PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1167–1172 (1975)

Effect of Butyldiiodohydroxybenzoate on Pituitary-Thyroid Interplay (38710)

YASUSHI IWASA, TOMOMICHI TSUKUI, YUTAKA KANNO, YOICHI KOIZUMI, AKIRA KOJIMA, AND TAKASHI YAMADA

Department of Medicine, Institute of Adaptation Medicine, School of Medicine, Shinshu University, Matsumoto, Japan

Sheahan *et al.* (1) have shown that an analogue of thyroxine (T_4), *n*-butyl-3,5-diiodo-4-hydroxybenzoate (BHDB), depressed the calorigenic action of T_4 in mice. They suggested that inhibition of T_4 action occurred as a result of competition between T_4 and BHDB for a site on an enzyme surface. Subsequent study further suggested that BHDB competed with T_4 for an enzyme which was responsible for the deiodination and conversion of T_4 to triiodothyronine (T_3) (2). If BHDB manifested its action by inhibiting deiodination of T_4, one might expect an increase of plasma T_4 concentration in animals treated with BHDB. In contrast to this speculation, Van Arsdel and Williams (3) have clearly indicated that BHDB markedly depressed plasma labeled T_4 concentration on one hand and increased liver concentration and fecal loss of labeled T_4 on the other hand in rats injected with labeled T_4. Flock *et al.* (4) have suggested that this increase of biliary-fecal excretion of T_4 was due to an increased conjugation of T_4 produced by BHDB. As a possible cause of an increased biliary-fecal excretion of T_4, Hershman further indicated that BHDB competed with T_4 for the binding sites of plasma protein *in vitro* (5).

Since a number of aspects of T_4 metabolism are thus affected by BHDB, it can be expected that pituitary-thyroid interplay may also be affected by BHDB. We have investigated the effect of BHDB on plasma TSH, T_4 and T_3 concentrations and goiter development in rats. To analyze pituitary-thyroid interplay, we have also measured fecal loss of T_4 and T_4-plasma protein interaction under the influence of BHDB.

Materials and Methods. One hundred and seventy-one male Wistar rats, weighing 116–160 g, were used in the experiments. The animals were fed a moderately low iodine diet[1] (MLID) with or without added BHDB (0.05%), methimazole (0.05%), BHDB + methimazole, KI (0.037%) and KI + methimazole for 2–14 days. Radioiodine (0.1 μCi of ^{131}I) was injected intraperitoneally 4 hr before the autopsy. At autopsy, blood was obtained by cardiac puncture using heparinized syringes, and the separated plasma was kept at $-20°$ until use. Thyroid was cleanly dissected out and expressed as mg/100 g body wt. After measurement of thyroidal radioiodine uptake, thyroids were homogenized, and the homogenates were precipitated with 1 ml of 5% trichloroacetic acid (TCA) containing KI. The precipitate was washed three times with a similar volume of TCA. Radioactivity of PB^{131}I was expressed as the percent of total radioactivity of the homogenates. Plasma concentrations of TSH and T_3 were measured by the radioimmunoassays reported previously (6). Plasma T_4 concentration was measured by competitive binding assay (7). Plasma protein-T_4 interaction was measured by muscle (8) and resin uptake of labeled T_4 or assessed by measuring free T_4 (9). Labeled T_4 (^{131}I-T_4) was obtained from Abbott laboratories. The specific activity was 50 mc/mg.

In experiment 4, beginning 14 days after surgical thyroidectomy, 20 animals were injected with 1 ml of T_4 solution containing 2 μg T_4 and 0.5 μCi of labeled T_4 for 3 days and fed a MLID with or without BHDB (0.05%). Twenty-four hour after the first, second and third injection of T_4 solution, feces were collected and blood (0.2 ml) was obtained by cardiac puncture. Amounts of T_4 excreted into feces and plasma T_4 concentration were calculated from the specific activity of the original T_4 solution (10).

[1] Composition of this diet was reported in Endocrinology 79, 138 (1966).

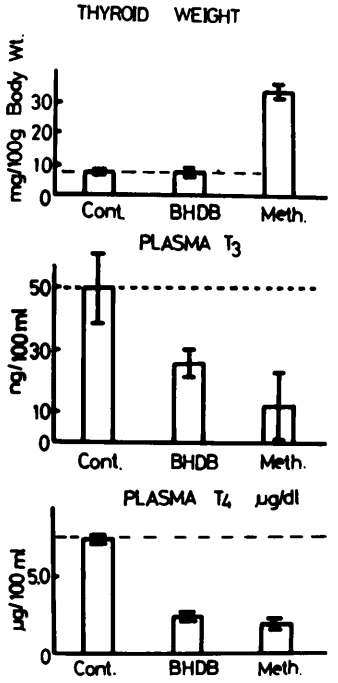

FIG. 1. Effect of BHDB (0.05% in the diet) or methimazole (0.05% in the diet) feeding on thyroid weight and plasma T_3 and T_4 concentrations. Bars and vertical lines indicate mean ± SE calculated from seven determinations. Contr. = control animals, BHDB = animals were fed BHDB for 14 days, Meth. = animals were fed methimazole for 14 days. Statistical analysis: Thyroid weight Cont. vs Meth. $P < 0.001$. Plasma T_3 Cont. vs BHDB $P < 0.05$. Cont. vs Meth. $P < 0.05$. Plasma T_4 Cont. vs BHDB $P < 0.01$. Cont. vs Meth. $P < 0.01$.

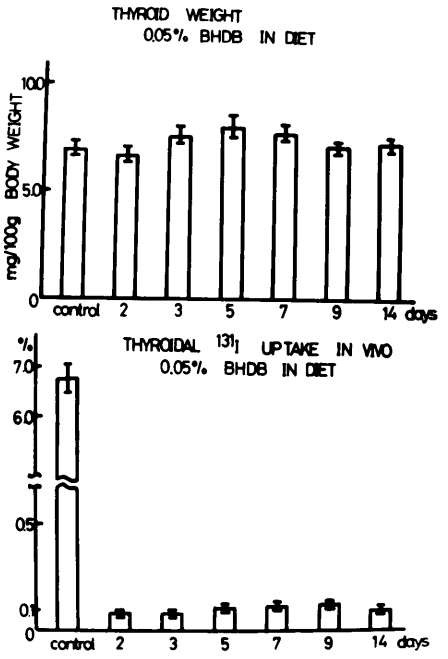

FIG. 2. Animals were fed BHDB for 2–14 days. Radioiodine was injected intraperitoneally 4 hr before autopsy. Bars and vertical lines indicate mean ± SE calculated from five determinations.

Results. Experiment 1. Effects of BHDB and methimazole on thyroid weight and plasma thyroid hormone concentration. In the first part of this experiment, 21 animals were divided into three equal groups and fed a MLID with or without added BHDB (0.05%) or methimazole (0.05%) for 2 wk. BHDB did not affect thyroid weight but significantly depressed plasma T_4 and T_3 concentrations (Fig. 1). Since BHDB contained iodide in its molecule, a possible increase of plasma iodide was measured by autoanalyzer. Since plasma total iodide concentration was 7489 µg/100 ml, and since plasma T_4 or T_3 concentration was less than 3 µg/100 ml, plasma iodide concentration could be extremely high. Methimazole produced goiter and depressed plasma T_4 and T_3 concentrations. T_3/T_4 ratio of methimazole group was not significantly different from that of BHDB group.

In the second step, 35 animals were divided into seven equal groups and fed BHDB (0.05% in the diet) for 2–14 days. As shown in Fig. 2, 4-hr thyroidal radioiodine uptake was depressed markedly throughout the experimental period. However, no significant change in thyroid weight was found throughout the experimental period.

Experiment 2. Effect of BHDB on organic binding of radioiodine by the thyroid gland. Since thyroidal radioiodine uptake was extremely low in BHDB fed animals, thyroidal $PB^{131}I$ was used to assess organic binding of radioiodine. Twenty-five animals were divided into five groups, and fed a MLID with or without BHDB (0.05%) for 1–7 days. In the two groups of the control animals (Fig. 3), more than 92% of total thyroidal radioactivity was $PB^{131}I$. In contrast, 19.8% of total thyroidal radioactivity was $PB^{131}I$ in the group fed BHDB for 1 day. $PB^{131}I$ was about 80% at the second day of

BHDB feeding, and was completely normal at seventh day.

Experiment 3. Effect of BHDB on plasma protein-thyroxine interaction. In the first part of this experiment, 15 pieces of 100 mg diaphragm each were obtained from five rats, and *in vitro* uptake of labeled T_4 by diaphragm was measured in the presence of dilute rat plasma. In the control group, 30% of labeled T_4 was taken up by the muscle. The uptake of labeled T_4 by muscle increased markedly when BHDB was present in the incubation medium (0.1 or 0.5 mg/ml) (Fig. 4).

In the second part of this experiment, plasma was obtained from five rats. BHDB was added to the plasma to obtain concentrations of 2×10^{-6} mole and 2×10^{-4} mole. In the control group, resin uptake of labeled T_4 was 9.07%. A low concentration of BHDB did not affect resin uptake of labeled T_4 but a high concentration of BHDB markedly augmented resin uptake of labeled T_4.

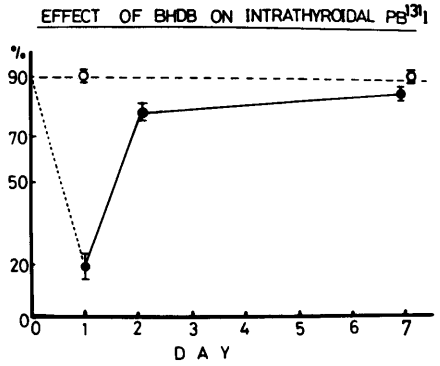

FIG. 3. Animals were fed BHDB for 1–7 days. Radioiodine was injected 4 hr before autopsy. Intrathyroidal $PB^{131}I$ is expressed as percent of total thyroidal radioactivity. Circles and vertical lines indicate mean ± SE calculated from five determinations. Solid circles indicate BHDB group, and open circles indicate control groups.

In the final step, an increase of free T_4 in response to BHDB was studied by using pooled rat plasma. BHDB was added to the plasma to obtain concentrations of 2×10^{-6} mole and 2×10^{-4} mole. A large dose of BHDB elevated markedly the free fraction of T_4, but a small dose of BHDB was without effect.

Experiment 4. Effect of BHDB on fecal loss of thyroxine and plasma thyroxine concentration in thyroidectomized rats treated with thyroxine. Twenty four hours after the second and third injections of T_4, blood was obtained to assess plasma T_4 concentration. As shown in Fig. 5, plasma T_4 concentration was significantly less in BHDB group than in the control.

In the first day, fecal radioactivity was slightly more in BHDB group than in the control group. In the second and third day of the experiment, fecal radioactivity was apparently more in the BHDB group than in the control.

Experiment 5. Effect of combined use of BHDB and methimazole on plasma TSH and goiter development. In the first part of this experiment, 24 animals were divided into four equal groups and were fed a MLID with or without methimazole, BHDB and BHDB + methimazole for 14 days. As shown in the upper panel of Fig. 6, methimazole administration markedly increased plasma concentration of TSH. As the result, an increase of thyroid weight was apparent.

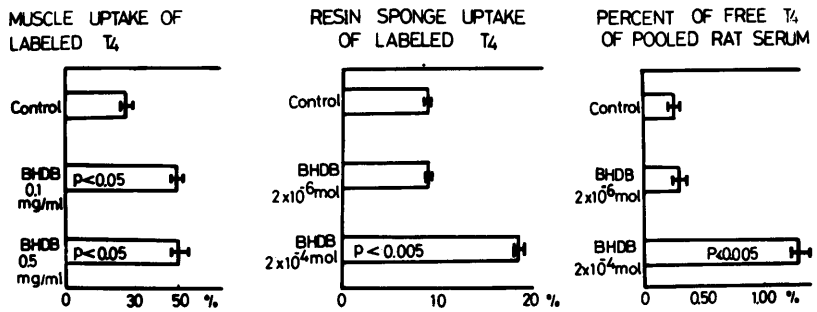

FIG. 4. Bars and horizontal lines indicate mean ± SE calculated from five to seven determinations. *P* value indicates the significance of difference when compared with control.

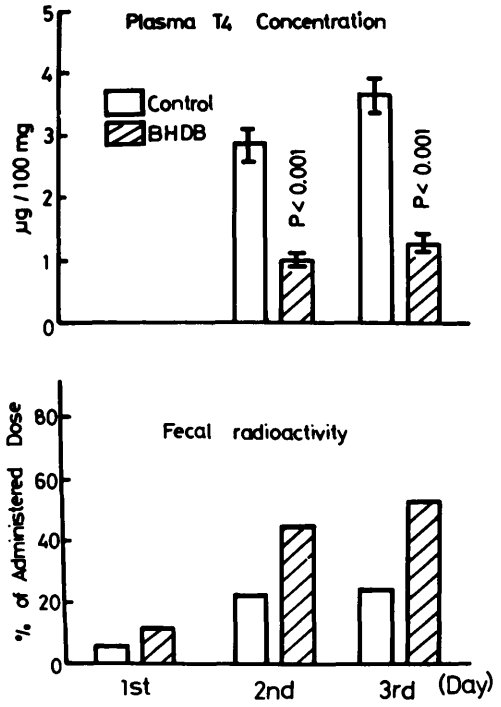

FIG. 5. Effect of BHDB feeding on plasma T_4 concentration and fecal loss of T_4 in thyroidectomized animals treated with T_4. In upper panel, bars and vertical lines indicate mean ± SE calculated from 10 determinations. In lower panel, bars indicate mean of the determinations.

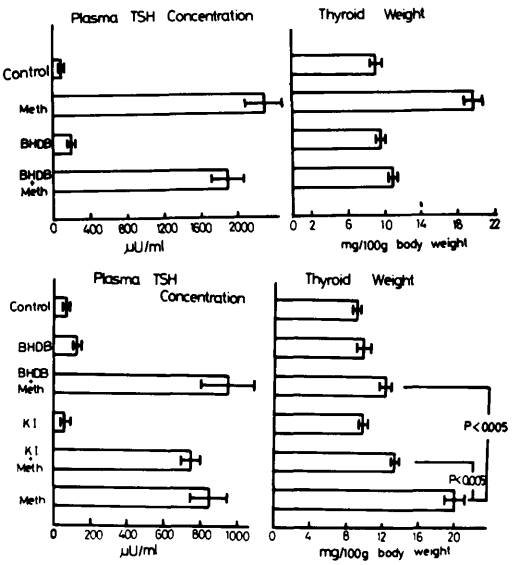

FIG. 6. Effect of methimazole, BHDB, BHDB + methimazole, KI, KI + methimazole on plasma TSH and thyroid weight. Meth = animals were fed methimazole (0.05%) for 10–14 days, BHDB + Meth = animals were fed BHDB (0.05%) and methimazole for a similar period. Bars and horizontal lines indicate mean ± SE calculated from six determinations.

BHDB slightly increased plasma TSH concentration and thyroid weight, but the difference was not statistically significant. When both drugs were combined, plasma TSH concentration was comparable to that produced by methimazole alone. In spite of this increase of plasma TSH, thyroid weight did not significantly increase.

In the second part of this experiment, 36 animals were divided into six equal groups and were fed a MLID with or without added drug for 10 days. Again plasma TSH concentration was slightly more in BHDB group. As expected, methimazole increased plasma TSH concentration and thyroid weight (lower panel of Fig. 7). Combined use of methimazole with BHDB also produced an increase of plasma TSH similar to that produced by methimazole alone. However, thyroid weight was markedly less than that produced by methimazole. Combined use of methimazole with KI produced an increase of plasma TSH concentration which was comparable to that found in the methimazole group. Thyroid weight was significantly less than that produced by methimazole.

Discussion. Our present study clearly indicated that BHDB, an analogue of T_4, depressed plasma T_4 and T_3 concentrations, the magnitude of decrease and T_3/T_4 ratio being comparable to those produced by methimazole. Like methimazole (11, 12), BHDB suppressed thyroidal radioiodine uptake significantly. In contrast to methimazole, however, BHDB failed to increase plasma TSH concentration and to produce goiter in the presence of low plasma T_4 and T_3 concentrations. These data suggested that BHDB lowered plasma thyroid hormone concentrations in a manner quite different from that of methimazole.

To confirm this hypothesis, our first attempt was to study whether BHDB blocked organic binding of iodine by the thyroid gland or not. Since thyroidal radioiodine

uptake was extremely low in BHDB-fed animals, thyroidal $PB^{131}I$ was used to assess organic binding of iodine. As shown in Fig. 3, thyroidal organic binding of iodine was blocked only for 1 day and this block disappeared thereafter in spite of continuous administration of BHDB. Since BHDB contained iodide in its molecule, we checked plasma iodide concentration and found that tremendous amounts of iodide were circulating in the blood in rats treated with BHDB for 14 days. Under such condition, it is reasonably suspected that excess iodide blocked organic binding of iodine for a short period as reported previously (13, 14). It is therefore concluded that BHDB lowered plasma T_4 and T_3 concentrations without blocking organic binding of iodine in the thyroid.

Second, we considered the possibility that BHDB lowered plasma T_4 and T_3 concentrations by displacing T_4 from the binding sites of plasma protein, since free T_4 is available for degradation and excretion. Our *in vitro* study indicated that BHDB augmented muscle uptake and resin uptake of labeled T_4 in the presence of plasma protein. In agreement with the previous report (5), this indicated that BHDB displaced T_4 from the binding site of plasma protein. In fact it was found that BHDB increased plasma free fraction of T_4 *in vitro*. Since previous studies (15, 16) have shown that a number of compounds reduced plasma thyroid hormone concentration by augmenting fecal loss of free T_4, we have measured fecal loss of T_4 in thyroidectomized rats treated with T_4. As expected (3, 4), BHDB apparently lowered plasma T_4 concentration and augmented fecal loss of thyroid hormone. It is therefore concluded that BHDB lowered plasma thyroid hormone concentration by augmenting fecal loss of thyroid hormone. Since plasma TSH concentration was normal even under such low plasma thyroid hormone concentrations, it seems that plasma free thyroid hormone concentrations were normal because of displacement of thyroid hormone from the binding protein.

Finally, we considered the possibility that BHDB does show some influence on the development of goiter produced by methimazole because of the unique action of excess iodide on the thyroid. Quite interestingly, BHDB depressed the development of goiter produced by methimazole without affecting plasma TSH concentration. Since Abbassi and McKenzie (17) have found that excess iodide produced a similar effect, and since plasma iodide concentration was extremely high in rats fed BHDB, we have further studied the effect of excess iodide on the development of goiter produced by methimazole. In agreement with the report by Abbassi and McKenzie (17), excess iodide depressed the development of goiter produced by methimazole without affecting plasma TSH concentration. It is therefore concluded that BHDB depressed the development of goiter by releasing excess iodide from the degradation of BHDB.

Summary. The effect of BHDB, an analogue of thyroxine, on the pituitary-thyroid system was studied in the rat. BHDB produced low plasma T_4 and T_3 concentrations similar to those produced by methimazole, but failed to elevate plasma TSH and to produce goiter because of displacement of T_4 from the binding protein. Low plasma thyroid hormone concentrations were due to an increase of fecal loss of thyroid hormones. By releasing excess iodide, BHDB blocked the development of goiter produced by methimazole.

1. Sheahan, M. M., Wilkinson, J. H., and Maclagan, N. F., Biochem. J. **48**, 188 (1951).
2. Wilkinson, J. H., Sprott, W. E., Bowden, C. H., and Maclagan, N. F., Biochem. J. **56**, 215 (1954).
3. Van Arsdel, P. P., Jr., and Williams, R. H., J. Biol. Chem. **223**, 431 (1956).
4. Flock, E. V., Bollman, J. L., Owen, C. A., Jr., and Zollman, P. E., Endocrinology **77**, 303 (1965).
5. Hershman, J. M., Endocrinology **72**, 799 (1963).
6. Kojima, A., Hershman, J. M., Azukizawa, M., and DiStefano, J. J., III, Endocrinology **95**, 599 (1974).
7. Murphy, B. P., J. Lab. Clin. Med. **66**, 161 (1965).
8. Yamada, T., Takemura, Y., Kobayashi, I., and Shichijo, K., Endocrinology **79**, 849 (1966).
9. Ingbar, S. H., Braverman, L. E., Dawber, N. A., and Lee, G. Y., J. Clin. Invest. **44**, 1679 (1965).

10. Yamada, T., Endocrinology **82**, 327 (1968).
11. Astwood, E. B., Bissell, A., and Hughes, A. M., Endocrinology **37**, 456 (1945).
12. Iino, S., Acta Endocrinol. **36**, 212 (1961).
13. Wolff, J., and Chaikoff, I. L., J. Biol. Chem. **172**, 855 (1948).
14. Yamada, T., Iino, S., and Shichijo, K., Endocrinology **72**, 83 (1963).
15. Van Middlesworth, L., Endocrinology **61**, 570 (1957).
16. Tsukui, T., Takasu, N., Onaya, T., and Yamada, T., Proc. Soc. Exp. Biol. Med. **146**, 494 (1974).
17. Abbassi, V., and McKenzie, J. M., Endocrinology **81**, 871 (1967).

Received September 6, 1974. P.S.E.B.M. 1975, Vol. 148.