EFFECTS OF TWO DIFFERENT PCDD-COCKTAILS ON ACTIVITIES OF THYROID-RESPONSIVE AND THYROID-NONRESPONSIVE HEPATIC ENZYMES IN EUTHYROID OR HYPOTHYROID C57BL/6J MICE

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) administered to euthyroid animals are known to act as agonists as well as antagonists of thyroid hormones, resulting in a hyperthyroid and a hypothyroid state respectively[1]. Thyroid hormones exert a pervasive control on metabolic processes in the sense that a small dose of a hormone produces anabolic effects, whereas a large dose produces opposite, catabolic effects. A similar biphasic phenomenon is observed when PCDDs are administered over a wide dose range. Most scientists investigating the mechanism of action of PCDDs on intermediary metabolism have used high, acutely toxic doses leading to anorexia, wasting and death of the animals. Lower doses of PCDDs, however, exert anabolic effects in certain organs as for example in the liver, where an increased protein synthesis and cell hypertrophy can be seen. The purpose of the present study was to examine the effects of single relatively low doses of PCDDs on hepatic key enzymes of carbohydrate metabolism in dioxin-sensitive C57BL/6J mice. It was of further interest to determine, if and how these effects were modulated by the thyroid.

Materials and Methods

Female C57BL/6J mice were raised either under normal conditions or were functionally thyroidectomized with 7.5 mCi ^{131}J/kg given i.p. at the age of 7 weeks. Thyroxine (T_4) in serum was determined by radioimmunoassay. 2,3,7,8-TCDD alone or in combination with two different mixtures of PCDDs were applied in a single dosing regimen. PCDD mix I was composed of 4CDD (1%), 5CDD (6%), 6CDD (12%), 7CDD (26%) and OCDD (55%). The composition of PCDD mix II was: 4CDD (5%), 5CDD (17%), 6CDD (31%), 7CDD (34%) and OCDD (13%). 100 µg of PCDD I contained 0.65 µg I-TE, 100 µg of PCDD II 1.4 µg I-TE. The mice were dosed by oral gavage after dissolving the substances in peanut oil. 10 euthyroid and 10 thyroidectomized mice per dosing group were given either 15 µg/kg 2,3,7,8-TCDD, 15 µg/kg 2,3,7,8-TCDD plus 1250 µg/kg PCDD I or 15 µg/kg 2,3,7,8-TCDD plus 500 µg/kg PCDD II. Food and water were offered ad libitum. Mice in the respective experimental groups were sacrificed at day 4,

day 7 or day 30 after dosing. The activities of the following enzymes in high speed supernatant of homogenates were determined: pyruvate kinase (PK; EC 2.7.1.40), enolase (EN; EC 4.2.1.11), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), malate dehydrogenase (=malic enzyme, ME; EC 1.4.1.3), NAD-dependent glycerol-3-phosphate dehydrogenase (G3PDH; EC 1.1.1.8), fructose-1,6-bisphosphatase (FBP; EC 3.1.3.11), alanine aminotransferase (ALT; EC 2.6.1.2), glutamate dehydrogenase (GLDH; EC 1.4.1.3), aspartate aminotransferase (AST; EC 2.6.1.1), lactate dehydrogenase (LDH; EC 1.1.1.27) and alkaline phosphatase (AP; EC 3.1.3.1). At the respective time points the following parameters in serum were also measured: AST, GLDH, LDH, FBP, ME, glucose, triglycerides, cholesterol, ß-hydroxybutyrate, acetoacetate, lactate, glutamate, glutamine. Data were analysed using the single factor analysis of variance followed by multiple comparison procedure (Tukey test) at various significance levels.

Results

Administration of PCDDs to mice in all treatment groups caused no decrease in body weight. In both euthyroid and thyroidectomized animals an increase in liver mass and a decline of thymus mass was apparent at day 4 in all treatment groups persisting up to day 30. Thyroidectomy lowered the thyroxine concentration in serum from 75 nmol/l to about 25 nmol/l. A further decrease due to PCDD application was observed in thyroidectomized animals. Cholesterol in serum was approximately twice as high in thyroidectomized controls, triglycerides however had only half the concentration as compared to euthyroid controls. The activities of **PK** and **EN**, which are important enzymes for substrate-level phosphorylation within the glycolysis, were significantly lowered by PCDDs in both euthyroid and hypothyroid mice. The effect on **PK** occurred earlier than that on EN (Figures 1, 2). For both enzymes it persisted up to day 30. **FBP** activity was increased following all PCDD treatments and for mice of either thyroid state. The maximal activity appeared on day 4. It diminished slightly through day 7 to day 30 (Figure 3). The increased activity of **FBP** in serum was most pronounced on day 7. In euthyroid mice liver **G6PDH** was more active than in thyroidectomized animals and a significant increase compared to respective controls was observed in livers of thyroidectomized animals at all time points after dosing, reaching maximal values at day 30 (Figure 4). The activity of **ME** was increased by PCDDs in a similar fashion but also only in thyroidectomized animals (Figure 5). In addition to this increase in the high-speed supernatant of liver homogenates, an increase in serum activity of **ME** could be observed in hypothyroid mice but only on day 7. **G3PDH** activity was higher in thyroidectomized than in euthyroid mice. In both thyroid states, as well as in all treatment groups, its activity decreased beginning at day 4 (Figure 6). **ALT** and **AST** had higher activities in thyroidectomized than in euthyroid mice. No dosing regimen altered the activity of **ALT** in animals of either thyroid state or at any time point. In the case of **AST**, there was an initial decrease in activity in the liver homogenate of euthyroid

Legend for Figures 1 to 6

Left part of the figures compares the enzyme activity of undosed euthyroid and thyroidectomized mice at the time of sacrifice. Right part of the figures shows the relative enzyme activity at the indicated days after dosing in comparison to the respective controls. 1= 15 µg 2,3,7,8-TCDD; 2= 15 µg 2,3,7,8-TCDD + 1250 µg PCDD I (=23,16 µg I-TE); 3= 15 µg 2,3,7,8-TCDD + 500 µg PCDD II (=22,0 µg I-TE).
Significance level: · $p < 0.05$; • $p < 0.01$; • $p < 0.001$.

Fig. 1: PK

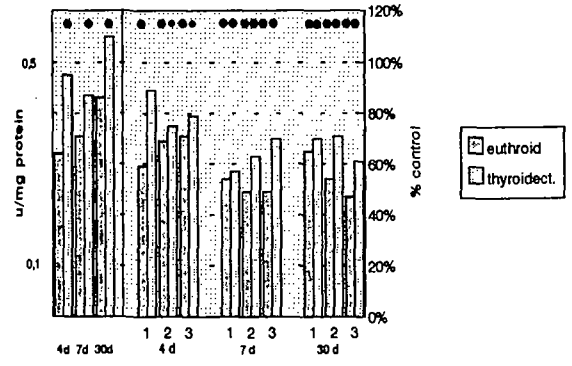


Fig. 2: EN

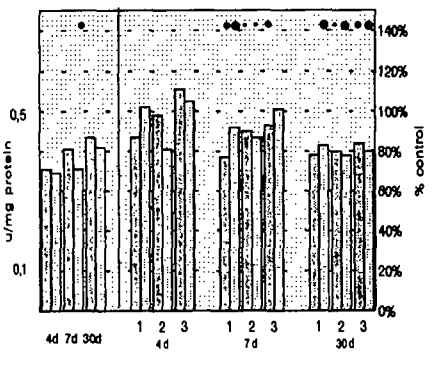


Fig. 3: FBP

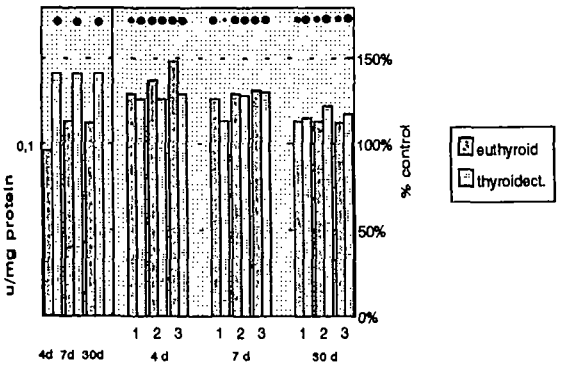


Fig. 4: G6PDH

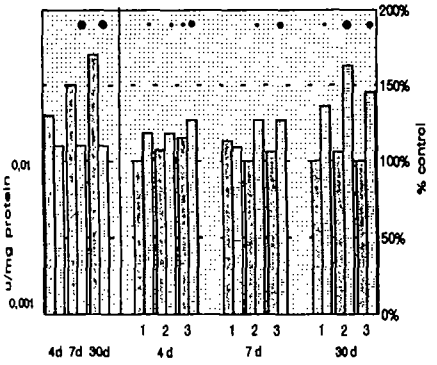


Fig. 5: ME

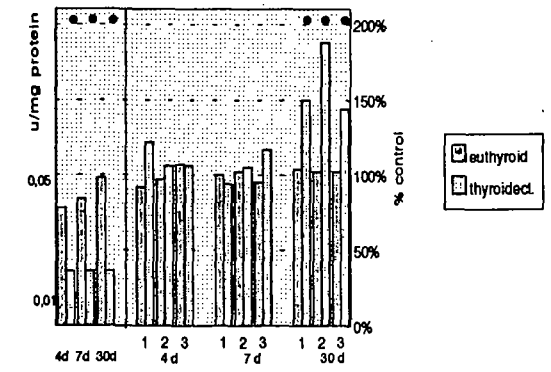
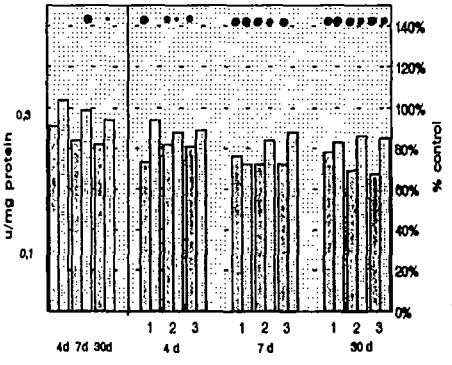


Fig. 6: G3PDH



animals. The activity of **GLDH** was lowered significantly in the liver homogenate of thyroidectomized animals in all dose groups, with the maximal decrease at the end of the experimental period. The thyroid-nonresponsive activities of **AP** or **LDH** were not altered by PCDDs.

Conclusions

The results of this study indicate that PCDDs act on the thyroid hormone-responsive enzymes **G6PDH** or **ME** in thyroidectomized mice as thyroid hormone agonists. The results with subtoxic amounts of TCDD or PCDDs further indicate that the key enzymes of glycolysis, **En** and **PK**, were inhibited already by single doses of these substances. This corresponds to the results obtained with a treatment schedule of administering low doses of PCDDs up to their steady state concentration in the liver[2]. The inhibition of these enzymes will impair the flux of glycolytic intermediates directed towards pyruvate. Due to the PCDD-caused increase of **FBP** activity in euthyroid as well as in thyroidectomized mice, this important enzyme in gluconeogenesis will direct the glycolytic intermediates towards glucose-6-phosphate rather than to pyruvate. PCDDs act on **FBP** as thyroid antagonists which reduce the efficiency of glycolysis. The increased activities of **ME** and **G6PDH** in thyroidectomized mice generate higher amounts of NADPH, providing reducing equivalents for lipid synthesis as well as for hepatic monooxygenation. **G3PDH**, whose activity is impaired by PCDDs in the sense of thyroid hormone antagonists and irrespective of the thyroid state of mice, is a key enzyme favoring the ketogenic rate from glycerol. However, since the amounts of ß-ketoglutarate and acetoacetate are not increased in serum, the reuse of glycerol-3-phosphate for the synthesis of glycerides or phosphatides is more plausible than its entrance into the glycolytic pathway. Euthyroid mice appear to gain reducing equivalents for synthetic processes from the reaction of **GLDH** coupled to the transaminase reactions rather than through the reactions catalyzed by **ME** or **G6PDH**. The promoting actions of PCDDs on the activity of enzymes in the sense of thyroid hormone agonists or antagonists may be caused by an altered gene expression as is known for some protooncogenes by thyroid hormones[3]. In some cellular models, a transformation of protooncogenes by chemicals is permitted by thyroid hormones[4]. Since PCDDs also induce various protooncogenic products, the altered metabolic rate as induced by these substances may be the basis for their carcinogenic co-action.

References

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