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The H_2O_2-Generating System Modulates Protein Iodination and the Activity of the Pentose Phosphate Pathway in Dog Thyroid*

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ABSTRACT. Iodide oxidation and binding to proteins require a thyroperoxidase and an ill defined H_2O_2-generating system. The $NADP^+$ supply and, thus, NADPH oxidation are the limiting steps of the pentose phosphate pathway. The purpose of this work was to test the hypothesis that H_2O_2 generation is a limiting step of iodination and NADPH oxidation and, therefore, of the pentose phosphate pathway. H_2O_2 produced by dog thyroid slices was measured with the homovanillic fluorescence assay. Our data show that H_2O_2 generation is stimulated by both the cAMP cascade [as activated by TSH, forskolin and $(Bu)_2cAMP$] and the Ca^{2+}-phosphatidylinositol cascade (as activated by carbamylcholine, ionomycin, and 12-*O*-tetradecanoylphorbol-13-acetate). We used several physiological and pharmacological agents that modulate iodide organification. In all cases there was a strict parallelism between effects on H_2O_2 generation, iodide binding to proteins, and pentose phosphate pathway activity. Moreover, in TSH- or carbamylcholine-stimulated slices, glucose or Ca^{2+} depletion, which greatly depressed H_2O_2 generation, also greatly decreased iodide organification and the activity of the pentose phosphate pathway. The glutathione peroxidase-catalyzed H_2O_2 reduction in the cytosol, which involves NADPH oxidation and, therefore, increases the NADP supply, also enhances the activity of the pentose phosphate pathway.

All of these data strongly support the hypothesis that H_2O_2 generation in dog thyroid controls iodination of proteins; through the NADPH oxidation resulting from H_2O_2 production and reduction, hydrogen peroxide also regulates the activity of the pentose phosphate pathway. (*Endocrinology* **128:** 779–785, 1991)

THE IODINATING system of the thyroid consists of a thyroperoxidase which oxidizes iodide in the presence of H_2O_2 and an ill defined H_2O_2-generating system using $NADPH_2$ as coenzyme (1, 2). Iodination can be much increased by exogenous H_2O_2 in thyroid slices and acellular systems (3, 4). On the other hand, TSH stimulates both iodination and H_2O_2 generation (5, 6). On this basis, it had been proposed that H_2O_2 generation is the limiting step in iodination in the thyroid cell (7). TSH also activates the pentose phosphate pathway in the thyroid. $NADP^+$ supply and, thus, NADPH oxidation limit the activity of this pathway (8). As the generation of H_2O_2 requires NADPH oxidation, it was also hypothesized that the stimulation of the pentose phosphate pathway might result from activation of the H_2O_2-generating system (7). The purpose of this work was to clarify the relationships among H_2O_2 generation, protein iodination, and glucose oxidation in dog thyroid slices.

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Materials and Methods

Products

Horseradish peroxidase type II, homovanillic acid, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), and TRH were purchased from Sigma Chemical Co. (St. Louis, MO), carbamylcholine (Cchol) from K and K (Plain View, NY), ionomycin (Iono) from Calbiochem-Behring (La Jolla, CA), forskolin (FSK) from Hoechst Pharmaceuticals (Bombay, India), bovine TSH (Thytropar) from Armour Pharmaceutical Co (Phoenix, AZ), catalase and $(Bu)_2cAMP$ from Boehringer Pharma (Mannheim, Germany), prostaglandin F_2 (PGF_2) from Upjohn Co. (Kalamazoo, MI), sodium fluoride from Merck (Darmstadt, Germany), and D-[1-^{14}C]glucose and D-[6-^{14}C]glucose from Amersham (Amersham, England). All other reagents were of the purest grade commercially available.

On the day of the experiment, dogs were anesthetized with pentobarbital, and the thyroid lobes were resected and sliced

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at room temperature with a Stadie-Riggs microtome (Arthur Thomas, Philadelphia, PA). Thyroid slices (30–50 mg) were weighed and then incubated at 37 C in 2 ml Krebs-HEPES buffer (KRH) or Krebs-Ringer bicarbonate (KRB), pH 7.4, supplemented with glucose 8×10^{-3} M and 0.5 g/L BSA. For KRB medium the gas phase was 95% O_2-5% CO_2; for KRH it was air.

H_2O_2 was estimated according to the method of Benard and Brault (6) based on the conversion of the nonfluorescent substrate homovanillic acid to a fluorescent derivative in the presence of H_2O_2 and horseradish peroxidase.

Except when indicated, thyroid slices were always preincubated for 1 h in KRH medium and then transferred to fresh medium containing horseradish peroxidase (type II; 0.1 mg/ml), homovanillic acid (0.44 mM), and various agonists. Slices were incubated for 90 min, and at the end of the incubation the medium was collected on ice. The fluorescence of the incubation medium was measured in a Perkin-Elmer LS3 fluorimeter (λ-excitation, 315 nM; λ-emission 425 nM). Fluorescence is stable for several hours at 4 C. The H_2O_2 concentrations measured represent the accumulation of H_2O_2 generated, which has diffused from the cells and reacted with homovanillic acid. They do not allow calculation of the real concentration of H_2O_2 at the iodination site.

Iodide organification was measured as described by Rodesch *et al.* (9). Briefly, slices were incubated for 45 min in KRB or KRH medium supplemented with KI (4×10^{-5} M) and ^{131}I (0.5 μCi/ml) and homogenized in a methimazole solution (2×10^{-3} M). The proteins were precipitated with 10% trichloroacetic acid and counted in a well-type Packard autogamma counter (Downers Grove, IL). Data were expressed as picomoles of iodide organified per 100 mg wet wt tissue/45 min. Glucose oxidation was determined by the method of Dumont (8). After preincubation, the slices were transferred to fresh medium containing 0.5 μCi/ml [1-^{14}C]glucose or [6-^{14}C]glucose and various agonists.

Inositol triphosphate (IP_3) was measured as described previously (10), and cAMP was determined as described by Van Sande and Dumont (11). The activity of the glutathione peroxidase was determined by the method of Ganther *et al.* (12).

Results are expressed as the mean ± SEM of at least three pooled experiments, and in each experiment incubation was performed in triplicate with three pooled thyroids. For statistical analysis the results of individual experiments were pooled and analyzed by analysis of variance, followed by Dunnet's or Newman-Keuls multiple comparison test (13). Two-sample comparisons were performed by Student's paired t test. To permit comparison where there was a large variation between experiments, the effects of the agonists were expressed as a percentage of the control values.

Results

Control dog thyroid slices generated H_2O_2 in the incubation medium. Figure 1 shows that this basal H_2O_2 production was linear for incubation times varying from 30 min to 3 h. Addition of catalase (32,500 U/ml) to the incubation medium reduced H_2O_2 by more than 80%

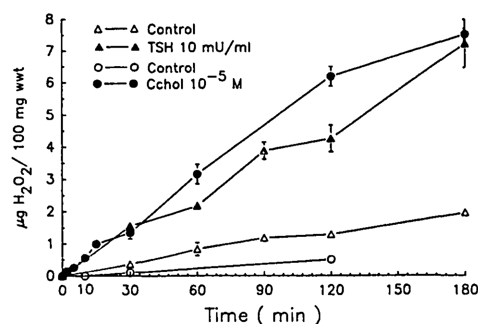

FIG. 1. Time course of H_2O_2 generation in control, TSH (10 mU/ml)-stimulated, and Cchol (10^{-5} M)-stimulated slices.

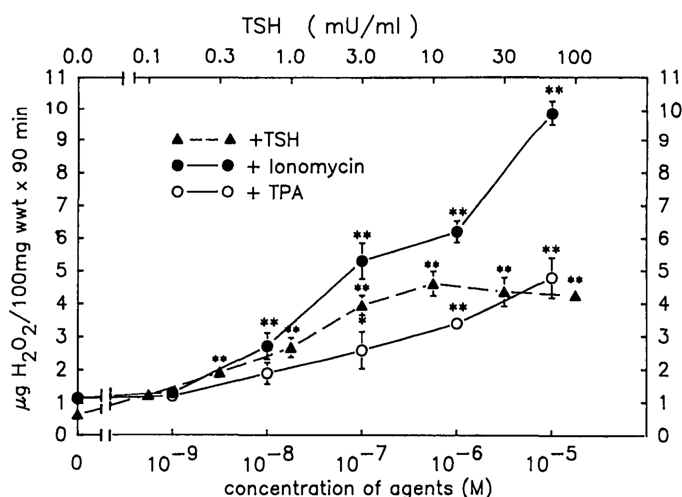

FIG. 2. Concentration-response curve of H_2O_2 production in TSH-, TPA-, and ionomycin (IONO)-stimulated slices (incubation time, 90 min). *, $P < 0.05$; **, $P < 0.01$ (compared to the control).

(from 0.37 ± 0.04 μg H_2O_2/100 mg wet wt·90 min to 0.055 ± 0.015 with catalase; $P < 0.002$, by Student's paired t test).

TSH stimulated H_2O_2 generation in the slices. This effect was linear with time up to 3 h (Fig. 1). It was also greatly reduced by the addition of catalase (32,500 U/ml) to the medium (from 6.05 ± 0.05 μg/100 mg wet wt 90 min to 1.17 ± 0.1 with catalase; $P < 0.0005$, by Student's paired t test). The effect of TSH was concentration dependent (Fig. 2); a significant effect was observed with 0.3 mU/ml, while the maximum was reached with 10 mU/ml TSH. Table 1 shows that this effect could be reproduced by FSK (10^{-5} M; an adenylate cyclase activator) and $(Bu)_2cAMP$ (5×10^{-4} M; a stable analog of cAMP).

Cchol, the stable analog of acetylcholine, stimulates the Ca^{2+} phosphatidylinositol cascade in dog thyroid slices (14). This agent also greatly enhanced H_2O_2 production (Table 1). This effect was linear for up to 3 h (Fig. 1) and was concentration dependent (Fig. 3). A significant effect was observed at 10^{-6} M. The effect of Cchol on iodide organification was strictly parallel with its effect on H_2O_2 generation. As with other effects of

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TABLE 1. Comparison of the effects of various agonists on H_2O_2 production, iodide organification, and [1-^{14}C]glucose oxidation in dog thyroid slices

Addition	H_2O_2 (% of control)	Iodide organification (% of control)	[1-^{14}C]Glucose oxidation (% of control)
None	100	100	100
TSH_1	497 ± 123 (4)	480 ± 75 (10)	335 ± 46 (5)
TSH_{10}	763 ± 79 (13)	859 ± 107 (7)	517 ± 33 (6)
$(Bu)_2cAMP$	605 ± 70 (6)	358 ± 62 (5)	483 ± 44 (3)
FSK	748 ± 137 (5)	429 ± 102 (7)	ND
Cchol	954 ± 98 (8)	686 ± 188 (7)	811 ± 111 (4)
Ionomycin	828 ± 147 (7)	641 ± 141 (5)	553 ± 64 (5)
TPA	562 ± 64 (8)	453 ± 106 (5)	629 ± 37 (3)

Values are the mean ± SEM; the number of experiments for each condition is given in parentheses. ND, Not done. Agonists were used at the following doses: TSH_1, TSH 1 mU/ml; TSH_{10}, TSH 10 mU/ml; $(Bu)_2cAMP$, 5×10^{-4} M; FSK, 10^{-5} M; Cchol, 10^{-5} M; ionomycin, 10^{-6} M; and TPA, 5×10^{-6} M. The stimulatory effect of all the agonists used in Table 1 was significant ($P < 0.05$).

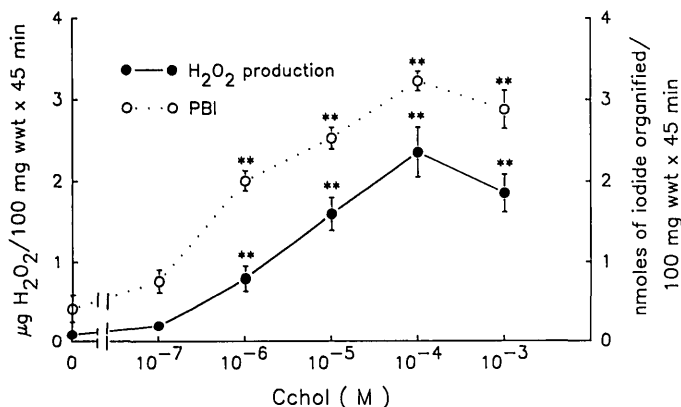

FIG. 3. Concentration-response curve of H_2O_2 generation and iodide organification in Cchol-stimulated slices incubated for 45 min in KRB medium. **, $P < 0.01$ compared to the control.

the drug, this could be mimicked by ionomycin (10^{-6} M), a divalent cation ionophore. It was also mimicked by TPA (5×10^{-6} M), a pharmacological probe for diacylglycerol-regulated protein kinase-C. Figure 2 shows that the effects of ionomycin and TPA are concentration dependent.

If NADPH supply is necesssary for H_2O_2 generation, a decrease in H_2O_2 generation would be expected under conditions of reduced activity of the main NADPH-generating pathway, the pentose phosphate pathway. Increasingly severe glucose depletion conditions were obtained either by glucose omission in KRH or KRB incubation medium or by glucose omission in KRH or KRB preincubation and incubation media. Figure 4 shows that H_2O_2 production stimulated by either TSH (10 mU/ml) or the cAMP enhancers [10^{-5} M FSK and 5×10^{-4} M $(Bu)_2cAMP$] decreased with glucose depletion. Similar results were obtained when H_2O_2 generation was

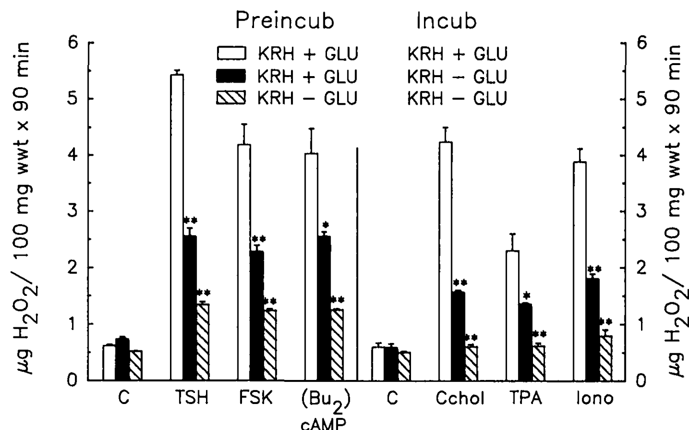

FIG. 4. Effect of glucose omission on H_2O_2 generation in control slices and slices stimulated by TSH (10 mU/ml), FSK (10^{-5} M), $(Bu)_2cAMP$ (5×10^{-4} M), Cchol (10^{-5} M), TPA (5×10^{-6} M), and ionomycin (10^{-6} M). After 60-min preincubation, the slices were incubated for 90 min in KRH medium. KRH + GLU, 8×10^{-3} M glucose in the medium; KRH-GLU, no glucose in the medium. *, $P < 0.05$; **, $P < 0.01$ [compared to the respective controls (C; 8×10^{-3} M glucose in the preincubation and incubation media)].

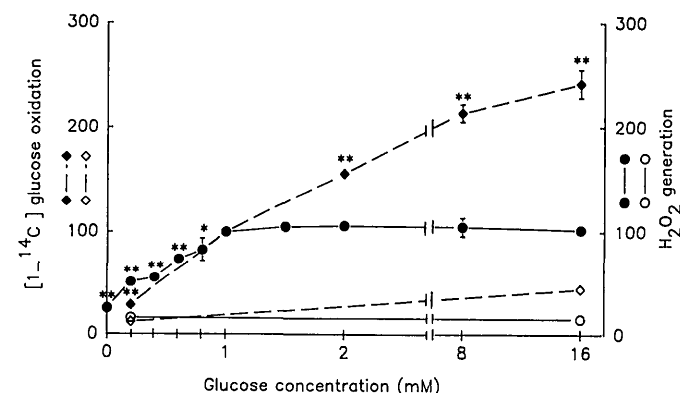

FIG. 5. Effect of increasing medium glucose concentration on H_2O_2 generation and [1-^{14}C]glucose oxidation in control (○ and ◇) and TSH (● and ◆; 10 mU/ml)-stimulated slices incubated for 60 min in KRH medium after 60-min preincubation. *, $P < 0.05$; **, $P < 0.01$ (compared to the respective 100% value at 1 mM glucose with 10 mU/ml TSH).

stimulated by Cchol (10^{-5} M) or probes of the Ca^{2+} phosphatidylinositol cascade [10^{-6} M ionomycin or 5×10^{-6} M TPA]. As, under our conditions of incubation, [1-^{14}C]glucose oxidation is more than 10 times higher than [6-^{14}C]glucose oxidation (not shown), the 1-$^{14}CO_2$ released from [1-^{14}C]glucose may be considered a good estimation of the activity of the pentose phosphate pathway (15). The half-maximum effect of glucose on TSH (10 mU/ml)-induced H_2O_2 generation was reached at a concentration of 5×10^{-4} M. H_2O_2 generation plateaued above 1×10^{-3} M (Fig. 5), while increasing the glucose concentration from 1×10^{-3} to 1.6×10^{-2} M still doubled the activity of the pentose phosphate pathway (Fig. 5). Glucose deprivation in the preincubation and incubation media decreased in parallel H_2O_2 generation and iodide organification stimulated by TSH (10 mU/ml) or Cchol

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(10^{-5} M) (Fig. 6). The absence of glucose had no significant effect on iodide transport (calculated as the ratio of ^{131}I in 100 mg methimazole-treated slices to that in 100 μl medium; not shown) and on the intracellular signals generated in response to TSH (10 mU/ml) and Cchol (10^{-5} M). TSH (10 mU/ml)-induced cAMP accumulation was 1,780 ± 80 pmol cAMP/100 mg wet wt in the presence and 1,480 ± 125 in the absence of glucose [$P > 0.1$ (NS), by Student's paired t test], and Cchol-induced IP_3 generation was 43,000 ± 17,100 cpm IP_3/100 mg wet wt in the presence and 57,600 ± 19,200 in the absence of glucose in the medium [$P > 0.1$ (NS), by Student's paired t test].

Increasing Ca^{2+} depletion conditions were obtained either by simple Ca^{2+} omission in KRH preincubation and incubation media or by addition of EGTA (10^{-4} M) in the Ca^{2+}-free preincubation medium, followed by incubation in the absence of Ca^{2+}. Figure 7 shows that H_2O_2 (basal or stimulated by 10 mU/ml TSH) decreased with progressive Ca^{2+} depletion. Mn^{2+}, a competitor of Ca^{2+} (16), stimulated H_2O_2 production, especially in Ca^{2+}-depleted slices. Similar results were obtained for iodide organification.

H_2O_2 and iodide binding to proteins was measured at varying pH. Figure 8 shows that for extracellular pH varying between 6.8–8.0, H_2O_2 production and iodide organification increased in parallel with alkalinization of the medium.

Iodide inhibits H_2O_2 generation stimulated by 10 mU/ml TSH (17). We show here that this effect was maximal at 10^{-3} M (Fig. 9). This effect was prevented by adding methimazole (10^{-4} M) to the incubation medium.

NaF, PGF_2, and TRH stimulate protein iodination and oxidation of [1-^{14}C]glucose (18–20). In a typical experiment these agents also stimulated H_2O_2 generation from

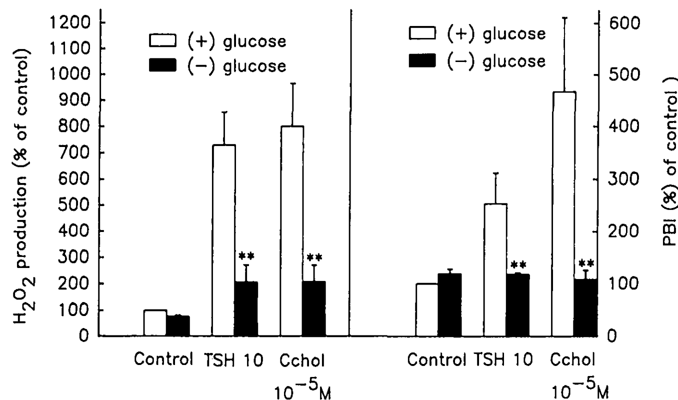

FIG. 6. Effect of glucose omission on H_2O_2 generation and iodide organification in control slices and slices stimulated by 10 mU/ml TSH or 10^{-5} M Cchol. After 60-min preincubation, slices were incubated for 45 min in KRH medium. (+) glucose, 8×10^{-3} M glucose in the preincubation and incubation media; (-) glucose, no glucose in the preincubation and incubation media. **, $P < 0.01$ compared to the respective control with glucose.

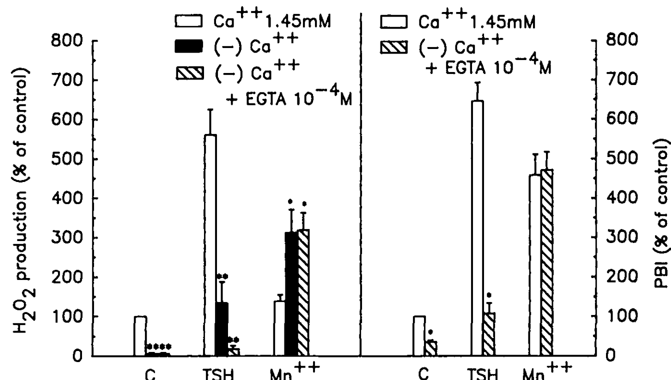

FIG. 7. Effect of Ca^{2+} depletion on H_2O_2 generation and iodide organification in control slices, slices stimulated by 10 mU/ml TSH, and slices incubated in the presence of 1.45×10^{-3} M Mn^{2+}. Slices were preincubated for 60 min in KRH medium with 1.45×10^{-3} M Ca^{2+} (□), without Ca^{2+} (■), or without Ca^{2+} in the presence of 10^{-4} M EGTA (▨). Slices were incubated for 45 min in KRH medium with (□) or without (■ and ▨) Ca^{2+}. *, $P < 0.05$; **, $P < 0.01$ [compared to the respective control (C) with Ca^{2+}].

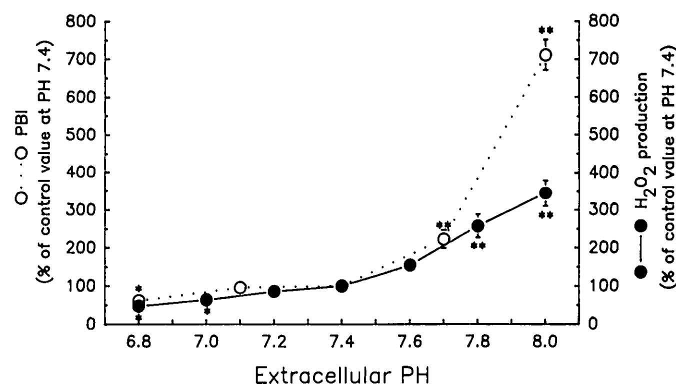

FIG. 8. Effect of extracellular pH on H_2O_2 generation and iodide organification. For H_2O_2 measurement, slices were incubated for 90 min in KRH medium. For PBI measurement, slices were incubated for 45 min in KRH medium supplemented with $NaHCO_3$ (10^{-2} M). *, $P < 0.05$; **, $P < 0.01$ (compared to the respective control at pH 7.4).

0.27 ± 0.02 μg/100 mg wet wt·90 min for control slices to 2.08 ± 0.37 in the presence of NaF (10^{-5} M), 0.61 ± 0.05 in the presence of PGF_2 (10 μg/ml), and 0.56 ± 0.06 in the presence of TRH (5×10^{-6} M).

As in other tissues, H_2O_2 is reduced by glutathione (GSH) peroxidase in the thyroid. For each H_2O_2 reduced, one NADPH is oxidized to $NADP^+$ (21). H_2O_2 addition to thyroid slices should increases $NADP^+$ generation and, thus, the activity of the pentose phosphate pathway. Indeed, H_2O_2 addition stimulates [1-^{14}C]glucose oxidation (Table 2). In dog thyroid homogenate the mean GSH peroxidase activity in three pooled experiments was 4.2 pmol NADPH oxidized/h·100 mg wet wt. The quantitative estimates of the various metabolic fluxes derived from the experimental data are summarized in Table 3.

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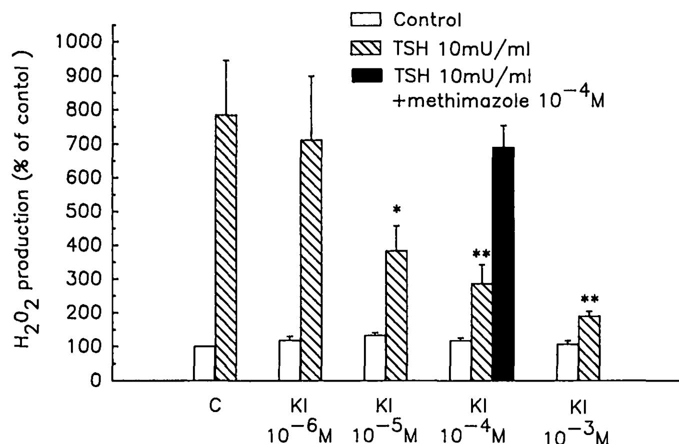

FIG. 9. Effect of increasing concentration of iodide in the medium on H_2O_2 generation in control slices and TSH (10 mU/ml)-stimulated slices. After 60-min preincubation, the slices were incubated for 90 min in the presence of increasing concentrations of iodide with or without methimazole (10^{-4} M). *, $P < 0.05$; **, $P < 0.01$ [compared to the respective control (C) without iodide].

TABLE 2. Effect of increasing H_2O_2 concentration on oxidation of [1-^{14}C]glucose

Addition	[1-^{14}C]Glucose oxidation (% of control)
None	100
TSH (10 mU/ml)	462 ± 13[a]
H_2O_2 (0.02 mM)	114 ± 10
H_2O_2 (0.2 mM)	224 ± 19[a]
H_2O_2 (2 mM)	364 ± 28[a]

Values are the mean ± SEM. After 60-min preincubation, slices were incubated for 45 min in KRH medium. Hydrogen peroxide (0.02, 0.2, or 2 mM) was added during the 45-min incubation period once immediately before transferring the slices and after 15 and 30 min of incubation.

[a] $P < 0.01$ compared to the control.

Discussion

The purpose of this work was to investigate whether the H_2O_2-generating system is a limiting step in the control of protein iodination and the pentose phosphate pathway in the thyroid (Fig. 10). Iodide oxidation, protein iodination, and thyroid hormone synthesis in the thyroid are catalyzed by a peroxidase coupled to an H_2O_2-generating system which provides it with its substrate (2, 22). The H_2O_2-generating system uses NADPH as a coenzyme (1, 23). In acellular systems H_2O_2 is limiting for iodination (2). The following facts suggest that this also applies to intact follicles. 1) Exogenous supply of H_2O_2 greatly enhances iodination in thyroid slices (3, 24). 2) TSH, which enhances iodination in dog thyroid, also activates H_2O_2 generation in this system (5).

The present results establish by pharmacological means that iodination of proteins in dog thyroid slices is controlled by H_2O_2 generation. Qualitatively, all conditions or agents that enhanced protein iodination also

TABLE 3. Comparison between H_2O_2 produced, iodide organified, and [1-^{14}C]glucose oxidation in dog thyroid slices

Units	Control	TSH (10 mU/ml)
nmol H_2O_2 produced/100 mg wet wt·h	27	189
nmol NADPH oxidized by the H_2O_2-generating system/100 mg wet wt·h	27	189
nmol iodide organified/100 mg wet wt·h	0.3	2.3
nmol glucose oxidized through the pentose phosphate pathway/100 mg wet wt·h	48	240
nmol $NADP^+$ reduced by the pentose phosphate pathway/100 mg wet wt·h	288	1440

Maximal activity of GSH peroxidase: 4200 nmol $NADPH_2$ oxidized/100 mg wet wt·h or 4200 nmol H_2O_2 reduced/100 mg wet wt·h. The results for H_2O_2 generation, iodide organification, and pentose phosphate pathway activity were expressed as the mean of three typical experiments. To allow the various estimations, the following assertions were made: 1 nmol H_2O_2 = 34 ng H_2O_2, the stoechiometry between H_2O_2 generation and NADPH oxidation is 1 (31), and 6 nmol $NADP^+$ are reduced for each 1 nmol glucose oxidized through the pentose phosphate pathway (7). The quantitative activity of the pentose phosphate pathway was estimated as described previously (29).

activated H_2O_2 generation in this unique experimental model [alkalinization of the medium or TSH, $(Bu)_2cAMP$, FSK, Cchol, ionomycin, TPA, and PGF_2, NaF, TRH]. Conversely, several conditions or agents that depressed H_2O_2 generation, *i.e.* iodide excess (Wolf-Chaikoff effect) (17), glucose depletion, Ca^{2+} depletion, and acidification of the incubation medium, were also associated with a large decrease in protein iodination. The inhibitory effect of excess iodide on H_2O_2 generation was relieved by methimazole, which suggests that the effect of iodide on this metabolism, as in other regulatory pathways, is due to an organified derivative (11, 25). Quantitatively, the concentration-effect curves for all of the agents studied on iodide organification and H_2O_2 generation were parallel. Similar results for some of these conditions had been obtained previously (26–28). Thus, in thyroid cells, protein iodination is enhanced by exogenous H_2O_2. H_2O_2 generation and protein iodination are controlled qualitatively and quantitatively in parallel. These facts strongly support the hypothesis that H_2O_2 generation is the controlling step in iodination (Fig. 10).

The pentose phosphate pathway is limited by $NADP^+$ supply, *i.e.* by NADPH oxidation in the thyroid, as in other tissues (7, 8). Any factor increasing NADPH oxidation enhances the activity of this pathway (7). Moreover, all factors, including TSH, that increase the $NADP^+$/NADPH ratio in dog thyroid cells (7) also enhance the activity of the pentose phosphate pathway. As NADPH is the coenzyme used by the H_2O_2-generating

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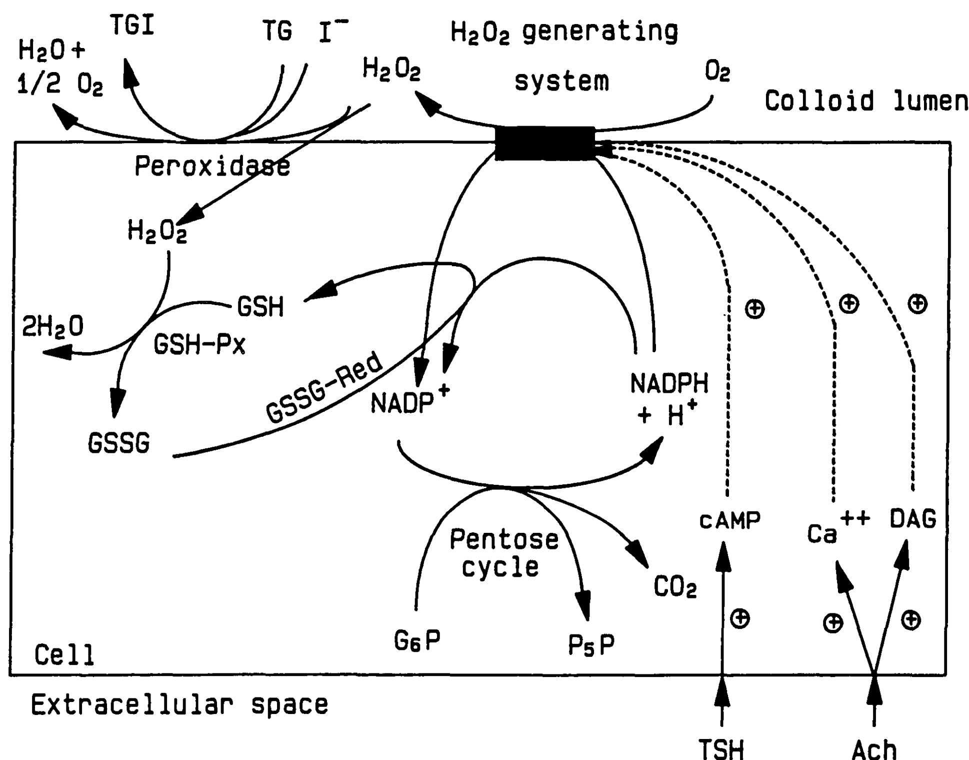

FIG. 10. Proposed scheme on the control of H_2O_2 generation, iodide organification, and pentose phosphate pathway activity in dog thyroid. *Dotted arrows*, Positive controls; GSH Px, GSH peroxidase; G6P, glucose-6-phosphate; P5P, pentose-5-phosphate; DAG, diacylglycerol; GSSG-Red, GSH reductase; Ach, acetylcholine; TG, thyroglobulin.

system, activation of this system should induce NADPH oxidation and activation of the pentose phosphate pathway. In this work we show that, indeed, a variety of physiological and pharmacological agents that activate H_2O_2 generation and, thus, protein iodination also stimulate the activity of the pentose phosphate pathway (see Table 1).

Obviously other processes are involved in NADPH oxidation and activation of the pentose phosphate pathway, such as the reduction in the cell of H_2O_2 spilling in the cytosol by GSH peroxidase (Fig. 10). H_2O_2 indeed stimulated the activity of the pentose phosphate pathway (Table 2). The maximal activity of the GSH peroxidase was sufficient to account for the reduction of great amounts of H_2O_2 (see Table 3). Thus, both H_2O_2 generation and the reduction of H_2O_2 spilling over into the cell drive the activity of the pentose phosphate pathway (Fig. 10).

The requirement of pentose phosphate pathway activity for H_2O_2 generation and protein iodination is demonstrated by the fact that in the absence of glucose in the medium, *i.e.* under conditions where the activity of the pentose phosphate is minimal (29), these two metabolic functions were almost abolished whatever the stimulus used. Indeed, whether stimulated by the cAMP pathway [by TSH, $(Bu)_2$cAMP, or FSK] or the Ca^{2+} phosphatidylinositol cascade (by Cchol, ionomycin, or TPA), H_2O_2 generation and protein iodination were greatly decreased in the absence of glucose. A similar requirement for glucose in protein iodination induced by TSH had been shown in dog thyroid slices (30). Moreover, there is a semiquantitative parallelism between the intensity of glucose depletion (length of incubation in the absence of glucose) and the depression of H_2O_2 generation.

Thus, glucose supply may become limiting for both protein iodination and H_2O_2 generation. Nevertheless, in physiological concentrations of glucose, NADPH generation, *i.e.* the activity of the pentose phosphate pathway, exceeds the requirement of H_2O_2 generation. Above 1×10^{-3} M glucose, TSH-induced H_2O_2 generation plateaus, while the activity of the pentose phosphate pathway and, thus, NADPH generation can still be multiplied by a factor of 2.5. Pushing further the activity of the pathway and $NADPH_2$ generation does not increase H_2O_2 generation and iodination. The intracellular level of NADPH estimated at 9×10^{-5} M (7) is indeed greater than the apparent K_m of the H_2O_2-generating system for NADPH (3×10^{-5} M) (31). Quantitative comparison between the data is also consistent with the model, in that NADPH generation is largely sufficient to provide and reduce the H_2O_2 generated, and H_2O_2 generation is in excess with regard to iodination (Table 3).

NADPH generation exceeds the requirement for H_2O_2 formation and is, therefore, not limiting. However, the consumption of NADPH by the H_2O_2-generating system represents a significant part of the $NADP^+$ needed for the pentose phosphate pathway activity. This part is still underestimated, as the fraction of the H_2O_2 degraded in the peroxidase reaction or by H_2O_2 detoxifying systems (catalase, GSH peroxidase) in the cell is not measured. Moreover, reduction of H_2O_2 by GSH peroxidase also

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contributes to NADPH oxidation (Fig. 10). In this regard it should be noted that maximal activity of GSH peroxidase would be largely sufficient to reduce all of the H_2O_2 produced. As for any enzymatic reaction, the substrate (here H_2O_2) is produced in large excess for protein iodination (Table 3), but as the apparent K_m of thyroperoxidase for H_2O_2 is high ($\sim 3 \times 10^{-4}$ M) (32), H_2O_2 does not saturate the enzyme and is, therefore, limiting. As demonstrated in this work in tissue preparations or homogenates (33), when iodide and thyroglobulin concentrations are kept constant, H_2O_2 regulates the activity of thyroperoxidase, *i.e.* iodination.

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