RADIOMETRIC DETERMINATION OF THYROTOXIC EFFECTS OF SOME XENOBIOTICS*

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Abstract. *Recently, we have shown in the isolated rat thyroids marked effects of excessive exogenous bromide and perchlorate ions on the activity of thyroid peroxidase (TPO), the key enzyme in thyroid hormones (TH) metabolism. Here, we studied in more details, with the aid of several radioanalytical methods, the effects of an enhanced bromide and/or perchlorate intake on various aspects of iodine metabolism and, consequently, on TH metabolism in the rat. Goitrogenic and thyrotoxic effects of excessive bromide and perchlorate ions were followed in adult male rats, maintained on diets with various iodine content, ensuring either sufficient iodine supply or mild to severe iodine deficiency. In rats administered with these xenobiotics, we measured a consistent increase in relative weight of the thyroids with increasing time and concentration of applied bromide, and a sharp reduction of the 24-h uptake of [^{131}I]-iodide by their thyroids. In these animals, we also determined a steady decline in serum total thyroxine concentration. At the molecular level, we found, unexpectedly, that the influence of exogenous bromide on the TPO enzyme activity in the rat thyroids was not simply inhibitory. It was more complex, biphasic with regard to the extent of bromide intake in the animals. With the use of several radioanalytical methods, including adapted radiometric determination of TPO enzyme activity, we therefore confirmed and quantified the presumed thyrotoxic effects of xenobiotics bromide and perchlorate ions.*

Key words*: Bromide, perchlorate, thyroid hormones, radiometric determination, xenobiotics*

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1. INTRODUCTION

Iodine forms an irreplaceable part of thyroid hormones (TH) and is one of the essential substances inevitable for the proper development of young mammals. In contrast, not enough information is available on biological function and metabolism of similar halogen bromine. This ubiquitous trace element has not been conclusively shown to perform any essential function in animals, plants or microorganisms (for review see 1, 2). Bromide has been introduced in large amounts into the environment as a salt mining waste and a degradation product of bromine containing fumigants. The main origin of the observed pharmacological and toxicological effects of bromide ion appears to be some interference with the action of other halides, chloride and iodide. Interestingly, the biological behavior of bromide in the thyroid gland, in contrast with other organs and tissues, is more similar to the biological behavior of iodide, rather than of chloride (3). Therefore, goitrogenic effects of bromide may be assumed (4), similar to those of another inorganic goitrogenic agent perchlorate. However, the nature of the toxic effects of bromide on the thyroid gland and the molecular mechanisms of its interference with the biosynthesis of TH has not been explained so far.

Recently, we have shown in the isolated rat thyroids (5) marked effects of exogenous bromide and perchlorate ions on the activity of the key enzyme in TH metabolism, on thyroid peroxidase (TPO). Here, we followed in more details, with the aid of several radioanalytical methods, including an improved radiometric enzyme assay for TPO, the influence of an enhanced bromide and/or perchlorate intakes in the animals, maintained on diets with diverse iodine content, on various aspects of iodine metabolism and, consequently, on TH metabolism in the rat.

2. EXPERIMENTAL

2.1. *Radiometric determination of TPO specific enzyme activity*

For studies at the molecular level, we adapted the radiometric enzyme assay for TPO (6). Firstly, we established proper assay conditions for determination of specific TPO activity in isolated thyroid glands of iodine-deficient rats. The procedure of the radiometric assay for TPO *in vitro* (in isolated microsomal fractions of the rat thyroids) was based on the ability of TPO to oxidize [^{125}I]- or [^{131}I]-iodide in the presence of H_2O_2, which was the immediate oxidant for this reaction and was generated *in situ* by glucose oxidase. TPO further catalyzed subsequent iodination of specific tyrosyl residues bound in a large glycoprotein thyroglobulin, added in the incubated reaction mixtures (7).

2.2. *Experiments performed at the level of whole organism*

Presumed goitrogenic and thyrotoxic effects of excessive bromide and perchlorate ions were followed in adult Wistar rats, maintained under conditions of sufficient iodine supply (on a standard

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diet B) and of iodine deficiency (on a very low-iodine diet R). The animals were administered subchronicaly (in drinking water, for 7 up to 56 days) either with bromide alone (in solutions of Br^- with concentrations of 1, 3 and 5 g L^{-1}) or Br^- in combination with perchlorate (in solution with concentration of 10 g L^{-1}).

The influence of an extremely high bromide intake (> 160 mg bromide per animal per day), and also of lower intakes, on the uptake of [^{131}I]-iodide by the rat thyroids was determined by *in vivo* gamma-spectrometry (using Pb collimator). At the end of each experiment, after decapitation of the animals, relative weights of the isolated thyroids were measured. In blood sera of the rats, concentrations of total thyroxine (tT_4) were determined with the use of commercial radioimmunoassay (RIA) kits.

3. Results and Discussion

3.1. *The influence of exogenous bromide and perchlorate on the TPO enzyme activity*

Our improved radiometric enzyme assay for TPO employed as the measure of peroxidase activity in microsomal fractions of the rat thyroids the amount of radioiodine, incorporated into added pig thyroglobulin during the incubation of microsomes with [^{131}I]-labeled iodide in the presence of hydrogen peroxide, produced *in situ* by glucose oxidase generating system. The incorporated portion of radioiodine was determined by the measurement of ^{131}I radioactivity in the separated fractions: either after TLC separation of the unconsumed ^{131}I-iodide in aliquots of the incubated reaction mixtures containing measured samples, by sophisticated quantification of the corresponding radio-chromatograms (*see* Fig. 1); or simply after precipitation of radiolabeled thyroglobulin with an excess of 10% trichloroacetic acid and separation of the sediment by brief centrifugation. Practically the same results were obtained with the use of both procedures.

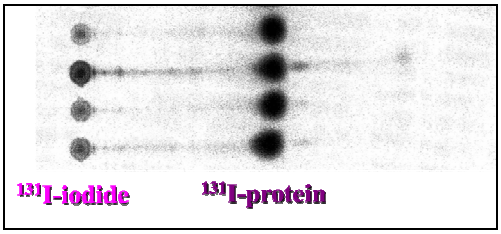

Figure 1. A section of radiochromatogram of four different incubated reaction mixtures containing iodinated thyroglobulin and unconsumed [^{131}I]-radioiodide.

Van Leeuwen et al. (8) stated that excessive exogenous bromide inhibited the TPO activity in the rat thyroid glands. With the aid of the described radiometric assay for TPO, we found that the influence of exogenous bromide on the TPO activity in the rat thyroids was biphasic, in relation to the extent of bromide intake in the animals. An increase (up to 3-fold) in TPO activity was measured in rats with a low or moderate bromide intake (below ca. 60 mg per animal per day), while in animals with very high bromide intake (over ca. 160 mg per animal per day) its thyrotoxic effects prevailed and TPO activity was reduced (Fig. 2).

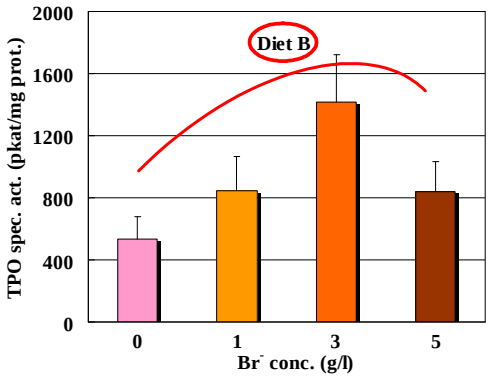

Figure 2. Thyroid peroxidase (TPO) specific enzyme activity (pkat/mg prot.), determined in microsomal fractions of the thyroid glands of rats maintained for up to 56 days on the iodine-sufficient diet B, in dependence on the extent of bromide intake. The rats permanently drank solutions of bromide with different concentrations of 0, 1, 3 or 5 g/l. The results are means ± SD for n = 6–8 male rats.

The inhibitory effect of bromide was markedly increased in animals maintained on very low-iodine diet R (Fig. 3). Interestingly, perchlorate administered subchronically in even higher amounts than the highest quantity of bromide caused in all cases of the rat thyroids elevation of the TPO activity. If used in combination with bromide, perchlorate partially counteracted the effects of bromide (not shown).

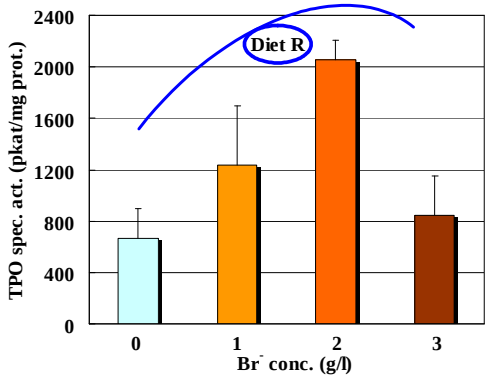

Figure 3. Thyroid peroxidase (TPO) specific enzyme activity (pkat/mg prot.), determined in microsomal fractions of the thyroid glands of rats maintained for up to 56 days on very low-iodine diet R, in dependence on the extent of bromide intake. The rats permanently drank solutions of bromide with concentrations of 0, 1, 2 or 3 g/l. The results are means ± SD for n = 6–8 male rats.

3.2. *Goitrogenic and thyrotoxic effects of bromide and perchlorate ions*

We measured a sharp reduction of the 24-h uptake of [^{131}I]-iodide by the thyroids of rats administered with bromide and perchlorate ions. The suppressive effect of perchlorate on the uptake

of radioiodide was much more pronounced (not shown). A consistent increase in relative weight of the rats' thyroid glands with increasing time and concentration of applied bromide was observed (Fig. 4 and 5).

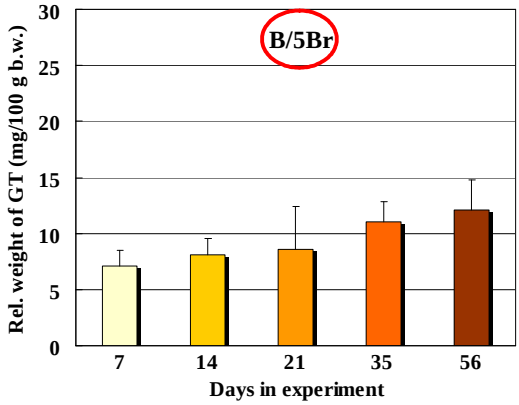

Figure 4. Relative weight (mg/100 g b.w.) of the thyroid glands of rats maintained for up to 56 days on the iodine-sufficient diet B, in dependence on duration of the treatment. The rats drank permanently water containing bromide 5 g/l. The results are means ± SD for n = 8 male rats.

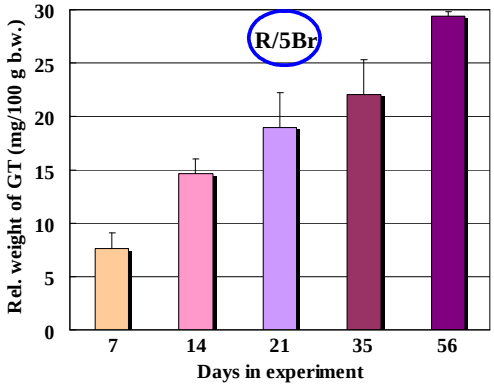

Figure 5. Relative weight (mg/100 g b.w.) of the thyroid glands of rats maintained for up to 56 days on very low-iodine diet R, in dependence on duration of the treatment. The rats drank permanently water containing bromide 5 g/l. The results are means ± SD for n = 8 male rats.

This goitrogenic effect of bromide was much more pronounced in rats maintained on very low-iodine diet R (compare Fig. 4 *vs.* 5). In all animals, we also determined a steady decline in serum total thyroxine concentration. The inhibitory influence of subchronic administration of very high amounts of bromide and perchlorate, or their combination, on the production of TH in the rats is documented in Fig. 6 for animals maintained on the standard diet B and in Fig. 7 for animals maintained on the very low-iodine diet R. Again, the inhibitory effect of perchlorate alone was more pronounced in comparison with bromide.

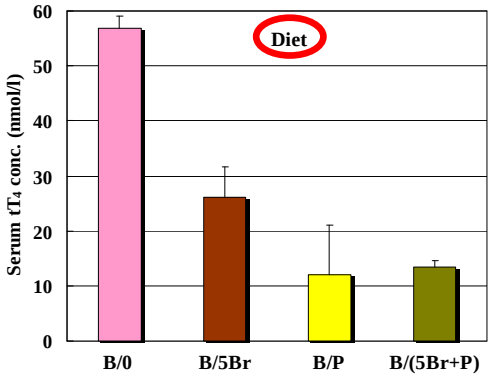

Figure 6. Serum total thyroxine concentrations (tT_4, nmol/l) in rats maintained for up to 56 days on the iodine-sufficient diet B, and drinking water containing bromide, 0, 3 or 5 g/l (0; 3Br; 5Br) or perchlorate, 10 g/l (P), or their combination (5Br+P). The results are means ± SD for n = 6–8 male rats.

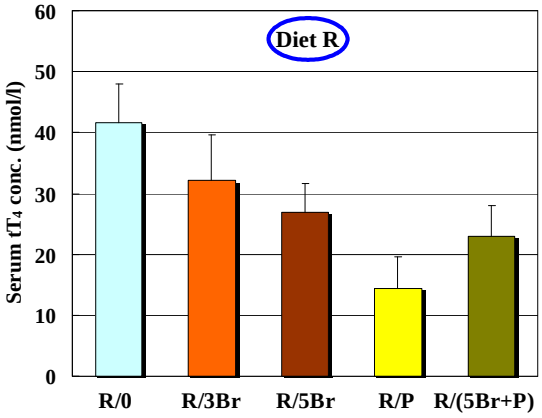

Figure 7. Serum total thyroxine concentrations (tT_4, nmol/l) in rats maintained for up to 56 days on the very low-iodine diet R, and drinking water containing bromide, 0, 3 or 5 g/l (0; 3Br; 5Br) or perchlorate, 10 g/l (P), or their combination (5Br+P). The results are means ± SD for n = 6–8 male rats.

4. Conclusions

The presumed thyrotoxic effects of exogenous bromide and perchlorate ions have been confirmed and quantified. Excessive bromide exerted a biphasic effect on the enzyme activity of TPO, depending on the extent of bromide intake in the animals, and also of iodine content in their diet. In contrast, all the rats that were administered with high amounts of perchlorate were found with elevated TPO activities.

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REFERENCES

1. S. Pavelka, "Bromine" (Chapter 9.3), in Elements and Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance, 2nd ed., vol. III, E. Merian, M. Anke, M. Ihnat and M. Stoeppler, Eds. Weinheim: Wiley-VCH Verlag, 2004, pp. 1445-1455.
2. S. Pavelka, "Metabolism of bromide and its interference with the metabolism of iodine", *Physiol. Res.*, vol. 53 (Suppl. 1), pp. S81-S90, 2004.
3. S. Pavelka, "Iodine transfer through mother's milk: The influence of bromide" (Chapter 20), in Comprehensive Handbook of Iodine, V.R. Preedy, G.N. Burrow and R. Watson, Eds. Oxford: Academic Press, 2009, pp. 199-206.
4. S. Pavelka, "Bromide interference with iodine metabolism: Goitrogenic and whole-body effects of excessive inorganic bromide in the rat" (Chapter 61), in Comprehensive Handbook of Iodine, V.R. Preedy, G.N. Burrow and R. Watson, Eds. Oxford: Academic Press, 2009, pp. 587-595.
5. S. Pavelka, "Radioiodine tracers as useful tools in studies of thyrotoxic effects of exogenous bromide and perchlorate ions", *J. Radioanal. Nucl. Chem.*, vol. 291, pp. 405-408, 2012.
6. T. Nakashima and A. Taurog, "Improved assay procedures for thyroid peroxidase: application to normal and adenomatous human thyroid tissue", *Clin. Chim. Acta*, vol. 83, pp. 129-140, 1978.
7. L.E. Braverman and R.D. Utiger (Eds.), Werner and Ingbar's the Thyroid. A Fundamental and Clinical Text, 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2000.
8. F.X.R. Van Leeuwen, R. Hanemaaijer and J.G. Loeber, "The effect of sodium bromide on thyroid function", *Arch. Toxicol.*, vol. 12 (Suppl.), pp. 93-97, 1988.