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Different Tissue Distribution, Elimination, and Kinetics of Thyroxine and Its Conformational Analog, the Synthetic Flavonoid EMD 49209 in the Rat*

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ABSTRACT

The synthetic flavonoids EMD 23188 and EMD 49209, developed as T_4 analogs, displace T_4 from transthyretin, and *in vitro* they inhibit 5′-deiodinase activity. *In vivo* EMD 21388 causes tissue-specific changes in thyroid hormone metabolism. In tissues that are dependent on T_3 locally produced from T_4, total T_3 was diminished. It was not known whether it was the presence of EMD interfering with 5′-deiodinase type II in tissues or the decreased T_4 (substrate) availability that caused the lowered T_3. To study whether the flavonoids enter tissues and, if this were the case, whether they enter tissues similarly, [^{125}I]EMD 49209 together with [^{131}I]T_4 were injected into female rats and rats pretreated with EMD 21388. Tissues were extracted and submitted to HPLC. [^{125}I]EMD 49209 disappeared quickly from plasma and enters peripheral tissues; peak values were reached after 0.25–0.5 h. Then [^{125}I]EMD 49209 appeared in the intestines (after 6 h 40% of the dose). Tissue uptake of [^{131}I]T_4 was very rapid. EMD 21388 pretreatment caused an increase in the excretion of [^{125}I]EMD 49209 into the intestines (40% after 0.25 h). The uptake of [^{131}I]T_4 increased, but not high enough to ensure normal tissue T_4 concentrations. In the 5′-deiodinase type II-expressing tissues, no [^{125}I]EMD 49209 could be detected. We conclude that the decrease in T_3 locally produced from T_4 is caused by the shortage of T_4 as substrate and not to a direct effect of EMD on the activity of 5′-deiodinases I and II. (*Endocrinology* **138:** 79–84, 1997)

FLAVONOIDS, naturally occurring secondary metabolites and dyes in plants, have been shown to be the main constituents of folk remedies used for the treatment of thyroid and other hormonal disorders (1–4). Early studies indicated that natural flavonoids are able to induce goiter. Together with an increase in thyroid weight, a reduced iodide organification was found (5). Recently, it has been shown that flavonoids as constituents in a staple food, the African millet, are the cause of goiter and associated disorders (6). To study the interactions of flavonoids with the iodine-thyroid hormone system, a model was chosen in the form of synthetic flavonoids. These flavonoids were developed by means of molecular drug design (1, 2, 4). The synthetic flavonoids 3-methyl-4′,6-dihydroxy 3′,5′-dibromo-flavone (EMD 21388) and 3-methyl-4′,6-dihydroxy 3′,5′-diiodo-flavone (EMD 49209), both T_4 analogs, are potent inhibitors of the binding of T_4 to transthyretin (TTR), but not to other plasma proteins (1, 2, 7, 8). These specific TTR competitors are useful tools to study distribution and kinetics of T_4, as the synthetic flavonoids redistribute endogenous T_4 without the need to add exogenous (pharmacological) T_4.

EMD 21388 *in vitro* inhibits 5′-deiodinase type I in rat liver microsomes (9). *In vivo* studies with long term treatment of rats with EMD 21388 iv (20 μmol/kg BW·day) showed that this synthetic flavonoid interferes with thyroid hormone secretion, turnover, and metabolism in several tissues in different ways (10–13). From the double isotope equilibrium study (10), it was clear that in all tissues investigated T_4 concentrations decreased. In those tissues that express the 5′-iodothyronine deiodinase isoforms, liver, pituitary, testis, thymus, brown adipose tissue, brain, cerebellum, and hypothalamus, the amounts of T_3 locally produced from T_4 were decreased. The ratio of [^{125}I]T_3/[^{125}I]T_4 was unchanged. It was not known whether these effects of EMD 21388 were due to inhibition of deiodinase isoforms or to a shortage of the amount of substrate, T_4. As there were tissue-specific changes that differed from the effects expected from work *in vitro*, especially regarding the activity of the different 5′-deiodinase isoforms, it appeared important to assess whether the flavonoid enters into all organs and, if so, whether it does so similarly in all tissues.

To investigate this phenomenon we studied the distribution of EMD 49209, a congener of EMD 21388 in which bromide is replaced by iodide substituents on the phenolic ring. These iodide atoms were radiolabeled by an exchange labeling reaction with ^{125}I. We used this [^{125}I]radioactive flavonoid together with [^{131}I]T_4 to address the question of the changes in the different T_4 distribution pools in the intact animal during long term treatment with the flavonoid. We compared their tissue distributions for up to 6 h after a bolus injection in rats pretreated with EMD 21388 iv for 14 days and in rats receiving the vehicle without EMD 21388 pretreatment.

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Materials and Methods

Animals

The experiments were approved by the local committee on animal care. Two groups of female Wistar rats (CPB/WU, Iffa Credo, Brussels, Belgium), about 180 g BW, were used. The rats were individually housed at 22 C, with alternating 14-h light and 10-h dark periods. They were fed the American Institute of Nutrition Diet (14). Potassium iodide was added to the drinking water (10 mg/liter). One group (Exp B; n = 7) was given EMD 21388 twice a day iv via a cannula that was inserted into the right jugular vein and extended to the right atrium (20 μmol/kg BW·day) for 14 days. EMD 21388 (42.6 mg) was dissolved in 100 μl sodium hydroxide (0.18 M), and saline was added directly to obtain 10 ml; the other group (Exp A; n = 7) received the vehicle without EMD 21388 (10).

Preparation of radiolabeled compounds

EMD 49209 (10 μg/10 μl dimethylsulfoxide) was diluted with 40 μl phosphate buffer (0.25 M; pH 6.5). To 30 μl phosphate buffer (0.05 M; pH 7.4) were added chloramine-T (10 μg/10 μl H_2O) and 7.5 μl $Na^{125}I$ (375 μCi); the mixture was vortexed for 2 min at room temperature. EMD 49209 was added immediately and vortexed for 1 min at room temperature; the radiolabeling was stopped by the addition of 20 μg sodium bisulfite in 10 μl H_2O, followed by 800 μl H_2O. This mixture was purified by HPLC [Waters Associates, Milford, MA; with UV and radioactivity detectors; Eurosfer-100C10, 5 μm, JF 17 column; flow rate, 1 ml/min; gradient elutions A (5% acetonitril and 0.1% trifluoroacetic acid) and B (95% acetonitril and 0.1% trifluoroacetic acid) with gradient program 0–30% B in 1.3 min, 30–90% B in 1.3–30 min, 90–100% B in 30–31 min]. EMD 49209 and its ^{125}I-labeled derivate eluted at 20.8 min; a compound containing an additional iodine substituent eluted at 23.6 min. The respective fractions were collected and appropriately diluted. The specific activity of [^{125}I]EMD 49209 was about 20 μCi/μg with a yield of about 53%.

[^{131}I]T_4 was prepared freshly in our laboratory (15). The purity of the radioactivity was assessed by HPLC just before use. ^{125}I or percent ^{131}I was less than 0.1%, and no other labeled metabolites could be detected.

Experiments

On day 14 the rats received a bolus iv injection of 400 μl [^{125}I]EMD 49209 (3 μCi) and [^{131}I]T_4 (5 μCi) in saline containing 5% normal rat serum. This bolus injection of [^{125}I]EMD 49209 and [^{131}I]T_4 was given 1 h after the last iv injection of EMD 21388 (Exp B) or vehicle (Exp A); 0.25, 0.5, 1, 2, 3, 4, and 6 h after the injection the rats were bled and perfused with saline. Tissues were removed, weighed, and counted. To investigate whether [^{125}I]EMD 49209 is metabolized, plasma and tissues were extracted and subjected to HPLC chromatography following the same extraction procedure and HPLC protocol as those used for the separation of iodothyronines (16). In all tissues analyzed, [^{125}I]EMD 49209 appeared in the same retention time. With the exception of free ^{125}I, no other metabolites were detected. The extraction efficiency for the ^{125}I and ^{131}I activities from all tissues was greater than 95%.

Plasma [^{125}I]EMD 49209 and [^{131}I]T_4 disappearance rates and tissue contents of [^{125}I]EMD 49209 and [^{131}I]T_4 were determined. The percent doses in tissues were corrected for trapped plasma (17). The intestines of the rats were divided into five segments: three equal lengths of small intestine, cecum, and colon; feces and urine were collected, and for all of these fractions, the percent doses of ^{125}I and ^{131}I were determined.

In total, 30 different organs were dissected and processed. For the sake of clarity, only those organs that contributed substantially to the changes in the distribution of the radioactivity are shown, the other organs are taken together and are shown as "rest." The percent doses in total blood, muscle, fat, skeleton, and skin were calculated according to the relative contribution to body weight (18). The total contents of radioactivity in the intestinal segments and feces were also calculated.

After decay of the ^{131}I initially present in the plasma samples, the concentrations of T_4 and T_3 were assessed by rat RIA, using ^{131}I-labeled T_4 and T_3, respectively, as tracers (19). Free T_4 levels were measured by ultrafiltration using Microcon 10 (Amicon, Danvers, MA) filters and freshly labeled [^{131}I]T_4 (20). Plasma TSH was measured by the specific RIA developed for the rat by the NIDDK (NIH, Bethesda, MD). RP-2 was used as a standard.

Results

Both groups of rats had a normal increase in body weight. No toxic effects of EMD 21388 were observed (Exp B). Plasma T_4 and T_3 decreased in the EMD 21388-treated rats by approximately 55% and 28%, respectively. Although the percentage of free T_4 was higher, free T_4 concentrations were the same because of the lowered total plasma T_4 levels. Plasma TSH levels did not change (Table 1).

Exp A: controls or vehicle-treated rats

Distribution of [^{125}I]EMD 49209. Figure 1A shows the disappearance curves of [^{125}I]EMD 49209 from plasma. Fifteen minutes after the bolus injection, [^{125}I]EMD 49209 appeared in tissues (Table 2A). Peak values were reached between 15–30 min (Table 2A). The percent dose of [^{125}I]EMD 49209 decreased in tissues with time. After 6 h, 45% of the dose of [^{125}I]EMD 49209 was found in the intestines (Table 2A). In many tissues, such as brain, cerebellum, hypothalamus, and medulla, the percent dose of ^{125}I radioactivity is very low, ranging from 0.0001–0.070% of the dose (Table 3). After HPLC analysis, this activity appears to be iodide. In none of these tissues could [^{125}I]EMD 49209 be detected. The calculated tissue/plasma ratios of the individual tissues were more or less constant during the 6 h, indicating that there was no accumulation for [^{125}I]EMD 49209 (data not shown).

Distribution of [^{131}I]T_4. Figure 1B shows the disappearance of [^{131}I]T_4 from plasma. [^{131}I]T_4 disappeared rapidly, within the first 5–10 min. The uptake of [^{131}I]T_4 by tissues was rapid, especially that by liver (Table 2B). The percent dose of [^{131}I]T_4 in tissues decreased with time (Table 2B). In the intestines, about 22% was found after 6 h (Table 2B).

Comparison between [^{125}I]EMD 49209 and [^{131}I]T_4 distribution. Clear differences in kinetic behavior were evident (Fig. 1C). [^{131}I]T_4 disappeared more rapidly from plasma, within the first 5–10 min. At the start, the percent dose of [^{125}I]EMD 49209 was higher than that of [^{131}I]T_4, and then disappeared at a slower rate than T_4, but after 5–6 h, they were at the same level.

The uptake of [^{125}I]EMD 49209 in tissues was much less and slower than that of T_4. There was a striking difference in uptake in liver (Table 2, A and B). With time, there was a

TABLE 1. Body weight before and after treatment

	Vehicle-treated rats	EMD 21388-treated rats
BW (g)	180 ± 4	179 ± 5
BW after Rx	204 ± 5	205 ± 5
T_4 (nM)	38 ± 4	17 ± 4[a]
% Free T_4	0.047 ± 0.004	0.092 ± 0.004[a]
Free T_4 (pM)	18 ± 2	16 ± 2
T_3 (nM)	0.85 ± 0.05	0.61 ± 0.03[a]
TSH (ng/ml)	0.55 ± 0.07	0.60 ± 0.11

Plasma thyroid hormone and TSH values of vehicle-treated and EMD 21388-treated rats. $n = 7$ for both groups. Values are the mean ± SE.

[a] At least $P < 0.05$.

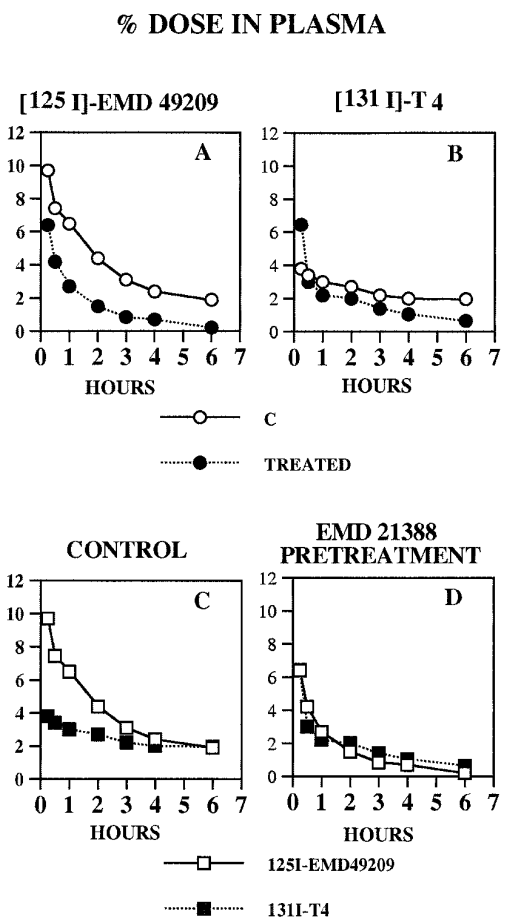

FIG. 1. Disappearance of [^{125}I]EMD 49209 and [^{131}I]T_4 from plasma. A, [^{125}I]EMD 49209 in vehicle-treated and EMD 21388-pretreated rats. B, [^{131}I]T_4 in vehicle-treated and pretreated EMD 21388 rats. C, [^{125}I]EMD 49209 and [^{131}I]T_4 in vehicle-treated rats. D, [^{125}I]EMD 49209 and [^{131}I]T_4 in EMD 21388-pretreated rats.

small increase in the uptake of [^{125}I]EMD 49209 in muscle and skin, comparable with that of [^{131}I]T_4.

After 6 h the greatest differences were found between the contents of [^{125}I]EMD 49209 and [^{131}I]T_4 in liver and intestines.

Exp B: pretreatment of rats with EMD 21388

Distribution of [^{125}I]EMD 49209. The disappearance of [^{125}I]EMD 49209 from plasma was greatly enhanced by pretreatment with EMD 21388 (Fig. 1A). EMD 21388 treatment resulted in an increase in the excretion of [^{125}I]EMD 49209 into the intestines as well as into the urine (Table 2C). At the first time point (0.25 h), 38% of the [^{125}I]EMD 49209 was already present in the intestines, mainly in the first part of the jejunum (Fig. 2, I, II, and III). After 6 h, most of the [^{125}I]EMD 49209 was found in the intestines (54%) and urine (31%; Table 2C). The radioactivity in the urine consisted mainly of iodide, indicating that this amount of [^{125}I]EMD 49209 was metabolized or at least deiodinated. The percent dose of [^{125}I]EMD 49209 in some organs was hardly detectable; after 6 h, no [^{125}I]EMD 49209 could be detected in brain, cerebellum, medulla, or hypothalamus. Table 2C shows the percent dose of [^{125}I]EMD 49209 at the different time points.

Distribution of [^{131}I]T_4. The disappearance of [^{131}I]T_4 from plasma was increased compared to the T_4 distribution in the vehicle-treated rats (Fig. 1B). After 15 min, the uptake of T_4 by liver was very high (Table 2D). After 6 h, a large part of the T_4 was still present in the liver and the various organs; the total excretion was 40% (intestines, 24%; urine, 16%). After pretreatment with EMD 21388, [^{131}I]T_4 distribution did not change markedly; there was less T_4 in the blood and more in the organs, and only a slight increase in excretion (Table 2D).

Comparison between [^{125}I]EMD 49209 and [^{131}I]T_4 distribution. Figure 1D shows that pretreatment with EMD 21388 abolished the difference in plasma disappearance of [^{125}I]EMD 49209 and [^{131}I]T_4 present during vehicle treatment. After 15 min, the uptake of [^{131}I]T_4 by liver was nearly 10 times higher than that of [^{125}I]EMD 49209 (Table 2, C and D). Figure 2 shows the presence of [^{125}I] (Fig. 2A) and [^{131}I] (Fig. 2B) activities in gut fractions with time. After 6 h, approximately 40% of the ^{125}I dose was found in intestinal contents and feces, whereas only 25% of ^{131}I was present. EMD 21388 treatment caused a shift in the appearance time of ^{125}I in intestinal fractions. At the first time point, 38% of the dose was already present in the intestines.

Discussion

The T_4 plasma disappearance and tissue distribution results for Exp A are in accordance with the data obtained by our group in earlier studies, in which T_4 metabolism was measured under several experimental conditions and other experimental set-ups, such as the double isotope equilibrium technique (10, 13, 15, 16).

The two flavonoids used in this study were developed by molecular drug design, directed toward the inhibition of the hepatic 5′-iodothyronine deiodinase. Both fulfill the criteria necessary for the optimal inhibitory potency (4). The only difference between these flavonoids is the two bromide atoms (EMD 21388) *vs.* the two iodide atoms (EMD 49209) in the 3′- and 5′-positions. This makes it possible that EMD 49209 can be deiodinated by deiodinases; debromination of EMD 21388 is less likely. That deiodination occurs can be seen from the free ^{125}I excreted in urine. This influences the kinetic behavior, which means that the disappearance rate of EMD 49209 is faster than that of EMD 21388; in the vehicle-treated rats, it is about 10% (Exp A), and in EMD 21388-pretreated rats, it is about 20% (Exp B).

From our study it is clear that the synthetic flavonoid, [^{125}I]EMD 49209, disappears very quickly from plasma and enters tissues. However, the percent dose of the flavonoid in the organs is never high and decreases with time. It can be concluded from the rapid uptake of both compounds by the liver that this organ functions as an essential part of the fast pool. However, there is a discrepancy between T_4 and the flavonoid. T_4 content as a percentage of the dose in the liver is much higher than that of the flavonoid (with a high excretion into the intestinal lumen). This means that in liver, many binding sites exist for T_4, but not for the flavonoid.

It appears that EMD 49209 does not bind to intracellular T_4 binding sites; there is a rapid transfer from plasma via the liver into the intestines. Also, EMD 21388 does not bind to nuclear thyroid hormone receptor (Bernal, J., personal com-

TABLE 2. Distribution of radioactivity, as percentage of the dose of [^{125}I]EMD 49209 or [^{131}I]T_4 from 0.25–6 h after bolus injection

	0.25	0.5	1	2	3	4	6
A) Distribution, as percentage of the dose of [^{125}I]EMD 49209, in vehicle-treated rats, from 0.25–6 h after the bolus injection							
Blood	72	60	53	42	31	28	18
Liver	7	9	6	6	4	3	2
Skin	4	6	7	8	8	10	9
Muscle	8	8	8	11	9	11	9
Fat	2	4	4	3	4	3	3
Skeleton	2	2	4	4	3	2	2
Rest	2	2	2	3	3	2	2
Intestines	5	9	17	25	32	33	45
Urine	0	0	0	1	6	8	10
B) Distribution, as percentage of the dose of [^{131}I]T_4, in vehicle-treated rats from 0.25–6 h after the bolus injection							
Blood	42	31	34	25	27	21	21
Liver	29	30	23	19	16	15	14
Kidney	2	2	2	2	2	2	1
Skin	4	7	7	9	11	9	11
Muscle	7	8	10	9	11	9	11
Fat	1	4	4	3	4	3	3
Skeleton	3	4	3	3	3	3	3
Rest	2	3	4	3	4	3	3
Intestines	10	12	14	23	17	26	22
Urine	0	0	0	4	5	10	12
C) Distribution, as percentage of the dose of [^{125}I]EMD 49209 after pretreatment with EMD 21388, from 0.25–6 h after the bolus injection							
Blood	37	22	15	14	13	9	3
Liver	5	6	2	2	2	2	1
Skin	7	8	6	9	8	9	2
Muscle	8	11	7	7	7	7	2
Fat	1	3	2	3	2	2	1
Skeleton	2	4	2	2	2	2	1
Rest	2	2	3	3	3	3	2
Intestines	39	46	50	53	45	48	56
Urine	0	0	13	8	20	18	31
D) Distribution, as percentage of the dose of [^{131}I]T_4, after pretreatment with EMD 21388 0.25–6 h after the bolus injection							
Blood	27	24	24	25	19	18	9
Liver	42	41	29	24	20	21	19
Kidney	4	4	4	3	3	3	1
Skin	6	6	8	10	11	11	7
Muscle	8	6	12	13	16	15	11
Fat	5	5	6	6	4	4	5
Skeleton	2	3	3	3	3	3	2
Rest	3	4	5	5	6	6	5
Intestines	4	4	10	11	15	16	24
Urine	0	0	1	1	4	4	16

All time points are shown. In total, 30 different organs were dissected and processed. For the sake of clarity, only those organs that contributed substantially to the changes in the distribution of the radioactivity are shown; the other organs are taken together and are shown as "Rest." The percent doses in total blood, muscle, fat, skeleton, and skin were calculated according to their relative contributions to the body weight (18). The total contents of radioactivity in the intestinal segments and feces were calculated.

munication) and does not activate the human 5′deiodinase thyroid hormone-responsive element in LLC-PK1 kidney cell line (Jacoks, T., personal communication). It is possible that without binding to the thyroid hormone-binding sites, there is no retention in tissues. Although these synthetic flavonoids were developed by molecular drug design as T_4 analogs (1–4), the *in vivo* kinetic behavior of [^{125}I]EMD 49209 is different from that of [^{131}I]T_4. This could be explained from the fact that EMD 21388 and EMD 49209 bind only to TTR and not to other plasma proteins, as is the case for T_4 (8, 21). This also can explain the differences in the behavior of [^{125}I]EMD 49209 between vehicle-treated and pretreated rats; in vehicle-treated rats, the tracer amount of [^{125}I]EMD 49209 will be totally bound to TTR, and its disappearance will be closely related not only to the binding to TTR, but also to the half-life of TTR. In the pretreated animals, the TTR is completely occupied by the EMD 21388, and the larger part of this flavonoid as well as [^{125}I]EMD 49209 will exist in plasma in the free form. It is surprising that the plasma disappearance curves of EMD and T_4 are very similar, whereas their tissue distributions are essentially different.

The higher amount of flavonoid present in the liver will induce hepatic enzymes, *i.e.* such as those involved in glucuronidation. This would lead to an increased metabolism not only of the flavonoid itself, but also that of T_4. It has been shown that the most substantial metabolic pathway of natural flavonoids in mammals is conjugation with glucuronic acid or sulfate (22, 23). The conjugates are excreted in bile and urine. The material excreted by the bile can be hydrolyzed in the intestines by the microflora; the liberated flavonoids may then be reabsorbed. We assume that this is also the case for our synthetic flavonoids.

TABLE 3. Percent doses of [^{125}I]EMD 49209 and [^{131}I]T_4 in various organs of rats

	% Dose of [^{125}I]EMD 49209		% Dose of [^{131}I]T_4	
	0.25	6	0.25	6
Vehicle-treated rats				
Brain	0.000	0.000	0.040	0.147
Cerebellum	0.000	0.000	0.007	0.021
Hypothalamus	0.000	0.000	0.001	0.007
Medulla	0.000	0.000	0.008	0.048
BAT	0.074	0.038	0.101	0.052
EMD 21388-treated rats				
Brain	0.000	0.000	0.067	0.150
Cerebellum	0.000	0.000	0.016	0.032
Hypothalamus	0.000	0.000	0.004	0.032
Medulla	0.000	0.000	0.014	0.036
BAT	0.037	0.010	0.076	0.116

The first time point after the bolus injection is 0.25 h; the last is 6 h. Rats were killed, bled, and perfused. Tissues were homogenized, counted, extracted, and submitted to HPLC. The percent dose in tissues is corrected for trapped plasma.

From our results it is clear that the flavonoid does not cross the blood-brain barrier; therefore, in our view this flavonoid cannot be held directly responsible for the inhibition of 5′-deiodinase type II in brain, cerebellum, hypothalamus, and medulla. Thus, the decrease in T_3 locally produced in those tissues should be due to the lowered amount of substrate (T_4) (10). The access of the flavonoid to the brain appears completely different from that of the peripheral tissues, indicating that T_4 conformational homology is not sufficient to gain access to the brain via the brain-blood barrier or the blood-choriod plexus-cerebrospinal fluid barrier.

Despite the high amount of EMD 21388 in the liver [calculated as: 0.305 (% dose [^{125}I]EMD 49209/g) $\times$ 1 μmol (100% dose) = 3.05 nmol/g] compared to that of T_4 (0.051 nmol/g) (24), there is no indication that inhibition of 5′-deiodinase type I occurs, as deduced from the unchanged Lc[^{125}I]T_3/[^{125}I]T_4 ratio in the liver (10). It seems likely that the flavonoids do not reach the deiodinase compartment in tissues despite high transient concentrations of reversible competitive binding of the flavonoid to deiodinases. This is in contrast to other known inhibitors, such as PTU and iopanoic acid, that bind irreversibly to the active site of the enzyme.

Acute and short term treatments with EMD 21388 demonstrate the effects on the displacement of T_4 from TTR, transiently increasing free T_4 and decreasing TSH (25–28), whereas long term treatment results in changes in the metabolic pathway of thyroid hormones that cannot only be accounted for by decreased binding of T_4 by TTR. Therefore, other mechanisms that have not yet been identified are likely to be responsible (10). The most pronounced change is the strong increase in intestinal excretion and the increased metabolism by the deiodinative pathway, as concluded from the increase in radioactivity in urine.

The differences between *in vivo* and *in vitro* effects of the flavonoids can be attributed to the presence of plasma binding proteins in the *in vivo* situation and their absence in the *in vitro* incubation system, leading to increased cellular flavonoid concentrations in the *in vitro* situation. The properties of the flavonoids make them valuable tools in studies of intracellular thyroid hormone regulation *in vitro*, but also *in vivo* by influencing T_4 production, metabolism, and excretion.

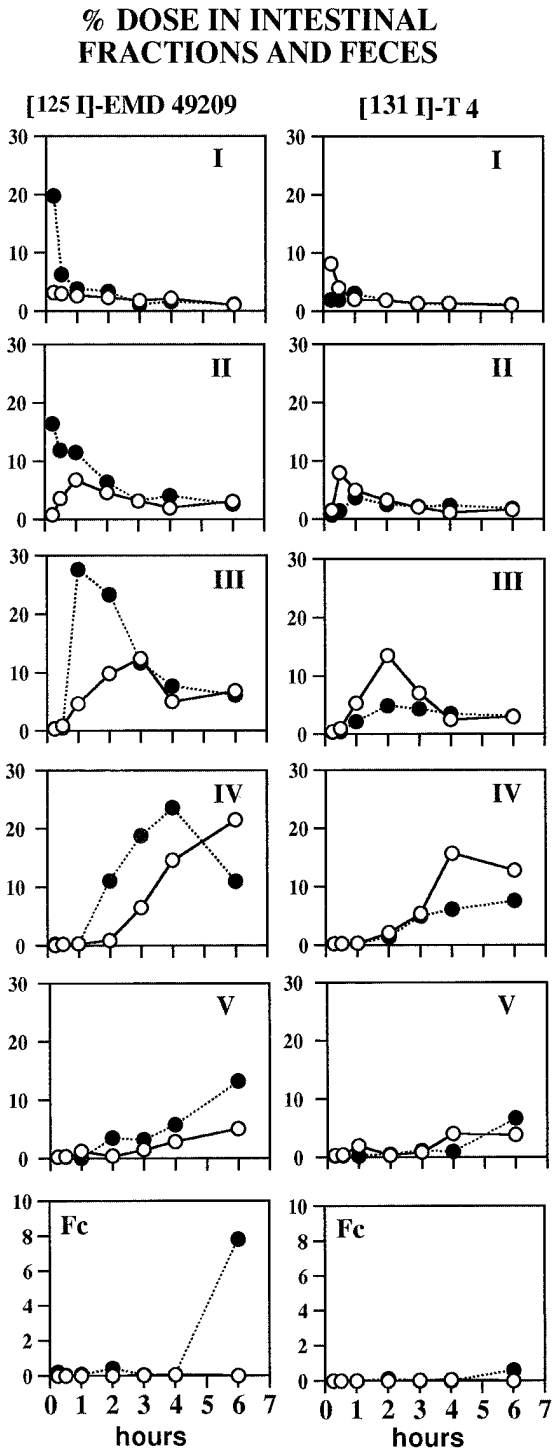

FIG. 2. The radioactivity in five fractions [three lengths of small intestines (I, II, III), cecum (IV), and colon (V)] of the intestines and feces (Fc) at seven time points after the bolus injection in vehicle-treated (C; *open circles*) and EMD 21388-pretreated (*closed circles*) rats. *Left row*, ^{125}I activity after a bolus injection of [^{125}I]EMD 49209. *Right row*, ^{131}I activity after a bolus injection of [^{131}I]T_4. Note the difference between the *y*-axis for the fractions and feces.

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