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INHIBITION BY IODIDE OF IODIDE BINDING TO PROTEINS : THE "WOLFF-CHAIKOFF" EFFECT IS CAUSED BY INHIBITION OF H_2O_2 GENERATION

Bernard Corvilain, Jacqueline Van Sande and Jacques E. Dumont

Institute of Interdisciplinary Research, School of Medicine,
University of Brussels, Campus Erasme, 1070 Brussels, Belgium

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H_2O_2 generation is limiting the oxidation and binding to proteins of iodide. In dog thyroid slices thyrotropin and carbamylcholine greatly enhance protein iodination and H_2O_2 generation. The action of thyrotropin is mimicked by dibutyryl cyclic AMP and forskolin which suggests that it is mediated by cyclic AMP. The action of carbamylcholine was mimicked by ionomycin and by phorbol myristate ester. This suggests that the effect of carbamylcholine is mediated by the two intracellular signals generated by the Ca^{++} phosphatidylinositol cascade : Ca^{++} and diacylglycerol.

The Wolff-Chaikoff effect is the inhibition by iodide of its own organification. In dog thyroid slices, iodide greatly inhibited H_2O_2 generation stimulated by thyrotropin and by carbamylcholine. Iodide decreased the production of intracellular signals induced by TSH and carbamylcholine but it also inhibited the action of probes of these intracellular signals (dibutyryl cAMP, forskolin, ionomycin, phorbol-myristate ester) on the H_2O_2 generating system itself. These effects were suppressed by methimazole an inhibitor of iodide oxidation.

The entire metabolism of iodide in the thyroid gland is set to make the most efficient use of scarce and intermittent supplies of iodide. When this supply becomes abundant, adaptation mechanisms, i.e. negative feedbacks, reminiscent of the end product inhibition and repression of pathways in bacteria, reduce this metabolism. The most immediate negative control is the inhibition by iodide of its own oxidation: the "Wolff-Chaikoff" effect (1). Iodide oxidation and binding to proteins require a thyroperoxidase and a still undefined H_2O_2 generating system. As iodide in excess inhibits its binding to

ABBREVIATIONS

Cchol : carbamylcholine; TSH : thyroid stimulating hormone; Methi : methimazole (2-mercapto-1-methyl-imidazol); HEPES : 4-(2-hydroxyethyl)-1-piperazine sulfonic acid; DBcA : dibutyryl cyclic adenosine 3'5' monophosphate, TPA : tetradecanoyl phorbol acetate; FK : forskolin.

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tyrosine by thyroperoxidase through competition with this aminoacid, it has been assumed until now that this effect in acellular systems accounts for the in vivo effect. We show here that iodide greatly inhibits the rate limiting step of its oxidation in the intact thyroid cell i.e. H_2O_2 generation. This mechanism is sufficient to explain the "Wolff-Chaikoff" effect.

MATERIALS AND METHODS

Horseradish peroxydase type II, homovanillic acid, phorbol myristate ester (TPA) were purchased from Sigma Chemical Co (St Louis, MO, USA), carbamylcholine from K and K (Plain View, NY, USA), ionomycin from Calbiochem-Behring (La Jolla, CA, USA), forskolin from Hoechst Pharmaceuticals (Bombay, India), bovine TSH (thytropar) from Armour Pharmaceutical Co (Phoenix AZ, USA), catalase and dibutyryl cyclic AMP from Boehringer Pharma (Mannheim, Germany). H_2O_2 production was estimated according to the method of Benard and Brault (2) based on the conversion of the non fluorescent substrate, homovanillic acid to a fluorescent derivative in the presence of H_2O_2 and peroxidase. Dog thyroids were sliced at room temperature and incubated at 37°C in 2 ml Krebs Hepes buffer pH 7.4 supplemented with glucose 5 x 10^{-3}M and bovine serum albumin 0.5g/l. For H_2O_2 measurements thyroid slices (30-50 mg) were always preincubated 1hr, then transferred to fresh medium containing horseradish peroxidase type II 0.1mg/ml, homovanillic acid 4.4 x 10^{-4}M and various agonists. Slices were incubated 90 min and at the end of the incubation, the medium was collected on ice and its fluorescence measured in a Perkin Elmer LS3 fluorimeter (λ excitation 315 nM, λ emission 425 nm). Fluorescence is stable for several hours at 4°C. Basal and stimulated H_2O_2 generation are linear up to three hours and inhibited by more than 80% by catalase (36.000U/ml). For the study of iodide action, to avoid direct effects of oxidized iodine on the measuring system, slices were preincubated two hours with iodide (5 x 10^{-5}M) then submitted to four 15 min washes to remove iodide in excess and finally transferred in appropriate medium for H_2O_2 determination for a 90 min incubation. For the measurement of iodide incorporation into proteins, slices were incubated for 120 min in medium supplemented with KI (10^{-7} to 10^{-3}M) and I^{131} (0.5µc/ml) and homogenized in a methimazole solution (2 x 10^{-3}M). The proteins were precipitated with 5% trichloroacetic acid and counted in a well type Packard autogamma counter (3). Data were expressed as picomoles of iodide organified per 100 mg wet weight tissue x 2h. Results are expressed as means $\pm$ SEM of triplicates in one typical experiment out of three.

RESULTS AND DISCUSSION

In the presence of increasing concentrations of iodide, the incorporation of iodide in proteins (expressed as picomoles iodide organified/100 mg tissue $\pm$ SEM) exhibits a biphasic curve with a decrease above a concentration of 10^{-4}M (fig. 1). This result corresponds to the classical in vivo observed Wolff-Chaikoff effect. In acellular system, H_2O_2 generation is limiting the oxidation and binding of iodide to proteins. In isolated thyroid cell catalase greatly inhibits iodination (4,5). As already shown previously (6,7)

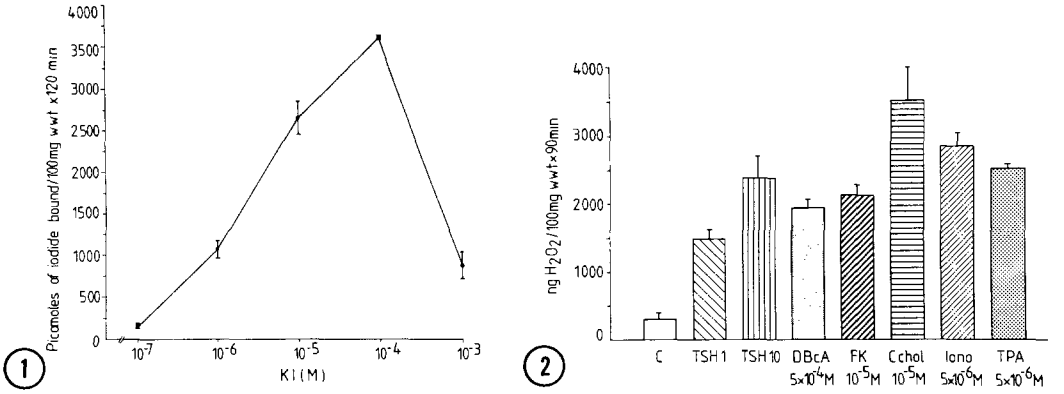


Fig. 1. Effect of increasing concentrations of iodide in the medium on iodide organification in dog thyroid slices. The slices were incubated two hours in presence of iodide (10^{-7}M to 10^{-3}M).

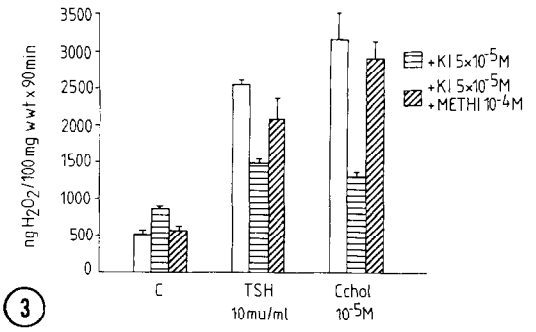
Fig. 2. Effect of thyrotropin 1mU/ml (TSH_1), 10 mU/ml (TSH_{10}), dibutyryl cyclic AMP 5 x 10^{-4}M (DBcA), Forskolin 10^{-5}M (FK), Carbamylcholine 10^{-5}M (Cch), Ionomycin 5 x 10^{-6}M (Iono), Tetradecanoyl phorbol acetate 5 x 10^{-6}M (TPA) on H_2O_2 generation in dog thyroid slices.
C : control.

addition of exogenous H_2O_2 or a system generating H_2O_2 greatly increases iodide binding to proteins in slices. For a concentration of 4 x 10^{-5}M iodide, the addition of 14 mU/ml D-aminoacid oxidase and 4.5 mg/ml D-alanine increases the incorporation ratio from 7% in the controls to 19% in dog thyroid slices (8). Thyrotropin 10 mU/ml and carbamylcholine 10^{-5}M which both greatly enhance iodide binding to proteins in dog thyroid slices also greatly stimulated H_2O_2 generation (fig. 2). Thus, in agreement with a previously stated conclusion (9) H_2O_2 generation is limiting protein iodination in dog thyroid slices as well as in acellular systems. We have shown previously (3) that the stimulation of protein iodination by thyrotropin is mediated by cyclic AMP. It is mimicked by the general adenylate cyclase activator forskolin and cholera toxin and by cyclic AMP analogues and inhibited by a cyclic AMP antagonist (10,11,12). Therefore, as expected, forskolin and dibutyryl cyclic AMP mimicked the effect of thyrotropin on H_2O_2 generation (fig. 2).

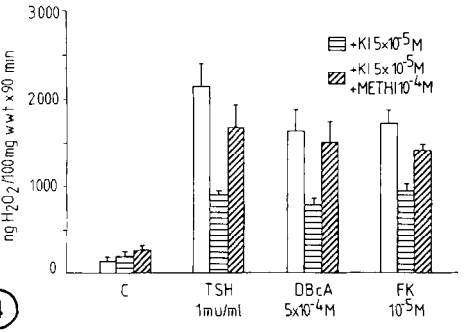
The stimulation of protein iodination by carbamylcholine, an activator of the phosphatidylinositol cascade is mediated by intracellular free Ca^{++} and to some extent by diacylglycerol. It is mimicked by a high extracellular calcium concentration, as by ionophore A23187 in the presence of calcium, and abolished by inhibitors of calcium channels and in Ca^{++} depleted slices (13). It is also activated by phorbolesters and diacylglycerol (14). The H_2O_2

generating system in isolated membranes of pig thyroid is also activated by Ca^{++} (15). Similarly H_2O_2 generation by calf thyroid slices (16) and by porcine open follicles (17) is stimulated by A23187 and inhibited in the absence of Ca^{++}. In dog thyroid slices the effect of carbamylcholine was also mimicked by ionomycin, a divalent cation ionophore and by phorbol myristate ester, a pharmacological probe for diacylglycerol regulated protein kinase C (fig. 2). Iodide inhibits both TSH induced activation of adenylate cyclase (18,19) and the carbamylcholine induced stimulation of inositol phosphates generation by phospholipase C (20). As these inhibitory effects are relieved by methimazole, a drug inhibiting thyroperoxidase and the oxidation of iodide, they have been ascribed to an unidentified postulated inhibitor(s). H_2O_2 generation in response to thyrotropin and carbamylcholine is also inhibited by iodide (fig. 3). The effects of iodide are already significant at 3 x 10^{-6}M and increase up to 10^{-4}M (not shown). They were both relieved when methimazole was added with iodide during the preincubation. Thus iodide, in an oxidized form, inhibits the rate limiting step in its oxidation : H_2O_2 generation. This inhibition is sufficient to explain the Wolff-Chaikoff effect.

Although, other supplementary mechanisms are not excluded, it is worth pointing out that inhibition of protein iodination by thyroperoxidase in vitro, requires very high unphysiological concentrations of iodide (10^{-3}M or more) (21,22).



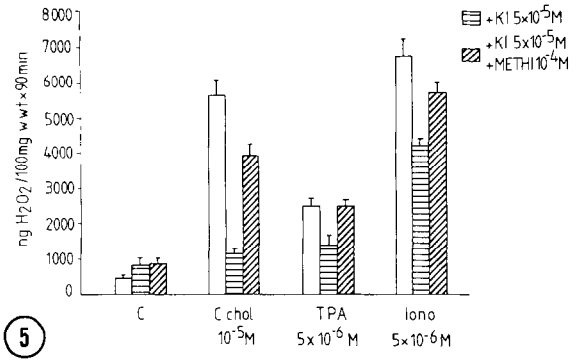
(3)



(4)

Fig. 3. Inhibitory effect of iodide on H_2O_2 production in dog thyroid slices stimulated by thyrotropin 10 mU/ml (TSH_{10}), or carbamylcholine 10^{-5}M (Cchol). Ki 5 x 10^{-5} M was present during the two hours preincubation with or without methimazole 10^{-4}M (METHI). C : control.

Fig. 4. Inhibitory effect of iodide on H_2O_2 production in dog thyroid slices stimulated by thyrotropin 1 mU/ml (TSH_1), Dibutyryl cyclic AMP 5x10^{-4}M (DBcA) and Forskolin 10^{-5}M (FK). KI 5 x 10^{-5}M was present during the two hours preincubation with or without methimazole 10^{-4}M (METHI). C : control.



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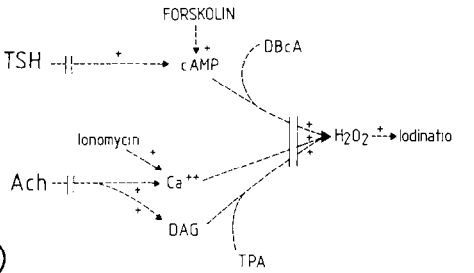

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Fig. 5. Inhibitory effect of iodide on H_2O_2 production in dog thyroid slices stimulated by carbamylcholine 10^{-5}M (Cchol), Tetradecanoyl phorbol acetate 5 x 10^{-6}M (TPA), Ionomycin 5 x 10^{-6}M (IONO). KI 5 x 10^{-5}M was present during the two hours preincubation with or without methimazole 10^{-4}M (METHI). C : control.

Fig. 6. Regulation of iodination in dog thyroid slices
--→ positive controls, stimulation
|| site of inhibition by an oxidized derivative of iodide

TSH	thyroid stimulating hormone
cAMP	cyclic adenosine 3'5' monophosphate
Ach	acetylcholine
DAG	diacylglycerol
Ca^{++}	calcium
TPA	tetradecanoyl phorbol acetate
DBcA	dibutyryl cyclic adenosine 3'5' monophosphate

The inhibition of H_2O_2 generation by iodide in thyrotropin or carbamylcholine stimulated dog thyroid slices bears at the level of intracellular signal generation but also on the action of these intracellular signals. H_2O_2 formation stimulated by activation of the cyclic AMP cascade by forskolin, downstream of the receptor or by dibutyryl cyclic AMP, downstream of cyclic AMP accumulation was also inhibited by iodide (fig. 4). Similarly, the action of phorbol myristate ester (TPA), a pharmacological analogue of diacylglycerol, as well as ionomycin, a probe of Ca^{++} responding systems, were also inhibited by iodide (fig. 5). All these effects of iodide were relieved by methimazole. They were of lower magnitude than those on slices stimulated by the extracellular signals thyrotropin and carbamylcholine. Iodide therefore inhibits H_2O_2 formation and thus its own oxidation, through unknown oxidized derivative(s) at both the level of intracellular signal generation (cyclic AMP, Ca^{++} and diacylglycerol) and of their action on the target system (fig. 6).

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