Antithyroid effect of a food or drug preservative: 4-hydroxybenzoic acid methyl ester

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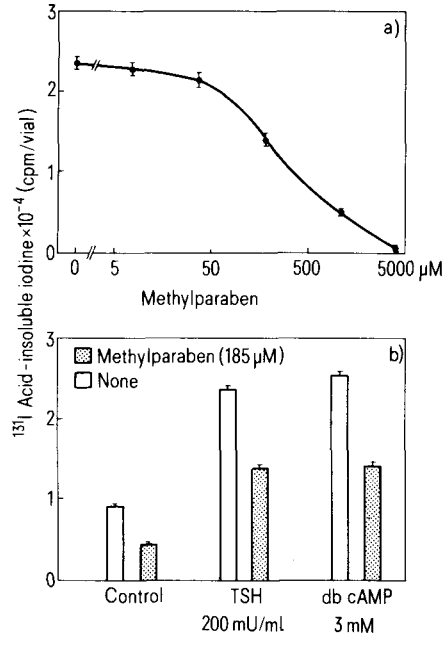
Summary. 4-hydroxybenzoic acid methyl ester (methylparaben) inhibits organification of iodide by isolated hog thyroid cells. The concentration which produces a 50% inhibition was about 2.0×10^{-4}M. A similar inhibition was observed in non-stimulated and TSH- or dibutyryl cyclic AMP-stimulated cells. Neither iodide uptake nor cyclic AMP generation were altered by methylparaben.

Antithyroid drugs refer to a group of compounds that block the synthesis of thyroid hormones. These compounds fall into 2 main categories: a) compounds that inhibit accumulation of iodide by the thyroid gland, b) compounds that interfere with the utilization of iodide into the gland. The compounds of the 2nd group exert their inhibitory effect by blocking one or several of the following reactions: oxydation of iodide, formation of iodotyrosines, coupling of iodotyrosines to form thyroid hormones. Besides, thionamides which are the most potent antithyroid agents, phenol and benzoic acid derivatives (resorcinol, p-aminobenzoic acid ...) are known to be efficient inhibitors of iodine metabolism both in vivo and in vitro. During the course of our study on the metabolism of iodide in dispersed thyroid cells, we found that 4-hydroxybenzoic acid methyl ester, a compound used as a food or drug preservative, prevented thyroid hormone synthesis. In this communication we report that this compound inhibits the organification of iodide without altering the iodide trapping mechanism.

Suspensions of dispersed hog thyroid cells were prepared as previously described[1]. Thyroid cells ($1-5 \times 10^7$) were incubated in Earle's balanced salt solution, pH 7.4, in the presence of 0.05 μM iodide labelled with 1–5 μCi ^{131}I, for 2 h at 37 °C with air as the gas phase and under constant shaking (60 cycles/min).

Iodide trapping, expressed in terms of ^{131}I-C/M ratio: the ratio between intracellular and medium labelled iodide concentration, was determined in the presence of 2 mM methimazole according to Rodesch and Dumont[2]. Iodide organification was assessed by measurements of ^{131}I-iodide incorporation into acid-insoluble material (mainly iodoproteins). Cell homogenates were supplemented with 0.1 ml 0.2 mM NaI and 0.1 ml 1% (w/v) bovine serum albumin and proteins were precipitated with 5% (w/v) trichloracetic acid. The 1500× g pellet was washed with 5% trichloracetic acid and counted for radioactivity. Cyclic AMP was assayed as previously described[3]. Thyrotropin (TSH),N^6,$O^{2'}$-dibutyryl-adenosine 3′:5′ monophosphate (db cAMP) and 4-hydroxybenzoic acid methyl ester were obtained from Armour (Kankakee, USA), Sigma (St. Louis, USA) and Organon (France), respectively.

4-hydroxybenzoic acid methyl ester: methylparaben (monograph number 5972 of the Merck index) inhibited ^{131}I-acid-insoluble iodine formation in a concentration-dependent manner (figure a). Above concentrations of 4 mM, iodide organification by dispersed thyroid cells was completely abolished. The concentration of methylparaben which induced a 50% inhibition was between 1.5 and 3.0×10^{-4} M. Methylparaben inhibited iodide organification both in basal and stimulated cells. The amount of ^{131}I-acid-insoluble iodine formed by control cells and by TSH- or db cAMP- stimulated cells was decreased respectively by 50%, 43% and 45%, in the presence of 185 μM methylparaben (figure b). That methylparaben exerts a selective effect on the iodide organification step is shown in the table. Neither iodide uptake (^{131}I-C/M) nor cyclic AMP



Inhibitory effect of 4-hydroxybenzoic acid methyl ester (methylparaben) on iodide organification in dispersed thyroid cells. Organified iodide was measured as ^{131}I-acid insoluble iodine. Cells were incubated with ^{131}I-iodide (0.05 μM) for 2 h. *A* Effect of increasing concentration of methylparaben; *B* effect of methylparaben on control cells or thyrotropin (TSH) or dibutyryl cyclic AMP (db cAMP) stimulated cells. Mean ± SEM of triplicate incubations.

Lack of effect of 4-hydroxybenzoic acid methyl ester (methylparaben) on iodide uptake (^{131}I-C/M) and cAMP generation in dispersed thyroid cells

Experiment	Parameter tested	Additions	Control	Methylparaben (185 µM)
A	^{131}I-C/M	-	20.0 ± 1.2	18.8 ± 0.9
B	cAMP accumulation	None	33.9 ± 4.6	27.9 ± 4.5
	(pmoles/100 µl cells)	TSH	200.0 ± 3.0	189.9 ± 3.4

A: Cells were incubated with ^{131}I-iodide (0.05 µM) in the presence of 2 mM methimazole with or without methylparaben for 2 h. B: Cells were preincubated with 3-isobutyl-1-methylxanthine for 15 min and incubated with or without thyrotropin (TSH) 200 mU/ml and with or without methylparaben for 15 min. Mean ± SEM of triplicate incubations.

generation were altered by methylparaben at a concentration which produced a 50% inhibition of iodide organification.

Previous studies[4-6] have shown that 4-aminobenzoic acid, 2:3- and 2:4-dihydroxybenzoic acid were antithyroid compounds in vivo in the rat at doses ranging from 0.1 to 1 mmole/100 g b.wt. In vitro, 4-aminobenzoic acid was found to be a very active inhibitor of the thyroid peroxidase catalyzed iodination[7]. The concentration of 4-aminobenzoic acid which produced a 50% inhibition was between 5 and 10 μM. This compound had the same potency as methimazole in this system. In the dispersed thyroid cell system, methylparaben is less potent than methimazole to block iodide organification. The concentrations which decrease iodide organification by 50% were about 200 μM and 8 μM for methylparaben and methimazole[8], respectively. Although, methylparaben seems to have a weak intrinsic antithyroid activity compared to that of a representative antithyroid agent, our results indicate that methylparaben included in drug preparation (generally at 0.1% w/v) or food is a potential inhibitor of thyroid functioning. However, it remains to establish its activity in in vivo conditions, where absorption properties and degradation pathways could increase or decrease the observed effect of the compound.

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Increase in the total reducing substances in the hemolymph of the freshwater crab, *Barytelphusa guerini*, produced by a pesticide (DDT) and an indolealkylamine (serotonin)

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Summary. DDT and serotonin produced significant increases in the total reducing substances in the hemolymph of intact crabs, *Barytelphusa guerini*, apparently by triggering release of the hyperglycemic hormone.

DDT and its breakdown products have been accumulating in the environment for many years, and crustaceans are being exposed constantly to chronic, if not lethal, concentrations of these compounds. But relatively little attention has been directed towards determining the effects of DDT on physiological and biochemical parameters in crustaceans.

The neurotransmitter serotonin (5-HT, 5-hydroxytryptamine) has been shown to produce hyperglycemia in crustaceans, apparently indirectly by triggering release of the hyperglycemic hormone (HGH)[1-5]. Evidence in support of this indirect action of 5-HT was obtained by Strolenberg and Van Herp[4] with the crayfish *Astacus leptodactylus* and by Martin[5] with the isopod *Porcellio dilatatus*. In both species not only did 5-HT produce hyperglycemia but also, as observed by electron microscopy, an increase of exocytotic figures in the sinus glands, the pair of neurohemal organs in these crustaceans from which the HGH is released. In view of the fact that DDT induces repetitive discharges in crustacean neurons[6], it is conceivable that DDT could produce secretion of HGH from the neurosecretory cells that synthesize it. This investigation was undertaken to determine a) whether a sublethal dose of DDT can indeed produce an increase in the quantity of total reducing substances (TRS), practically all, if not all, of which are sugars, in the hemolymph of the freshwater crab, *Barytelphusa guerini*, and b) if DDT does have such an effect whether it might involve stimulation or inhibition of the release of HGH.

Materials and methods. Adult, intermolt specimens of *Barytelphusa guerini*, collected in the area of Aurangabad were used in these experiments. The DDT was first dissolved in ethanol and diluted with distilled water so that the final concentration was 0.01 mg/0.05 ml of 0.5% ethanol. DDT control crabs received 0.5% ethanol alone. Reserpine (RSP) was prepared in distilled water in a concentration of 0.1 mg/0.05 ml. Serotonin creatinine sulfate (5-HT) was also dissolved in distilled water, the concentration being 0.01 mg/0.05 ml. Distilled water-injected crabs served as controls for the crabs receiving RSP or 5-HT. The volume of any solution or distilled water injected into each crab was always 0.05 ml.

TRS concentrations were determined by the Nelson-Somogyi method as modified by Varley[7]. All blood samples were removed the same time each day to obviate any possible effect of a circadian cycle in the concentration of the TRS. TRS concentrations were determined 2 h after any particular treatment. Student's t-test was used in the calculation of probability values.

Variations in the concentration of total reducing substances in the blood of *Barytelphusa guerini* after various treatments

Groups of crabs tested	Total reducing substances (mg%) ± SD
Intact crabs	26.2 ± 1.2
Distilled water-injected intact crabs (reserpine and serotonin controls)	33.3 ± 2.2
Reserpine-injected intact crabs	21.8 ± 3.8
Serotonin-injected intact crabs	38.5 ± 1.2
Ethanol-injected intact crabs (DDT control)	31.8 ± 1.7
DDT-injected intact crabs	41.0 ± 4.2
DDT + reserpine-injected intact crabs	28.8 ± 4.2
Eyestalkless crabs	22.4 ± 1.6
Distilled water-injected eyestalkless crabs	25.7 ± 7.5
Ethanol-injected eyestalkless crabs (DDT control)	27.2 ± 2.1
DDT-injected eyestalkless crabs	19.4 ± 3.5
Serotonin-injected eyestalkless crabs	29.5 ± 5.8