DISTRIBUTION OF THE FLAME RETARDANT TETRABROMO-BISPHENOL A IN PREGNANT AND FETAL RATS AND EFFECT ON THYROID HORMONE HOMEOSTASIS

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Introduction

Tetrabromobisphenol A (TBBPA) is the largest volume brominated flame retardant in use today. It has been detected in river sediments in Japan already in the 1980s[1], and recently in air sampled from office and computer rooms[2] and even in human serum[3]. Previous results from our laboratory revealed that TBBPA has a very high potency to competitively bind to human transthyretin (TTR) *in vitro*[4], the plasma transport protein for the thyroid hormone thyroxine (T_4). Since TTR is suggested to play a role in mediating the delivery of T_4 across the blood-brain barrier and in maternal-to-fetal transfer over the placenta[5,6], the question was raised whether the high affinity binding of TBBPA to TTR would lead to a selective delivery of this compound over the placenta to the fetal compartment and, especially, the brain. The aim of this study was to investigate (i) the distribution of TBBPA in the pregnant rat and (ii) the effect of *in utero* TBBPA exposure on thyroid hormone homeostasis.

Materials and Methods

Chemicals

[^{14}C]-Ring labelled TBBPA was prepared as described before[7]. Soluene-350 and Hionic Fluor were purchased from Packard, isopropanol and H_2O_2 from Merck.

Animal experiment

Pregnant Wistar WU rats (Charles River, Germany) were orally exposed to 5 mg [^{14}C]-TBBPA per kg body weight from day 10 to day 16 of gestation. Control rats received the vehicle (corn oil) only. Faeces and urine were collected daily, starting on day 10 of gestation (GD10). On GD20, rats were sacrificed under ether anaesthesia and maternal blood was collected via the *vena cava*. Several tissues and the carcasses from dams and fetuses were collected for radioactivity measurements.

Radioactivity measurements

Faecal samples were grind with a mortar under liquid nitrogen, and approximately 50 mg was dissolved in 1 ml Soluene-350 at 50ºC during 1 hour. After addition of 0.5 ml isopropanol and another two hours incubation at 50ºC the samples were bleached by H_2O_2, mixed with 20 ml

Hionic Fluor and radioactivity was measured by LSC. Tissues (60-100 mg) and plasma (25-50 µl) were solubilised in 1 ml Soluene-350, bleached with H_2O_2 and mixed with 20 ml Hionic Fluor prior to LSC.

Biochemical analyses

Plasma total T_4, free T_4 and total T_3 were measured using Amerlite chemiluminescence kits. Thyroid stimulating hormone (TSH) was measured with a specific rat TSH immunoassay. Both kits were purchased from Amersham, UK. Type II deiodinase activity in brain and T_4 uridine diphosphoglucuronyl transferase activity (T_4-UDPGT) in liver was measured as described before[8]. Plasma protein separation was performed by polyacrylamide gel electrophoresis (PAGE)[9], and *in vivo* ^{125}I-T_4-competition binding on transthyretin was determined in maternal and fetal plasma[10,11].

Results and Discussion

Most of the radioactivity (79.8%) was excreted in the faeces 48 hr after the last dose (table I). Limited amounts were excreted in urine (< 0.2% of the dose, not shown). Only 1.2% of the dose remained in the tissues of dams and fetuses, with distributions as shown in table I. These data are in agreement with earlier studies from Larsen *et al.* in non-pregnant rats[12]. Highest levels in maternal tissues could be detected in carcass (0.37%) and liver (0.26%). The total amount of radioactivity found in fetuses is about 40% of the maternal dose, with the highest levels also in carcass (0.07%) and liver (0.06%). There is no selective accumulation of TBBPA-related radioactivity in the fetal brain.

Table I. Distribution of ^{14}C-TBBPA derived radioactivity in pregnant rats orally dosed from GD10 to GD16 (data are expressed as percentage of total given dose). Note that amounts in tissues are given as $*10^{-3}$%).

Tissue/organ	Maternal tissue ($*10^{-3}$%)	Fetal tissue ($*10^{-3}$%)	Faeces (%, cumulative)
Carcass	368 ± 36	67.6 ± 3.31	GD11 22.2 ± 3.3
Liver	256 ± 1.39	62.1± 2.3	GD12 45.4 ± 28.7
Skeletal muscle	59.0 ± 46.0	n.d.	GD13 58.5 ± 15.7
Abdominal fat	15.6 ± 4.3	n.d.	GD14 64.0 ± 11.3
Placenta	14.4 ± 11.8	n.d.	GD15 75.3 ± 1.2
Total plasma	7.98 ± 4.5	0.097 ± 0.014	GD16 77.1 ± 2.4
Forebrain	1.50 ± 0.55	1.34 ± 1.11	GD17 76.7 ± 1.3
Kidney	1 ± 0.05	0.22 ± 0.07	GD18 79.8 ± 1.5
Lungs	0.5 ± 0.2	0.99 ± 0.61	GD19 80.4 ± 1.4
Spleen	0.44 ± 0.07	n.d.	GD20 80.6 ± 1.3
Heart	0.39 ± 0.18	0.12 ± 0.07	
Cerebellum	0.3 ± 0.2	0.63 ± 0.12	
Pancreas	0.2 ± 0.1	n.d.	
Thymus	0.08 ± 0.04	n.d.	
Total amount of radioactivity	830 ± 22	340 ± 130%	

No effects could be observed on maternal body weight, total litter size and number of resorptions after oral exposure to 5 mg TBBPA per kg body weight from gestation day 10 to 16. TBBPA exposed dams showed a significant increase in thymus to body weight ratio of 16.8% compared to

control. The average fetal weight of TBBPA treated animals was also slightly but significantly increased by 7.3%.

Despite the very high *in vitro* T_4-TTR binding competition potency (10.6 times more potent than thyroxine itself) no effects were observed on plasma total T_4 and free T_4 in both dams and fetuses, and also no effects on maternal total T_3 levels (table II). Brain type II deiodinase activity and hepatic T_4 UDPGT-activity were also not affected in both dams and fetuses.

Table II. Effect of maternal TBBPA exposure on thyroid hormone levels and TSH in the dams and fetuses. Data are presented as mean ± SEM (n = 4). * = significantly different ($p < 0.05$) from control).

	exposure	TT4 (nM)	FT4 (pM)	TT3 (nM)	TSH (ng/ml plasma)
Maternal	Corn oil	17.0 ± 2.3	9.3 ± 2.1	1.04 ± 0.13	18.14 ± 1.96
	TBBPA	16.1± 2.1	8.6 ± 1.5	0.87 ± 0.15	22.03 ± 3.72
Fetal	Corn oil	4.6 ± 0.1	2.7 ± 0.4	n.d.	1.64 ± 0.36
	TBBPA	4.2 ± 2.6	2.5 ± 0.2	n.d.	4.86 ± 1.37*

Strikingly, TSH levels were increased by 21.6% (not significantly) in maternal and even 196% (significantly) in fetal plasma. This may indicate that the TSH stimulation has lead to normal T_4 levels in fetal and maternal tissues. However, another compound with a very high *in vitro* T_4-TTR binding potency, the PCB-metabolite 4-OH,-2,3,3',4',5-pentachlorobiphenyl, was shown to decrease fetal T_4 levels by 90% in the same experimental setup[4], without an increase in TSH, suggesting that TBBPA acts via another mechanism. It is also possible that the label detected in the fetal and maternal tissues is not reflecting TBBPA-derived radioactivity, but a debrominated product of TBBPA, since we have not verified the nature of the label yet.

PAGE-analysis of fetal and maternal plasma revealed that no ^{14}C-label could be detected on transthyretin (data not shown). Also, the binding of ^{125}I-T_4 with fetal and maternal plasma proteins showed no shift in ^{125}I-radioactivity from the position of TTR to the front of the gel (figure 1), which would be expected if TBBPA was located on TTR. These results indicate that there is an apparent lack of TBBPA binding to TTR *in vivo*, and explain the normal levels in total and free T_4.

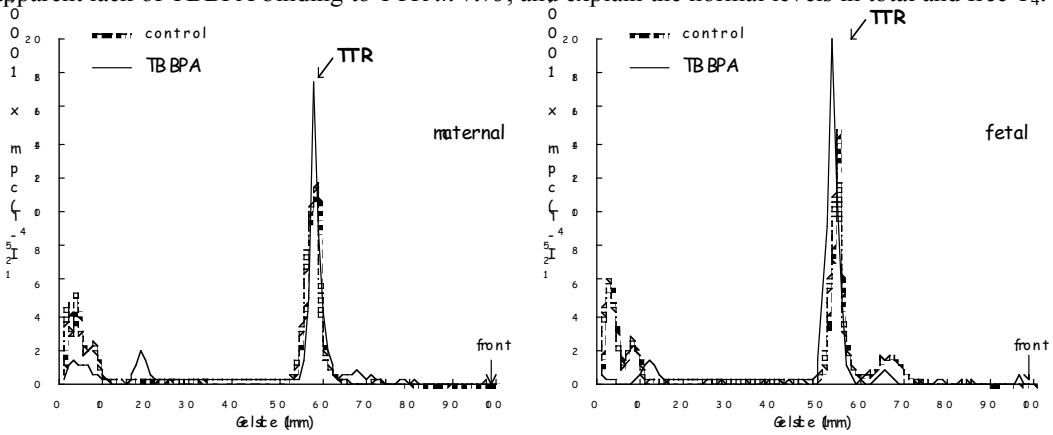

Figure 1. Distribution of radioactivity in maternal (left) and fetal (right) plasma after *in vitro* incubation with ^{125}I-T_4. Dotted line: plasma from corn oil treated animals; solid line: plasma from TBBPA treated animals. Position of the TTR reference is indicated in the figure.

In conclusion, these results indicate that despite a very high *in vitro* potency of TBBPA to compete with thyroxine for binding to TTR, this can not be observed *in vivo*. This most likely can be explained by the high faecal elimination of TBBPA via oral exposure. Of the administered dose, only 0.83% can be detected in maternal and 0.34% in fetal tissues. It may also be explained by the fact that TBBPA is rapidly debrominated and the label we detect in maternal and fetal tissues may not be derived from TBBPA itself but from its debrominated product. We are currently investigating the nature of the label found in maternal tissues to verify this.
The effects of TBBPA on the growth of the fetuses and maternal thymus weight can not be explained at the moment. Further research is needed to elucidate the observed effects of TBBPA on TSH production at the level of the pituitary (TSH secretion) or hypothalamus (secretion of thyrotropin releasing hormone, TRH).

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