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Comparison of Potential Protective Effects of Melatonin, Indole-3-Propionic Acid, and Propylthiouracil Against Lipid Peroxidation Caused by Potassium Bromate in the Thyroid Gland

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Abstract Potassium bromate ($KBrO_3$) is a prooxidant and carcinogen, inducing thyroid tumors. Melatonin and indole-3-propionic acid (IPA) are effective antioxidants. Some antioxidative effects of propylothiouracil (PTU)—a thyrostatic drug—have been found. The aim of the study was to compare protective effects of melatonin, IPA, and PTU against lipid peroxidation in the thyroids, collected from rats treated with $KBrO_3$, and in homogenates of porcine thyroids, incubated in the presence of $KBrO_3$. Wistar rats were administered $KBrO_3$ (110 mg/kg b.w., i.p., on the 10th day of the experiment) and/or melatonin, or IPA (0.0645 mmol/kg b.w., i.p., twice daily, for 10 days), or PTU (0.025% solution in drinking water, for 10 days). Homogenates of porcine thyroids were incubated for 30 min in the presence of $KBrO_3$ (5 mM) plus one of the antioxidants: melatonin (0.01, 0.1, 0.5, 1.0, 5.0, 7.5 mM), or IPA (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM), or PTU (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM). The level of lipid peroxidation products (MDA + 4-HDA) was measured spectrophotometrically in thyroid homogenates. In vivo pretreatment with either melatonin or with IPA or with PTU decreased lipid peroxidation caused by $KBrO_3$—injections in rat thyroid gland. Under in vitro conditions, PTU (5.0, 7.5, and 10.0 mM), but neither melatonin nor IPA, reduced $KBrO_3$-related lipid peroxidation in the homogenates of porcine thyroids. In conclusion, melatonin and IPA may be of great value as protective agents under conditions of exposure to $KBrO_3$. J. Cell. Biochem. 95: 131–138, 2005.

Key words: melatonin; indole-3-propionic acid; propylthiouracil; thyroid; potassium bromate; lipid peroxidation

Potassium bromate ($KBrO_3$) was used as a food additive in flour and barley processing and as a component in cold-wave hair lotions [FAO/WHO, 1979]. Although in 1992, the approval for its use in the treatment of flour was withdrawn [JECFA, 1992], there is still some concern, regarding the presence of this chemical compound in the environment. Bromate is frequently detected in tap and bottled water, since it is an inorganic oxyhalide by-product, formed during water disinfection by ozonation [van Dijk-Looijaard and van Genderen, 2000].

Potassium bromate has been classified as a compound, belonging to the group 2B of carcinogens (a possible human carcinogen) [IARC, 1986]. It has been demonstrated to cause renal tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid in rats [Kurokawa et al., 1990; Wolf et al., 1998].

Oxidative stress presumably plays a major role in the carcinogenic action of $KBrO_3$ [Sai et al., 1991]. This compound has been shown to cause oxidative modification of DNA bases, lipids, and proteins in kidneys [Chipman et al., 1998; Murata et al., 2001]. It has not been unambiguously defined what the mechanism

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of prooxidative action of $KBrO_3$ is. $KBrO_3$ is known to decrease the activity of an important antioxidative enzyme—glutathione peroxidase, and to increase the formation of the following free radicals and reactive oxygen species: peroxynitrite anion ($ONOO^-$), nitric oxide ($NO^{\cdot}$) and superoxide anion radical ($O_2^{-\cdot}$) [Watanabe et al., 2002]. On the other hand, experimental evidence suggests that the classic antioxidative enzymes, like superoxide dismutase and catalase, are not protective against $KBrO_3$-induced oxidative cellular DNA damage in vitro, and that the most reactive free radical—hydroxyl radical ($^{\cdot}OH$) and, additionally, $O_2^{-\cdot}$ and hydrogen peroxide (H_2O_2) are not involved, at least, directly in the prooxidative effects of $KBrO_3$ [Murata et al., 2001]. At the same time, the above cited authors found sulfhydryl compounds, like glutathione or cysteine, to be required for $KBrO_3$-induced oxidative DNA damage in vitro. The authors speculated that reduction of $KBrO_3$ by sulfhydryl compounds yields other bromine compounds, like oxobromate ions (BrO^-), bromine oxides (BrO, BrO_2), and bromine radicals ($Br^{\cdot}$), which oxidatively damage DNA and are responsible for carcinogenic effects of $KBrO_3$ [Murata et al., 2001]. That mechanism could possibly be involved in $KBrO_3$-induced lipid oxidative damage.

It is worth mentioning that oxidative stress plays a crucial role in physiological processes in the thyroid gland [Karbownik and Lewinski, 2003a].

Due to the prooxidative action of $KBrO_3$, antioxidants are expected to protect against carcinogenicity, caused by this compound. In fact, certain antioxidant substances, like ebselen [Watanabe et al., 2002], kolaviron [Farombi et al., 2002], and melatonin [El-Sokkary, 2000], have been shown to prevent oxidative damage to macromolecules, caused by $KBrO_3$ in kidneys.

Melatonin (*N*-acetyl-5-methoxytryptamine)—the pineal hormone—is an effective antioxidant and free radical scavenger [Reiter et al., 2000; Tan et al., 2003]. In numerous studies, it has been found to protect against oxidative damage to macromolecules by potential carcinogens [Karbownik et al., 2000a,b, 2001a,b,c; Karbownik and Reiter, 2000]. Since melatonin is known to inhibit growth processes in the thyroid [Lewinski and Karbownik, 2002; Lewinski et al., 2002], and since it protects against oxidative damage, induced by Fenton reaction (being the most classic reaction of oxidative stress) in thyroid homogenates [Karbownik and Lewinski, 2003b], the indoleamine may be assumed to protect against thyroid cancer. Indole-3-propionic acid (IPA) is an indole substance, which also reveals antioxidative properties, both in vivo [Karbownik et al., 2001b] and in vitro [Poeggeler et al., 1999; Karbownik et al., 2001a,d].

Recently, antioxidative effects of propylothiouracil (6-*n*-propyl-2-thiouracil; PTU), a thyrostatic drug, have been found [Hicks et al., 1992]. For example, PTU has been shown to decrease lipid peroxidation and the production of $NO^{\cdot}$, and to increase the activity of an antioxidant enzyme—superoxide dismutase [Seven et al., 2001].

The majority of studies on the prooxidative action of $KBrO_3$—the carcinogen—and on the potential protective effects of antioxidants against this process have been performed on kidneys, being the target organs for $KBrO_3$-induced carcinogenicity [Sai et al., 1991; Chipman et al., 1998; Murata et al., 2001]. No such studies have yet been carried out on the thyroid gland, which is the target tissue for the carcinogenic action of $KBrO_3$ as well [Kurokawa et al., 1990; Wolf et al., 1998].

The aim of the study was to compare the protective effects of melatonin, IPA, and PTU against lipid peroxidation in the thyroids, collected from rats pretreated with $KBrO_3$, and in homogenates of porcine thyroids incubated in vitro in the presence of $KBrO_3$.

MATERIALS AND METHODS

The procedures, used in the study, were approved by the Ethical Committee of the Medical University of Lodz.

Chemicals

Melatonin, IPA, PTU, and $KBrO_3$ were purchased from Sigma-Aldrich (St. Louis, MO). The LPO-586 kit for lipid peroxidation was purchased from Calbiochem (La Jolla, CA). Other chemicals were of analytical grade and came from commercial sources.

Experiment In Vivo

A total of 64 male Wistar rats (weighing about 160 g each) were used in the study. The animals were randomized into eight groups and administered: freshly prepared 0.9% NaCl:ethanol

(vol:vol, 10:1) (Control; n = 9), or $KBrO_3$ (110 mg/kg b.w., on the 10th day of the experiment; n = 9) in distilled H_2O, or melatonin [0.0645 mmol/kg (i.e., 15 mg/kg) b.w., two times daily, for 10 days; n = 6] in freshly prepared 0.9% NaCl:ethanol (vol:vol, 10:1), or IPA (0.0645 mmol/kg b.w., twice daily, for 10 days; n = 6) in freshly prepared 0.9% NaCl:ethanol (vol:vol, 10:1), or PTU (0.025% solution in drinking water, for 10 days; n = 9), or $KBrO_3$ + melatonin (n = 8), or $KBrO_3$ + IPA (n = 8), or $KBrO_3$ + PTU (n = 9). The control rats, which did not receive either $KBrO_3$ or antioxidants, were treated with their solvents, i.e., distilled H_2O or 0.9% NaCl:ethanol (vol:vol, 10:1) at the above mentioned time points; all the rats received water ad libitum. All the substances (except PTU) were administered i.p. in volumes of 0.5 ml/injection.

The rats were killed by decapitation 24 h after $KBrO_3$ injection. The thyroids were collected, frozen on solid CO_2 and stored at −80°C until assay. Just before lipid peroxidation assay, thyroid tissue from each animal was homogenized in ice cold 50 mM Tris-HCl buffer (pH 7.4) (2%, w/v– in final incubation volume).

Experiments In Vitro

Because the mass of the rat thyroid gland is very small, which would have required tissue collection from many animals, we used porcine thyroids in the in vitro study. Porcine thyroids were collected from 20 male animals (average age of 12 months) at a slaughterhouse, frozen on solid CO_2 and stored at −80°C until assay.

Thyroid tissue was homogenized in ice cold 50 mM Tris-HCl buffer (pH 7.4) (5%, w/v—in final incubation volume) and then, incubated for 30 min at 37°C in exposition to the studied substances.

In Experiment I (in vitro), thyroid homogenates were incubated in the presence of IPA (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM) or PTU (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM). The effect of melatonin (used in concentrations of 0.00001, 0.0001, 0.001, 0.01, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 mM) on the basal lipid peroxidation in homogenates of porcine thyroids was examined before [Karbownik and Lewinski, 2003b].

In Experiment II (in vitro), thyroid homogenates were incubated in the presence of $KBrO_3$, used in the following concentrations: 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 mM; a concentration-dependent increase in lipid peroxidation was observed, with statistical significance for the concentrations of 5 and 10 mM (Fig. 3). The concentration of 5 mM was selected for the subsequent experiment to induce lipid peroxidation.

Thus, in Experiment III (in vitro), thyroid homogenates were incubated in the presence of $KBrO_3$ (5 mM) in order to induce lipid peroxidation and, additionally, in the presence of one of the antioxidants: melatonin (0.01, 0.1, 0.5, 1.0, 5.0, 7.5 mM), or IPA (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM), or PTU (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM), to check their potential preventive effects.

The reactions were stopped by cooling the samples on ice. Each experiment was run in duplicate and repeated three times.

Measurement of Lipid Peroxidation Products

Concentrations of malondialdehyde + 4-hydroxyalkenals (MDA +4 -HDA), as the index of lipid peroxidation, were evaluated in the thyroid homogenates (either rat or porcine).

The homogenates were centrifuged at 3,000*g* for 10 min at 4°C. The supernatant was mixed with 650 μl of a methanol:acetonitrile (1:3, v/v) solution, containing a chromogenic reagent, *N*-methyl-2-phenylindole, and vortexed. After adding 150 μl of methanesulfonic acid (15.4M), the incubation was carried out at 45°C for 40 min. The reaction between MDA + 4-HDA and *N*-methyl-2-phenylindole yields a chromophore, which is spectrophotometrically measured at the absorbance of 586 nm, using a solution of 10 mM 4-hydroxynonenal as the standard. The level of lipid peroxidation was expressed as the amount of MDA + 4-HDA (nmol) per mg protein, and—in case of the in vivo experiment—additionally as the amount of MDA + 4-HDA (nmol) per mg tissue.

Measurement of Protein

Protein was measured, using the method of Bradford [1976], with bovine albumin as the standard.

Statistical Analysis

The data were statistically analyzed, using a one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls test. Statistical significance was determined at the level of $P < 0.05$. The results are presented as mean ± SEM.

RESULTS

Experiment In Vivo

The treatment of rats with $KBrO_3$ significantly increased lipid peroxidation in thyroids, collected 24 h later. The treatment with melatonin, IPA or PTU did not affect the basal lipid peroxidation (auto-oxidation). Co-treatment with melatonin, IPA or PTU prevented the $KBrO_3$-induced oxidative damage to lipids in rat thyroids (Fig. 1).

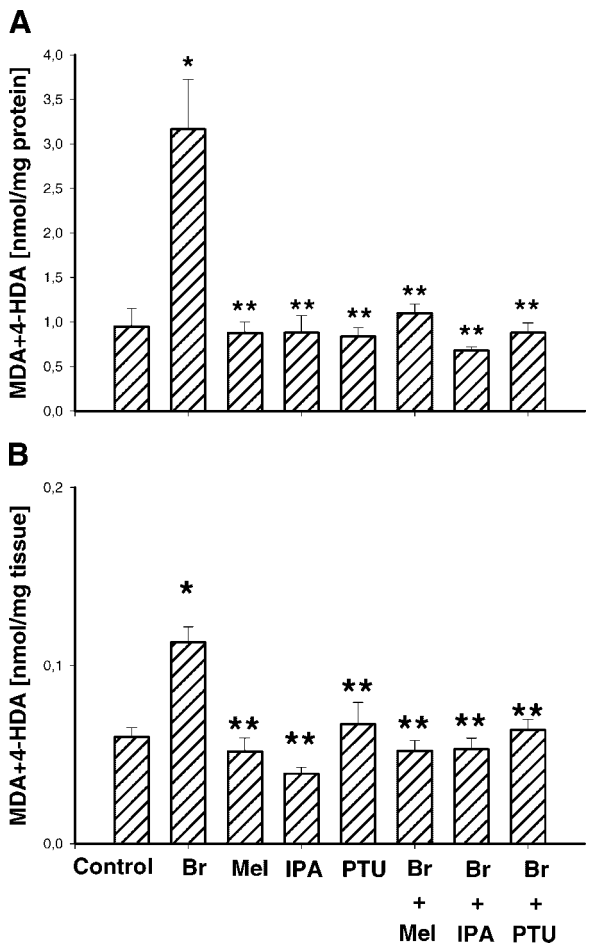

Fig. 1. Concentrations of malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA), expressed in nmol per mg protein (**A**) or in nmol per mg tissue (**B**) in the homogenates of thyroids, collected from rats administered 0.9% NaCl:ethanol (Control; n = 9), or $KBrO_3$ (110 mg/kg b.w., i.p., on the 10th day of the experiment; n = 9), or melatonin (0.0645 mmol/kg b.w., i.p., twice daily, for 10 days; n = 6), or IPA (0.0645 mmol/kg b.w., i.p., twice daily, for 10 days; n = 6), or PTU (0.025% solution in drinking water, for 10 days; n = 9), or $KBrO_3$ + melatonin (n = 8), or $KBrO_3$ + IPA (n = 8), or $KBrO_3$ + PTU (n = 9). Bars represent the mean ± SEM. *$P < 0.05$ versus Controls. **$P < 0.05$ versus $KBrO_3$.

Experiments In Vitro

The incubation of porcine thyroid homogenates in the presence of either IPA or PTU for 30 min did not affect the basal lipid peroxidation (data not shown). Similarly as in case of IPA and PTU in the present study, melatonin (used in the previous study in concentrations of 0.00001, 0.0001, 0.001, 0.01, 0.1, 0.25, 0.5, 1.0, 2.5, or 5.0 mM) failed to cause any changes in lipid peroxidation products in thyroid homogenates, even if the incubation time had been extended to 60 and 120 min [Karbownik and Lewinski, 2003b].

When the homogenates of porcine thyroids were incubated in the presence of $KBrO_3$, used in different concentrations, a concentration-dependent increase in lipid peroxidation was found, with statistical significance for the concentrations of 5 and of 10 mM (Fig. 2).

In the subsequent experiment in vitro, the homogenates of porcine thyroids were incubated in the presence of $KBrO_3$ (5 mM) plus one of the antioxidants: melatonin, IPA or PTU. Neither melatonin (Fig. 3) nor IPA (Fig. 4) prevented the increase in MDA + 4-HDA level, due to $KBrO_3$. Propylthiouracil (5.0, 7.5, and 10.0 mM) significantly reduced $KBrO_3$-induced lipid peroxidation in the homogenates of porcine thyroids (Fig. 5).

DISCUSSION

Our study is the first attempt, devoted to evaluate the prooxidative effects of $KBrO_3$ in

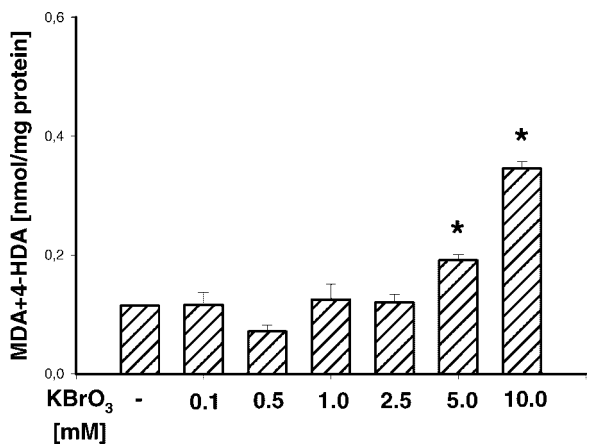

Fig. 2. Concentrations of MDA + 4-HDA in porcine thyroid homogenates, incubated for 30 min in the presence of $KBrO_3$ (0.1, 0.5, 1.0, 2.5, 5.0, 10.0 mM). Bars represent the mean ± SEM of three independent experiments run in duplicates. *$P < 0.05$ versus Controls.

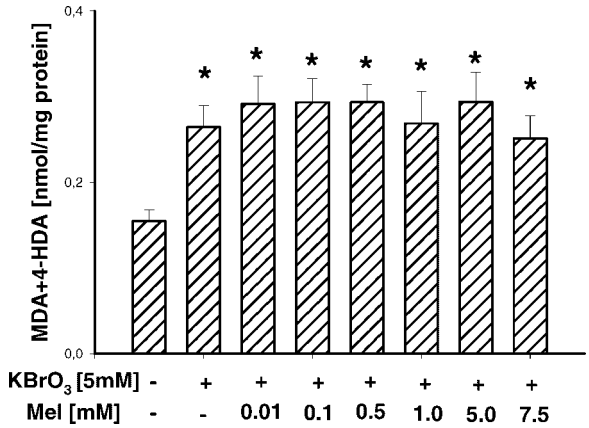

Fig. 3. Concentrations of MDA + 4-HDA in porcine thyroid homogenates, incubated for 30 min in the presence of $KBrO_3$ (5.0 mM) and melatonin (0.01, 0.1, 0.5, 1.0, 5.0, 7.5 mM). Bars represent the mean ± SEM of three independent experiments run in duplicates. *$P < 0.05$ versus Controls.

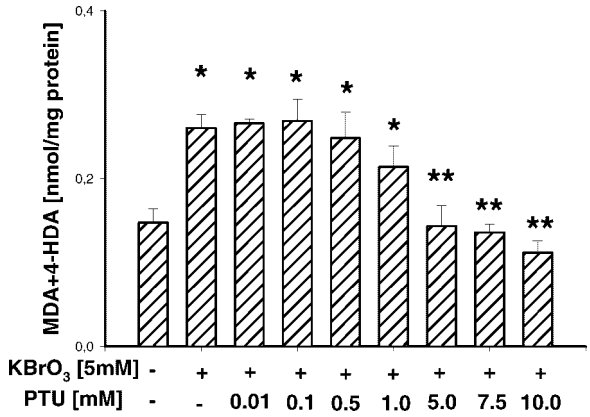

Fig. 5. Concentrations of MDA + 4-HDA in porcine thyroid homogenates, incubated for 30 min in the presence of $KBrO_3$ (5.0 mM) and PTU (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM). Bars represent the mean ± SEM of three independent experiments run in duplicates. *$P < 0.05$ versus Controls. **$P < 0.05$ versus $KBrO_3$.

the thyroid; lipid peroxidation was induced in the thyroid gland under both in vivo and in vitro conditions. Worth underlining is the fact that the increase of lipid peroxidation in the rat thyroid in vivo, followed by $KBrO_3$ injection, was spectacularly high, when compared to the results, found in other organs after treatment with other carcinogens [Karbownik et al., 2001c].

Our finding is in compliance with the assumption that oxidative stress plays a crucial role in the tumorogenic action of $KBrO_3$ not only in kidneys [Sai et al., 1991; Chipman et al., 1998; Murata et al., 2001], but also in the thyroid gland. Indeed, it is generally accepted that lipid peroxidation contributes to DNA damage and cancer initiation [Marnett, 1999].

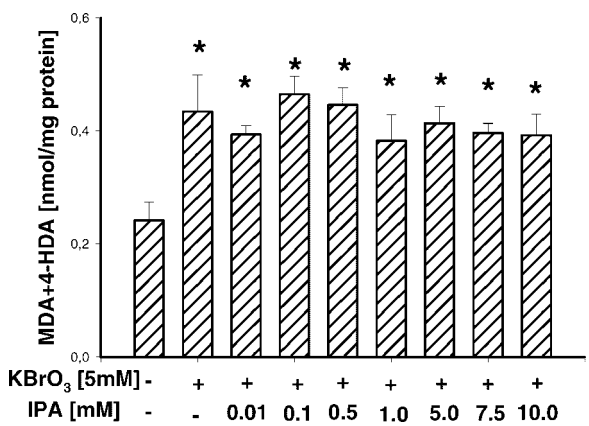

Fig. 4. Concentrations of MDA + 4-HDA in porcine thyroid homogenates, incubated for 30 min in the presence of $KBrO_3$ (5.0 mM) and IPA (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM). Bars represent the mean ± SEM of three independent experiments run in duplicates. *$P < 0.05$ versus Controls.

The induction of lipid peroxidation in the thyroid homogenates by using $KBrO_3$ only, and without H_2O_2, suggests that $KBrO_3$ acts—under in vitro conditions—via the mechanism different from Fenton reaction. Similarly, Murata et al. [2001] observed that H_2O_2 did not participate in $KBrO_3$-induced oxidative DNA lesion in human cultured cells. $KBrO_3$-induced lipid peroxidation in vitro might result from induction of such oxygen species like either $ONOO^-$ or $NO^{\cdot}$, or from suppressed activity of an important antioxidative enzyme—glutathione peroxidase [Watanabe et al., 2002]. Other mechanisms are also possible. As it has been mentioned before, other bromine compounds, like BrO^-, bromine oxides and $Br^{\cdot}$ could be responsible for carcinogenic effects of $KBrO_3$ [Murata et al., 2001]. In order to unambiguously evaluate the precise mechanism of $KBrO_3$, it would be worth comparing the in vitro and in vivo effects of BrO^- and/or other bromine compounds with those of $KBrO_3$ on lipid peroxidation in the thyroid gland. It should be mentioned, however, that $KBrO_3$ revealed a much stronger oxidative damaging effect on DNA than BrO^- when both were tested in the presence of glutathione in vitro [Murata et al., 2001].

Thus, a new in vitro model intended for the examination of $KBrO_3$-induced carcinogenesis has been described here, undergoing via the

mechanism of oxidative stress in the thyroid gland.

Concerning the potential protective effects of antioxidants against $KBrO_3$-induced lipid peroxidation in the thyroid, we did not find any concordant results, when comparing the in vivo and the in vitro conditions. Whereas all of the used antioxidants, given systemically, prevented lipid peroxidation, due to $KBrO_3$ treatment, PTU was the only agent revealing a certain protective effect under in vitro conditions.

The explanation of this apparent discrepancy may be as follows: first, the lack of protective effect of potential antioxidants in the in vitro conditions does not exclude their ability to reveal such an effect in living organisms; this observation has been confirmed for melatonin and IPA in the present study.

Second, the spectacular protective in vitro effects of potential antioxidants do not mean that such effects would occur in vivo, which has been documented for several substances. For example, *N*-acetylserotonin (NAS)—melatonin precursor—revealed a much stronger protective effect in vitro than melatonin against lipid peroxidation, caused by Fenton reaction in rat testes; at the same time, IPA was not protective factor at all [Karbownik et al., 2001a]. However, when these three indole substances were studied in vivo, only two of them—melatonin and IPA, but not NAS, revealed protective effects against the oxidative damage to kidney DNA, induced by prooxidant injection to hamsters [Karbownik et al., 2001b].

Molecules, like melatonin and IPA, act as endogenous electron donors, primarily detoxifying reactive oxygen species; this mechanism may be responsible for the high efficacy of antioxidative action of melatonin and IPA in vivo. Because these indoles do not posses the redox-active hydroxyl group at position 5 of the indole ring, they do not act as chain-breaking antioxidants and, therefore, they are either poorly protective or not effective in vitro [Chyan et al., 1999; Poeggeler et al., 1999].

Additionally, the discrepancy between in vivo and in vitro effects of melatonin may directly result from different mechanisms of $KBrO_3$ action in vivo and in vitro. Whereas $KBrO_3$-induced DNA oxidation was inhibited by glutathione, a well known intracellular antioxidant in vivo [Sai et al., 1992], in the conditions in vitro, glutathione activated $KBrO_3$ to form reactive species and to damage macromolecules [Parsons and Chipman, 2000]. Because melatonin increases glutathione synthesis [Reiter et al., 2000], it is able to protect against oxidative damage in vivo, caused by $KBrO_3$ via the mechanism, involving—among others—glutathione. In opposite, the increase of melatonin-induced glutathione synthesis in vitro may only enhance the damaging effects of $KBrO_3$, making the indoleamine impossible to prevent against $KBrO_3$-induced oxidative damage.

It should be stressed that the concentration of $KBrO_3$, reached in the rat thyroid gland after intraperitoneal injection (the injection of 110 mg/kg b.w. could result—in case of an equal distribution—in the concentration of approximately 0.66 mM), was probably much lower than that in the porcine thyroid homogenates, used in vitro (5 mM). Additionally, the substrate selectivity of the thyroid sodium/iodine (Na^+/I^-) symporter may further decrease $KBrO_3$ concentration in thyroid follicular cells after intraperitoneal injection, because $KBrO_3$ is a very poorly transported anion by this transporter [Eskandari et al., 1997]. Therefore, our in vivo and in vitro experiments are not necessarily comparable rather than discrepant.

However, it is worth stressing that the lack of protective effect of melatonin under in vitro conditions is very seldom observed, but this happened in case of $KBrO_3$ and the thyroid gland.

Another, and maybe the most important point is that potential antioxidants, which are able to reduce oxidative damage in vivo, and are considered to be used in humans, should be devoid of undesirable side effects. Considering the antioxidant factors, used in the current study, melatonin absolutely meets these requirements. Melatonin, even when used in very high pharmacological doses to either humans [Vijayalaxmi et al., 1996] or animals [Karbownik et al., 2000b], has never revealed any undesirable effects. This is not case of IPA; some differences in the chemical structure between IPA and melatonin make the former substance reveal prooxidative effects and some toxicity. Because PTU leads to hypothyroidism when used in euthyroid subjects, and, additionally, because it has also certain adverse effects, like toxic effects on the liver, agranulocytosis, vasculitis, etc. [Bandyopadhyay et al., 2002], it should not be recommended as an

antioxidative agent for cancer prevention in humans.

The mechanisms, by which melatonin protects against lipid peroxidation, most likely involve direct or indirect antioxidant and free radical scavenging activities of this indoleamine [Reiter et al., 2000; Tan et al., 2003]. The most important among them seems to be the ability of melatonin to scavenge such toxic species, like $ONOO^-$, which is sufficiently reactive to initiate the breakdown of lipids, and $NO^{\cdot}$ [Reiter et al., 2000; Tan et al., 2003], both being induced by $KBrO_3$. Although $KBrO_3$ has not been demonstrated to directly induce $^{\cdot}OH$, this highly reactive species is definitely involved—at least indirectly—in oxidative damage, due to $KBrO_3$ and, therefore, the high effectiveness of melatonin to scavenge $^{\cdot}OH$ is also of great importance [Reiter et al., 2000; Tan et al., 2003]. This indoleamine also neutralizes H_2O_2, $O_2^{-\cdot}$, and singlet oxygen ($^1O_2$). Melatonin, which is highly lipid soluble, is believed to be widely distributed in cellular membranes, where it may intercalate between the polar heads of fatty acids [Reiter et al., 2000; Tan et al., 2003]. The last property facilitates melatonin to reduce oxidative damage to lipids.

Additionally, it appears important that melatonin, under in vivo conditions, can stimulate the activities of antioxidative enzymes (γ-glutamylcysteine synthetase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, superoxide dismutase, and catalase), and inhibit the activity of nitric oxide synthase, i.e., a pro-oxidative enzyme [Reiter et al., 2000; Tan et al., 2003]. Scavenging effects of melatonin against bromine radical or other bromine reactive species have not yet been examined.

Since melatonin is known to inhibit growth processes in the thyroid [Lewinski and Karbownik, 2002; Lewinski et al., 2002], and since it can protect against oxidative damage in the thyroid, the indoleamine may be assumed to protect against thyroid cancer, as well.

Concerning IPA, this indole substance has been shown to be a potent scavenger of the $^{\cdot}OH$ [Poeggeler et al., 1999], to quench the $O_2^{-\cdot}$, [Hardeland et al., 1999] and to act synergistically with a well known intracellular antioxidant, glutathione [Poeggeler et al., 1999].

In conclusion, melatonin and, possibly, IPA, which protect against carcinogen $KBrO_3$-related lipid peroxidation in the thyroid gland, might be considered as pharmacological agents to be used for protection against potential carcinogens. The protective effects of PTU against $KBrO_3$-induced lipid peroxidation in the thyroid in vivo and in vitro allow proceeding into details of the antioxidative mechanism of PTU action, but—at present—when this drug is recommended for the therapy of hyperthyroidism, it is applied for its thyrostatic action and not for its possible cancer prevention properties.

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