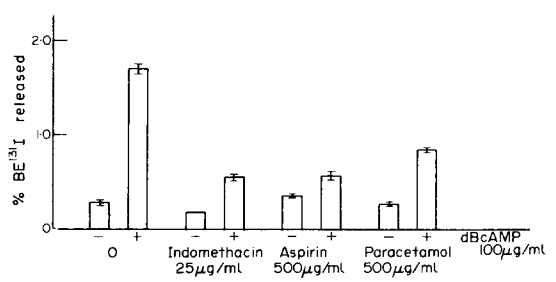

Fig. 3. Comparison between the effect of three prostaglandin synthesis inhibitors on dB cAMP-induced thyroid secretion. The drugs were dissolved in DM SO; control flasks also contained 1% DM SO, during both preincubation and test incubation. Results are expressed by the percentage of total ^{131}I radioactivity (slice + medium) present in the butanol extract of the incubation medium at the end of the test incubation (4 hr).

Table 2. Comparison of indomethacin effects on thyroid secretion and lactate production by dog thyroid slices

TSH 1·25 mU/ml	Indomethacin (μg/ml)	% BE^{131} I released	Lactate production (nM) mg tissue × 4 hr
–	0	0·62 ± 0·03	354 ± 71
+	0	1·57 ± 0·00	488 ± 42
+	25	0·98 ± 0·06	421 ± 9
+	50	0·71 ± 0·05	488 ± 41

The slices were first preincubated for 1 hr in the modified KRP medium containing indomethacin or DM SO 1% alone and then transferred to fresh media with the same composition, for a 4-hr incubation, in the presence or absence of TSH. Results are expressed as the percentage of the total ^{131}I radioactivity (slice + medium) present in the butanol extract of the incubation medium at the end of this incubation. Lactate was enzymatically assayed in the same medium.

similar inhibition of TSH effect was produced by 25 μg/ml (70 μM) indomethacin and 500 μg/ml (2·8 mM) aspirin (70 ± 5% and 81 ± 12% inhibition respectively). A significant inhibition was produced by aspirin concentrations as low as 20 μg/ml (26 per cent inhibition of the secretory effect of 1 mU/ml TSH, in one typical experiment).

Lactate production is enhanced by TSH [18]. This effect was not inhibited by indomethacin whereas thyroid secretion was suppressed (three experiments) (Table 2). None of the three tested drugs had a significant effect on the ATP content of control or TSH-stimulated thyroid slices (two experiments) (Table 3).

In the same range of concentrations which inhibit thyroid secretion, indomethacin did not inhibit or potentiate the stimulatory effect of TSH on cAMP accumulation in dog thyroid slices (three experiments). Paracetamol slightly potentiated and aspirin inhibited to some extent this action of TSH (two experiments) (Table 4).

Indomethacin decreased the iodide uptake as expressed by the *T/M* ratio. Concentrations of aspirin and paracetamol which produced an equivalent inhibition of thyroid secretion, had a similar but less important effect. As in other systems, TSH depressed the iodide *T/M* ratio [19] (Table 5).

Iodide incorporation into proteins was expressed by the TCA-precipitable ^{131}I radioactivity reported either to the total radioactivity of the slice or to the slice wet weight: in both cases similar results were obtained. Indomethacin had a slight stimulatory effect; on the contrary, aspirin and paracetamol were markedly inhibitory both in resting and stimulated slices (Fig. 4).

Table 3. Effect of three prostaglandin synthesis inhibitors on dog thyroid slices ATP concentrations

TSH (mU/ml)	ATP concn (μmoles/g tissue) 0	1·0
—	0·53 ± 0·05	0·60 ± 0·02
Indomethacin 25 μg/ml	0·56 ± 0·01	0·54 ± 0·03
Paracetamol 500 μg/ml	0·59 ± 0·03	0·58 ± 0·07
Aspirin 500 μg/ml	0·52 ± 0·02	0·51 ± 0·03

The slices were preincubated for 1 hr in KRP glucose medium in the presence of DM SO 1% with or without the drugs, then transferred to new incubation media of the same composition for an additional 30-min period.

Table 4. Comparison between the effects of three prostaglandin synthesis inhibitors on dog thyroid slices cAMP concentrations

TSH (mU/ml)	cAMP concn (pmoles/100 mg tissue) 0	1·0
—	21 ± 3	265 ± 14
Indomethacin 25 μg/ml	36 ± 0	231 ± 22
Paracetamol 500 μg/ml	35 ± 3	335 ± 20
Aspirin 500 μg/ml	29 ± 1	138 ± 25

The slices were preincubated for 1 hr in KRP glucose medium in the presence of DM SO 1% with or without the drugs, then transferred to new incubation media of the same composition for an additional 30-min period.

Table 5. Effects of three prostaglandin synthesis inhibitors and TSH on iodide uptake by dog thyroid slices, expressed by the iodide *T/M* (thyroid/medium) ratio

	T/M
—	19·4 ± 0·5
Indomethacin 25 μg/ml	7·1 ± 0·5
Aspirin 500 μg/ml	13·4 ± 0·9
Paracetamol 500 μg/ml	13·3 ± 0·9
TSH 12·5 mU/ml	7·7 ± 0·8

After a 1-hr preincubation in KRP glucose medium containing methimazole (2 mM), in the presence of the various drugs or of DM SO 1% alone, the slices were transferred to fresh incubation media with the same composition, but containing KI (2 μM) and ^{131}I (0·5 μCi/ml). TSH was then added to some flasks. This test incubation lasted 2 hr.

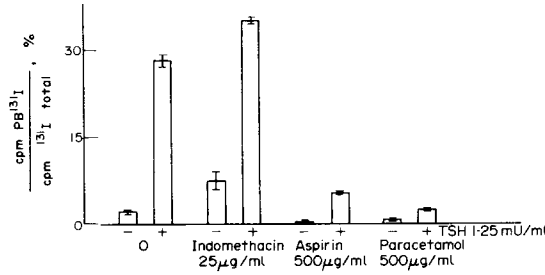

Fig. 4. Effect of three prostaglandin synthesis inhibitors on both basal and TSH-stimulated iodide incorporation in iodoproteins. Results are expressed as the ratio between TCA-precipitable and total ^{131}I radioactivities of the slices. The drugs were present during the 1-hr preincubation and the 1-hr test incubation, performed in the presence of 20 μM KI and 0·5 μc/ml ^{131}I.

Optic and transmission electron microscopic examination of dog thyroid slices treated with indomethacin and aspirin did not show any evidence of gross tissue damage.

DISCUSSION

Aspirin and indomethacin inhibit the prostaglandin synthetase activity of many tissues; paracetamol has the same effect in some of them [20, 21]. It has been shown that aspirin and indomethacin inhibit the thyroid synthesis of prostaglandins [6] and prevent the TSH-induced increase of thyroid cells prostaglandin concentration [22].

The TSH-induced dog thyroid secretion *in vitro* was inhibited by indomethacin, aspirin and paracetamol. The ID_{50} for indomethacin was 56 μM: this is a rather high concentration. Indeed, the indomethacin ID_{50} for prostaglandin synthetase, measured in acellular preparations, is about 0·5 μM for most tissues, but in some of them, it is as high as 38 μM [20]. As nothing is known about indomethacin transport and metabolism in the dog thyroid slices, it is impossible to determine the effective concentration of the drug. Indomethacin, aspirin and paracetamol are physicochemically unrelated drugs, sharing the common property of inhibiting prostaglandin synthetase, with the same order of potency in most tissues [20]: on a molar basis, indomethacin is far more potent than aspirin and paracetamol. The same order was observed in the case of thyroid secretion inhibition.

Since thyroid secretion is an energy requiring process, its inhibition by aspirin and indomethacin, which are both uncouplers of oxydative phosphorylation [20, 23], could be caused by a depletion of the cell ATP stores. This is unlikely since ATP levels were not modified by the drugs. Moreover, lactate formation, which contributes about 20 per cent to the ATP supply in the thyroid, but may play a special role in membrane processes such as hormone secretion [18], was not inhibited or enhanced by indomethacin.

Salicylates, but not indomethacin, have been reported to compete with cAMP for binding to protein kinases [24]. This effect has not been observed for thyroid protein kinases [25], so that there is little chance that it could explain the observed inhibition of thyroid secretion.

In conclusion, it is probable that the observed inhibition of TSH-induced thyroid secretion is caused by an inhibition of thyroid prostaglandin synthesis. This conclusion is supported by the fact that 7oxa-13 prostynoic acid, a prostaglandin competitive antagonist, also inhibits TSH- and dB cAMP-induced thyroid secretion*. Since dB cAMP and TSH stimulatory actions were inhibited to the same extent and as indomethacin did not inhibit TSH-promoted cAMP accumulation, the inhibition must bear on cAMP action rather than accumulation. It is therefore suggested that the cAMP-mediated stimulatory effect of TSH on dog thyroid secretion requires some prostaglandin. Wolff did not observe any inhibition of mouse thyroid secretion by indomethacin concentrations up to 400 μM [12]. Similar discrepancies between results obtained in thyroids of different animal species have already been reported [26].

No other consistent effect of the three prostaglandin synthesis inhibitors was observed. In particular, iodide incorporation in iodoproteins was depressed by aspirin and paracetamol, but not by indomethacin. Madaoui *et al.* [25] observed that TSH-stimulated iodide incorporation into proteins in rat thyroid was inhibited by indomethacin. However, in the absence of measurement of iodide trapping, it is not possible to know if in this case, the inhibition bore on iodide uptake or on iodide incorporation into proteins itself.

Indomethacin did not inhibit the cAMP accumulation produced by TSH in dog thyroid slices. This confirms results obtained by Haye *et al.* [6] and Mashiter *et al.* [27]. These data as well as the absence of inhibition of adenylate cyclase by indomethacin [12] and the difference in the patterns of adenylate cyclase activation by TSH and PGE_1 [1] do not support the hypothesis that TSH stimulatory effect on adenylate cyclase is mediated by an enhancement of thyroid prostaglandin synthesis. Nevertheless, the possibility is not excluded that TSH exerts this effect by releasing bound preformed prostaglandins.

In conclusion, we suggest that some prostaglandin plays a role in the process of dog thyroid secretion, which is not directly linked to cAMP metabolism. The identity of this prostaglandin and the step at which it acts remain to be defined. We do not exclude the possibility that prostaglandins could play other roles in the thyroid, particularly as local intercellular messengers. These points are currently investigated.

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