Stimulation by iodide of $\text{H}_2\text{O}_2$ generation in thyroid slices from several species

B. CORVILAIN, L. COLLYN, J. VAN SANDE, AND J. E. DUMONT

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Corvilain, B., L. Collyn, J. Van Sande, and J. E. Dumont. Stimulation by iodide of $\text{H}_2\text{O}_2$ generation in thyroid slices from several species. Am J Physiol Endocrinol Metab 278: E692–E699, 2000.—The regulation of thyroid metabolism by iodide involves numerous inhibitory effects. However, in unstimulated dog thyroid slices, a small inconstant stimulatory effect of iodide on $\text{H}_2\text{O}_2$ generation is observed. The only other stimulatory effect reported with iodide is on $[1-\text{I}^{14}\text{C}]$glucose oxidation, i.e., on the pentose phosphate pathway. Because we have recently demonstrated that the pentose phosphate pathway is controlled by $\text{H}_2\text{O}_2$ generation, we study here the effect of iodide on basal $\text{H}_2\text{O}_2$ generation in thyroid slices from several species. Our data show that in sheep, pig, bovine, and to a lesser extent dog thyroid, iodide had a stimulatory effect on $\text{H}_2\text{O}_2$ generation. In horse and human thyroid, an inconstant effect was observed. We demonstrate in dogs that the stimulatory effect of iodide is greater in thyroids deprived of iodide, raising the possibility that differences in thyroid iodide pool may account, at least in part, for the differences between the different species studied. This represents the first demonstration of an activation by iodide of a specialized thyroid function. In comparison with conditions in which an inhibitory effect of iodide on $\text{H}_2\text{O}_2$ generation is observed, the stimulating effect was observed for lower concentrations and for a shorter incubation time with iodide. Such a dual control of $\text{H}_2\text{O}_2$ generation by iodide has the physiological interest of promoting an efficient oxidation of iodide when the substrate is provided to a deficient gland and of avoiding excessive oxidation of iodide and thus synthesis of thyroid hormones when it is in excess. The activation of $\text{H}_2\text{O}_2$ generation may also explain the well described toxic effect of acute administration of iodide on iodine-depleted thyroids.

Wolff-Chaikoff effect; thyroid hormone synthesis; iodide toxicity; pentose phosphate pathway

THYROID HORMONE SYNTHESIS in the thyroid requires iodide, thyroglobulin, and an oxidation system to oxidize iodide and to iodinate tyrosyl groups in thyroglobulin and couple them into iodothyronines (13, 23). This oxidation system is constituted by a thyreroxidase that oxidizes iodide in the presence of $\text{H}_2\text{O}_2$ and an ill defined $\text{H}_2\text{O}_2$ generating system using NADPH as coenzyme.

The metabolism of iodide in the thyroid gland makes the most efficient use of an iodine supply that is often scarce and intermittent. But the thyroid also has adaptation mechanisms that reduce iodine metabolism when the supply is abundant, thus avoiding thyrotoxicosis. These include direct inhibitory effects of iodide in the thyroid itself, and inhibition by iodide of its own organification (Wolff-Chaikoff effect), its transport, thyroid hormone secretion, and formation in response to thyroid-stimulating hormone (TSH), and several other metabolic steps (29). We also previously observed an inhibitory effect of iodide on $\text{H}_2\text{O}_2$ generation in response to various agonists. Because, when iodide supply is sufficient, $\text{H}_2\text{O}_2$ generation is the limiting step for iodide organification, it was concluded that the Wolff-Chaikoff effect was caused by the inhibitory effect of iodide on $\text{H}_2\text{O}_2$ generation (11). However, in unstimulated dog thyroid slices, a small inconstant stimulatory effect of iodide on $\text{H}_2\text{O}_2$ generation was observed. Until then, the only stimulatory effect reported with iodide was on $[1-\text{I}^{14}\text{C}]$glucose oxidation in some species (sheep (14, 18), cattle (17), and to a lesser extent dogs (18, 25)). This effect was attributed to an increase in the NADP/ NADPH ratio, but its physiological significance was unknown (16).

The present study was initiated to determine whether the small iodide stimulatory effect on $\text{H}_2\text{O}_2$ generation previously observed in dog thyroid was also observed in other species. Because we have demonstrated recently in dog and human thyroid that the activity of the pentose phosphate pathway is controlled by the rate of NADPH oxidation, which itself depends on the rate of $\text{H}_2\text{O}_2$ generation (10, 12), we investigated mainly species in which a stimulatory effect of iodide on $[1-\text{I}^{14}\text{C}]$glucose oxidation had been demonstrated previously. We analyzed this effect for various iodide concentrations and various kinetics to compare those conditions with those in which the classical inhibitory effect on $\text{H}_2\text{O}_2$ generation is observed. Because it is well known that iodide depletion may influence thyroid response to iodide (6), we also studied in dog thyroids whether the size of the iodine pool, i.e., previous iodine supply, might influence the $\text{H}_2\text{O}_2$ response to iodide. The results suggest that iodide stimulates the generation of its cosubstrate $\text{H}_2\text{O}_2$, which limits its oxidation and thus its own metabolism.

MATERIALS AND METHODS

Products. Horseradish peroxidase type II, homovanillic acid, 12-O-tetradecanoylphorbol 13-acetate (TPA), and bovine

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TSH were purchased from Sigma Chemical (St. Louis, MO), carbamylcholine from K and K (Plainview, NY), ionomycin from Calbiochem-Berhing (La Jolla, CA), and forskolin (FSK) from Hoechst Pharmaceuticals (Bombay, India). All other reagents were of the purest grade commercially available.

Tissues. Human thyroid tissue was obtained from euthyroid patients undergoing lobectomies for resection of solitary cold nodules. Only the healthy normal-looking nonnodular tissue was used within 10–30 min of surgical resection.

Sheep, pig, bovine, and horse thyroid slices were obtained from freshly killed animals at a local slaughterhouse. Dog thyroids were obtained from dogs ≤ 10 kg otherwise used for cardiovascular experiments. Dogs with decreased iodide pool were obtained and treated for 6 wk with propylthiouracil (2 × 150 mg/day), strumazol (2 × 80 g/day), and NaClO₄ (2 × 1 g/day). To not interfere with iodide metabolism during the experiment, treatment with propylthiouracil and strumazol was stopped 48 h before and the treatment with NaClO₄ 24 h before the experiment. On the day of the experiment, dogs were anesthetized with pentobarbital, and the thyroid lobes were resected. Thyroids were cut into thin slices of ~50 mg wet weight (wet wt) with a Stadie-Riggs microtome.

H₂O₂ determinations. Slices were preincubated in Krebs-Ringer HEPES (KRH) medium supplemented with 8 mM glucose and 0.5 g/l of BSA and then transferred to fresh medium containing 0.1 mg/ml horseradish peroxidase type II, 440 µM homovanillic acid, and the tested agonists. The fluorescence of the medium was measured 90 min later except when stipulated otherwise (315 and 425 nm excitation and emission wavelengths, respectively) (2).

cAMP measurements. Slices were preincubated at 37°C for 1 h under an atmosphere of 95% O₂-5% CO₂ (v/v) in 2 ml of Krebs-Ringer bicarbonate (KRB) supplemented with 8 mM glucose and 0.5 g/l of BSA. For the test incubation of 1 h, medium was supplemented with 100 µM Ro 20-1724, a cAMP-specific phosphodiesterase inhibitor (22). cAMP was measured according to Brooker et al. (7).

Inositol phosphate measurements. Slices were preincubated for 4 h at 37°C in a medium similar to cAMP measurements but with 20 µCi/ml ³H-labeled inositol (specific activity 10–20 Ci/mmol, Amersham). The slices were then transferred to fresh unlabeled medium to which 10 mM lithium was added after 15 min. After an additional 5 min, the tested agents were added. The ³H-inositol phosphates were isolated by stepwise chromatography on a AG1-X8 resin (formate form, 100–200 µm mesh; Biorad, Watford, UK) Incorporation of ³H-inositol into the total phosphatidylinositol pool was quantitated after chloroform/methanol extraction of lipids from the pellet (3). Results are expressed as the percentage of radioactivity incorporated in inositol phosphates (IP₁ + IP₂ + IP₃) over the sum of radioactivity in inositol phosphates and phosphatidylinositol.

RESULTS

Effect of iodide on H₂O₂ generation in human, horse, dog, and dog thyroid slices. The initial experiments were performed on human thyroid slices and thyroid from three animal species. Various concentrations of iodide were added in the preincubation and incubation media for experiments performed on human, horse, and dog thyroid and only in the incubation medium for those performed on sheep thyroid. As shown in Table 1, the iodide effect on basal H₂O₂ generation depends on the species considered. In human and horse thyroid slices, iodide had only a small and inconstant stimulatory effect on H₂O₂ generation. In human thyroid slices, the basal production of H₂O₂ was very low and frequently below the detection limit. Only experiments in which the basal production of H₂O₂ was estimated to be reliable were taken into account (>20 ng H₂O₂ · 100 mg wet wt⁻¹ · 120 min⁻¹). Stimulation by iodide was significant in two experiments out of five with a maximal stimulation of 210% for an iodide concentration of 10⁻⁵ M. However, when all the experiments were pooled, the effect of iodide on H₂O₂ generation was not statistically significant whatever the concentration used. The same level of stimulation without statistically significant results was obtained in horse thyroid slices. In dog thyroid slices, iodide had a small but constant stimulatory effect on H₂O₂ generation. This effect was maximal for an iodide concentration of 10⁻³ M but remained

<table>
<thead>
<tr>
<th>Addition</th>
<th>Human</th>
<th>Horse</th>
<th>Dog</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>KI 10⁻⁶ M</td>
<td>132.7 ± 10.1 (5)</td>
<td>107.0 ± 5.1 (4)</td>
<td>114.2 ± 11.2 (5)</td>
<td>112.5 ± 10.2 (7)</td>
</tr>
<tr>
<td>KI 10⁻⁵ M</td>
<td>135.4 ± 19.2 (5)</td>
<td>130.1 ± 8.2 (5)</td>
<td>160.7 ± 19.1 (5)</td>
<td>185.7 ± 26.7 (8)</td>
</tr>
<tr>
<td>TSH 10 µM</td>
<td>110 ± 22.4 (5)</td>
<td>158.1 ± 11.8 (5)</td>
<td>ND</td>
<td>192.0 ± 30.5 (6)</td>
</tr>
<tr>
<td>KI 10⁻³ M</td>
<td>72.0 ± 18.4 (4)</td>
<td>119.7 ± 26.4 (5)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>KI 10⁻² M + MMI</td>
<td>89.5 ± 5.4 (4)</td>
<td>90.8 ± 4.1 (5)</td>
<td>90.0 ± 11.2 (7)</td>
<td>ND</td>
</tr>
<tr>
<td>TSH 10 µM/1l</td>
<td>718 ± 139.0 (5)</td>
<td>714.8 ± 118.2 (5)</td>
<td>356.4 ± 89.9 (8)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are means ± SE; number of experiments for each condition is given in parentheses. Length of incubation was 2 h for human, horse, and sheep and 1 h for dog thyroid slices. Basal values for H₂O₂ production for 100 mg wet wt (wwt) ranges were 21–61, 1040–2741, 261–731, and 43–1426 ng for human, horse, dog, and sheep thyroid slices, respectively. MMI, methimazole 10⁻⁴ M; ND, not done; 100, 100% of control value. *P < 0.05 vs. basal. Thyroid-stimulating hormone (TSH)-stimulated values are given only for comparison.
weak compared with that obtained with 10 mU/ml TSH. In sheep thyroid slices, an iodide concentration of 10^{-4} M nearly doubled H_2O_2 generation, which reached >50% of the value obtained with 10 mU/ml TSH. In all species, this stimulation was prevented by adding methimazole (10^{-4} M) to the preincubation and incubation media.

Effect of increasing time of preincubation with iodide on H_2O_2 generation. The data presented in Table 1 clearly show that the effect of iodide on H_2O_2 generation is dependent on its concentration and on the species studied. Because in human thyroid the basal level of H_2O_2 generation was too frequently below the detection limit, further experiments were performed on other species. The following set of experiments was performed to evaluate the kinetics of this effect in the three previous species and in two others (pig and bovine). The concentration of iodide for which the maximal stimulatory effect had been observed was used for those experiments (10^{-3} M for dog thyroid experiments and 10^{-4} M for other species). In all species but horse, preincubation with iodide increased its stimulatory effect on H_2O_2 generation. In horse thyroid slices, even a preincubation of 4 h did not increase its stimulatory effect (data not shown). In bovine thyroid slices, a preincubation of 4 h results in a quite undetectable basal level of H_2O_2 generation. The maximal measurable stimulation was therefore obtained after 2 h of preincubation and reached 444 ± 220% for an iodide concentration of 10^{-4} M (P < 0.05). In three species (dog, pig, and sheep) the kinetics of the iodide effect on H_2O_2 generation were analyzed in more detail (Fig. 1). In dog and pig thyroid slices, the maximal effect was nearly reached after 1 h of preincubation with iodide. Further increase of the length of preincubation resulted in only a minor additional increase of H_2O_2 generation. After 4 h of preincubation, the maximal effect reached 186.9 ± 18.3% of the control value in dog thyroid slices and 408 ± 58% of the control in pig thyroid slices. In sheep thyroid slices, the kinetic of preincubation with iodide showed a progressive increase of H_2O_2 generation with the time of preincubation. H_2O_2 generation after 4 h of preincubation reached 606 ± 76% of the control value. Compared with the stimulation obtained by TSH, the iodide stimulatory effect is weak in dog thyroids and of the same order of magnitude or even more potent in pig and sheep thyroid, respectively. As previously demonstrated, iodide inhibited H_2O_2 generation in dog TSH-stimulated slices (11), whereas in sheep and pig TSH-stimulated slices, iodide induced a further increase of H_2O_2 generation. Both effects were reversed in the presence of methimazole.

Effect of increasing iodide concentrations on H_2O_2 generation in dog and pig thyroid slices after preincubation of 4 h in presence of iodide. Because we had shown that increasing the time of preincubation may increase the stimulatory effect of iodide, we studied the effect of various concentrations of iodide after a preincubation of 4 h. Contrary to observations made with 1 h of preincubation, in dog thyroid, after 4 h of preincubation, an iodide concentration of 10^{-2} M nearly doubled H_2O_2 generation, which reached nearly 50% of the value obtained with 10 mU/ml TSH. In all species, this stimulation was prevented by adding methimazole (10^{-4} M) to the preincubation and incubation media.

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tion, H₂O₂ generation exhibited a biphasic curve in the presence of an increasing concentration of iodide (Fig. 2). As already shown in Fig. 1, a significant stimulatory effect of iodide could be obtained for an iodide concentration of 10⁻⁵ M. However, for a higher concentration of iodide, an inhibitory effect on H₂O₂ generation was observed that became statistically significant for an iodide concentration of 10⁻³ M. This effect was also reversed in the presence of methimazole. The situation was quite different in pig thyroid slices, where no inhibitory action of iodide after a 4-h preincubation was observed whatever the concentration of iodide used.

Effect of increasing time of preincubation with high concentration of iodide on H₂O₂ generation in dog thyroid slices.

In the presence of high concentrations of iodide (3 × 10⁻⁴ M), H₂O₂ generation exhibited a biphasic curve when plotted against the time of preincubation (Fig. 3). The production of H₂O₂ was maximal after 1 h of preincubation with iodide (136 ± 13% of the basal level) and then decreased to reach a maximal inhibition after 6 h (55 ± 10% of the basal level).

Effects of agents controlling either phosphatidylinositol P₂ or cAMP cascade on generation of H₂O₂ in sheep, pig, and dog thyroid slices. FSK was chosen to test the role of the cAMP cascade. Carbamylcholine was chosen to test the role of the whole phosphatidylinositol 4,5-biphosphate (PIP₂) cascade. Ionomycin, a divalent cation ionophore that allows extracellular Ca²⁺ to enter the cells, and TPA, a pharmacological probe for diaclylglycerol-regulated protein kinase C, were used to assess the separate effects of the activation of each branch of the PIP₂ cascade. In sheep thyroid slices, activation of the PIP₂ cascade by carbamylcholine, enhancement of Ca²⁺ intracellular levels by ionophore, and activation of protein kinase C by TPA all stimulated H₂O₂ generation. Conversely, the agents stimulating the cAMP cascade exerted an insignificant inhibition on H₂O₂ generation. TSH had a biphasic effect, inhibiting H₂O₂ generation at 1 mU/ml and increasing it at 10 mU/ml. Such biphasic effects previously observed in human thyroid slices corresponded to the stimulation of the cAMP cascade at the lower concentration of TSH and of the PIP₂-phospholipase C (PLC) cascade at the higher concentration, respectively (10). In pig thyroid, H₂O₂ generation was stimulated by low concentration of TSH (1 mU/ml), an insignificant stimulating effect was observed with FSK, and no effect was observed with carbamylcholine. In dog thyroid slices, as previously described (12), H₂O₂ generation was stimulated by both the cAMP cascade and the PIP₂-PLC cascade (Table 2).

Effect of iodide on PIP₂ cascade in sheep, pig, and dog thyroid slices. Because H₂O₂ generation in sheep and pig thyroid is controlled by the PIP₂ cascade, we studied here the effect of iodide on the [³H]inositol phosphate generation in those two species. Iodide (10⁻⁵ M) in the preincubation and incubation media did not modify [³H]inositol phosphate generation, whereas the effect of iodide on H₂O₂ generation in sheep and pig thyroid was clearly demonstrated. In the same set of experiments, 10 mU/ml TSH in pig and 10⁻⁵ M carbachol in sheep were moderate and potent stimulators, respectively, of the PIP₂ cascade. Even though both cascades positively control H₂O₂ generation in dog thyroid slices, we decided to test the effect of iodide only on the PIP₂
Table 2. Effects of agents controlling either PIP2 or cAMP cascade on generation of H2O2 in sheep, pig, and dog thyroid slices

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sheep</th>
<th>Pig</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 (14)</td>
<td>100 (5)</td>
<td>100 (10)</td>
</tr>
<tr>
<td>TSH (1 mU/ml)</td>
<td>85.8 ± 7.7 (9)</td>
<td>266.4 ± 71.7 (5)</td>
<td>367.9 ± 84.5 (3)</td>
</tr>
<tr>
<td>TSH (10 mU/ml)</td>
<td>189.7 ± 17.5 (9)</td>
<td>711.3 ± 108.9 (5)</td>
<td>670.9 ± 113.8 (5)</td>
</tr>
<tr>
<td>Cchol (10⁻⁵ M)</td>
<td>341.8 ± 58.9 (12)</td>
<td>116.4 ± 16.9 (5)</td>
<td>979.2 ± 354.7 (6)</td>
</tr>
<tr>
<td>FSK (10⁻³ M)</td>
<td>72.9 ± 9.6 (9)</td>
<td>131.0 ± 27.4 (5)</td>
<td>533.5 ± 90 (5)</td>
</tr>
<tr>
<td>Ionomycin (10⁻⁶ M)</td>
<td>608.0 ± 123.3 (9)</td>
<td>922.7 ± 182.0 (5)</td>
<td>772.9 ± 254.9 (5)</td>
</tr>
<tr>
<td>TPA (5 × 10⁻³ M)</td>
<td>238.4 ± 26.9 (9)</td>
<td>615.3 ± 54.7 (5)</td>
<td>570 ± 157.2 (5)</td>
</tr>
</tbody>
</table>

Results are means ± SE; number of experiments for each condition is given in parentheses. Ranges of basal values for H2O2 production for 100 mg wet wt were 381–1054 in sheep, 2700–2960 in pig, and 13349–21561 in dog thyroid slices, respectively. Ranges of basal values for inositol phosphate (IP) generation were 52–251 in sheep, 130–141 in pig, and 133–1148 in dog thyroid slices, respectively. H2O2 stimulated values are given only for comparison.

Table 3. Effects of iodide, TSH, and Cchol on inositol phosphate generation in sheep, pig, and dog thyroid slices

<table>
<thead>
<tr>
<th>Addition</th>
<th>Sheep</th>
<th>Pig</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>KI (10⁻⁴ M)</td>
<td>H2O2, %Control</td>
<td>313 ± 102</td>
<td>707 ± 508</td>
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<tr>
<td>TSH (10 mU/ml)</td>
<td>IP, %Control</td>
<td>119 ± 7</td>
<td>1158 ± 59</td>
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<tr>
<td>Cchol (10⁻³ M)</td>
<td>H2O2, %Control</td>
<td>425 ± 108</td>
<td>505 ± 185</td>
</tr>
<tr>
<td>TSH (10 mU/ml)</td>
<td>IP, %Control</td>
<td>104 ± 11</td>
<td>358 ± 43</td>
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<tr>
<td>Dog</td>
<td>H2O2, %Control</td>
<td>154 ± 25.5</td>
<td>800 ± 182</td>
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<tr>
<td>TSH (10 mU/ml)</td>
<td>IP, %Control</td>
<td>99 ± 4</td>
<td>948 ± 225</td>
</tr>
</tbody>
</table>

Results are means ± SE; n = 3 for sheep experiments and n = 2 for pig and dog experiments. *P < 0.05 vs. basal; †P < 0.01 vs. basal. Ranges of basal values for H2O2 generation (in ng/100 mg wet wt) were 52–251 in sheep, 130–141 in pig, and 133–1148 in dog thyroid slices, respectively. Ranges of basal values for inositol phosphate (IP) generation were (in cpm/100 mg wet wt) 384–1054 in sheep, 2700–2960 in pig, and 13349–21561 in dog thyroid slices, respectively. H2O2 stimulated values are given only for comparison.

DISCUSSION

Thyroid hormone synthesis is characterized by the requirement of a scarce substrate (iodide) and a toxic cofactor (H2O2). The thyroid cell generates large amounts of H2O2, which is toxic for any cell because of its transformation into various O2-derived free radicals (9, 19, 28). A process of apoptosis has also recently been described in vitro when the thyrocyte is exposed to H2O2 (24). The thyroid cell protects itself by regulating
its production of H$_2$O$_2$ according to what is required for thyroid hormone synthesis. It is able to increase its H$_2$O$_2$ production up to 10-fold in the presence of a high concentration of TSH or other agents that stimulate thyroid metabolism. H$_2$O$_2$ not used for thyroid hormone synthesis is destroyed in the thyroid, as in other cells, by several enzymes of the glutathione peroxidase family and by catalase (5). We had already demonstrated that large concentrations of iodide inhibited the H$_2$O$_2$ generation stimulated by various agonists, but until now, a possible action of iodide on basal H$_2$O$_2$ production had never been thoroughly investigated. All the effects of iodide on iodide metabolism described so far are inhibitory, apart from the effect on glucose metabolism. Actually, various data had already demonstrated a stimulatory effect of iodide on basal [1-14C]glucose oxidation in several species [strong in sheep and bovine thyroid (18, 14, 17), weaker in dog thyroid (18, 25)]. The present data establish that iodide can also stimulate H$_2$O$_2$ generation in several species (strong in sheep and bovine thyroid but weaker in human, dog, and horse thyroid). The stimulatory effect of iodide on H$_2$O$_2$ generation was prevented by methimazole, suggesting that it is mediated by an oxidative derivative of iodide. Apart from the fact that both require a functional iodide oxidation system, the stimulatory and inhibitory effects of iodide are clearly distinct; they are species dependent, observable in different experimental conditions, and have an opposite physiological meaning.

In some species (pig and sheep), the stimulatory effect of iodide predominates, and demonstration of the inhibitory effect requires special experimental procedures such as an increased incubation time. In those species, the strong iodide stimulation on H$_2$O$_2$ generation further increases the effect of TSH. Conversely, in other species (dog and human), the inhibitory effect predominates and the stimulatory effect of iodide is weaker. In these species, iodide inhibits the effect of TSH on H$_2$O$_2$ generation, as demonstrated here and previously (10, 12). This inhibition was attributed to the strong inhibitory effect of iodide on the activation of the cAMP cascade by TSH upstream and downstream of cAMP (10, 12). In species where both effects coexist, the inhibitory effect is observed earlier and for lower iodide concentrations. In dog thyroid, for a given preincubation time with iodide, iodide can stimulate or inhibit basal production of H$_2$O$_2$ according to its concentration. In the same experimental system, we had previously shown that high concentrations of iodide (>10$^{-4}$ M) are needed to demonstrate the Wolff-Chaikoff effect (11), the same as the concentration needed here to inhibit the basal production of H$_2$O$_2$ and a concentration 100 times higher than the concentration needed to observe a stimulatory effect. Finally, in dog and pig thyroid, the first stimulatory effect is followed much later by an inhibition. In dog thyroid, iodide can stimulate or inhibit basal production according to the length of preincubation with iodide. In pig thyroid slices, when the stimulatory effect of iodide is initiated, it persists after 5 h of washing in the absence of iodide. However, after 20 h of washing, an inhibitory effect can be observed, suggesting a late synthesis of an inhibitory compound. Because the inhibitory effect is partially relieved when the washing is done in the presence of methimazole, this suggests that the synthesis of this inhibitory compound requires a functional peroxidase.

The physiological role of the stimulatory and inhibitory effects is also different. Inhibition by excess iodide of H$_2$O$_2$ generation and, therefore, of iodide oxidation and thyroid hormone synthesis is a rapid mechanism to prevent excessive thyroid hormone secretion. It precedes the later classical negative feedback mechanism of excess thyroid hormones on TSH secretion and thyroid stimulation. On the other hand, activation of H$_2$O$_2$ generation at low concentrations of iodide, especially in iodide-deficient animals, will tie the generation of the toxic but necessary H$_2$O$_2$ with the availability of substrate and thus could represent a remarkable adaptation mechanism. Activation or induction of the enzymes necessary for the metabolism of a substrate by the substrate itself is a widespread strategy in bacterial and eukaryote metabolism.

To test this hypothesis we attempted in the current study to evaluate the extent to which iodide depletion or repletion may influence the H$_2$O$_2$ response of the gland to a small iodide load. Compared with control
dogs, the effect of iodide on H$_2$O$_2$ generation was strongly increased in thyroid with a decreased iodine pool but was absent in thyroid with an increased iodine pool. This suggests that thyroid iodide content is a major determinant of the H$_2$O$_2$ response of the gland to a small iodide load. However, we cannot exclude the possibility that other mechanisms may play a role, because in our model, iodine deprivation coexists with hypothyroidism and chronic stimulation by TSH.

We therefore addressed the question of the mechanisms involved in this action of iodide. As a first step, we defined, by using various agonists, which cascade is implicated in the control of H$_2$O$_2$ generation in sheep and pig thyroid. The present data establish that in sheep thyroid, as already observed in human (10), calf (20), and pig thyroid (4), H$_2$O$_2$ generation is activated only by the PI$_2$ cascade, whereas in these species the cAMP cascade has an unimportant (4) or even an inhibitory effect on H$_2$O$_2$ generation (10, 20). As already described, both cascades activate H$_2$O$_2$ generation in dog thyroid slices (12). We could not observe any effect of iodide on the PI$_2$ cascade in pig, sheep, and dog thyroid. It is unlikely, therefore, that the action of iodide on basal H$_2$O$_2$ generation is mediated by an activation of the PI$_2$ cascade. Iodide inhibited basal cAMP generation in sheep thyroid slices. Because cAMP exerts a small negative control on H$_2$O$_2$ generation in sheep thyroid, iodide may partly stimulate H$_2$O$_2$ generation by relieving this negative control. Even if this mechanism possibly plays a role in sheep thyroid, it cannot be involved in dog thyroid, in which the cAMP cascade positively controls H$_2$O$_2$ generation, or in pig thyroid, where cAMP cascade does not exert a negative control. It is possible that other untested metabolic pathways could be involved; therefore, the classical metabolic cascades are not involved in the stimulatory effect of iodide, contrary to what was observed previously for the inhibitory effect of iodide on H$_2$O$_2$ generation that obtains both at the level of intracellular signal generation and at the level of the H$_2$O$_2$ generating system (10, 12). This effect of iodide, like many others, is inhibited by methimazole, which suggests that the effect is not direct but is secondary to the generation of an oxidized form of iodine, previously called X-I (27).

Conversely, it is also possible that in vivo, the increase in H$_2$O$_2$ synthesis induced by iodide in iodine-depleted thyroid may have a toxic role in the cell. A necrosis of follicular cells was already described after administration of iodide to iodine-deficient dogs but not to control dogs (1). A necrotizing effect of iodide was also described in iodine-deficient rats and mice (8, 21). The toxicity of iodide was aggravated in cases of selenium deficiency, in a circumstance in which defenses against H$_2$O$_2$ are reduced due to a decreased activity of glutathione peroxidase (8). Our data are in keeping with the hypothesis that some of these toxic effects induced by iodide in iodine-deficient thyroids may be partly related to the toxicity of H$_2$O$_2$.

In conclusion, iodide stimulates H$_2$O$_2$ generation in several species. Our experiments suggest that in dog, bovine, and sheep thyroid, the previously observed iodide stimulatory effect on [1-14C]glucose oxidation actually results from an increased H$_2$O$_2$ generation through NADPH oxidation by the still unknown H$_2$O$_2$ generating system. As for [1-14C]glucose oxidation, great interspecies variation exists. The stimulatory effect was greater in sheep, pig, and bovine than in dog thyroids and inconstant in human and horse thyroids. This may be due to either genetic or environmental factors; however, because we demonstrated in dog thyroid that iodide pool greatly influences the H$_2$O$_2$ response to iodide, it seems quite possible that different diets may account, at least in part, for differences between species. Whatever the mechanism involved, the stimulatory action of iodide on H$_2$O$_2$ generation makes physiological sense. In the absence of iodide, there is no need to generate toxic H$_2$O$_2$. Conversely, in its presence, increased H$_2$O$_2$ generation stimulates a more efficient oxidation of iodide. When compared with the inhibitory actions of iodide, this effect is obtained earlier and for lower concentrations, suggesting a physiological role at least as important as that attributed to the classical inhibitory effects. All our results can be viewed teleologically as a means of gearing H$_2$O$_2$ generation to iodide supply. However, when the iodine thyroid pool is or becomes sufficient, as in dogs treated with lipiodol or after long preincubation time with iodide or for high concentration of iodide, the thyroid protects itself against excess iodine organification by decreasing H$_2$O$_2$ production, in keeping with the classical Wolff-Chaikoff effect.

We thank C. Maenhaut for organization of the in vivo treatments and C. Massart for excellent technical help.

This work was supported by the Fonds de la Recherche Scientifique Médicale, the Ministère de la Politique Scientifique (PAI) and the Fondation Tournaï-Solvay.

B. Corvilain is a fellow of the Erasmus Foundation (ULB, Brussels).

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Received 28 May 1999; accepted in final form 12 November 1999.

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