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EFFECT OF DEUTERIUM SUBSTITUTION ON THE DISPOSITION OF INTRAPERITONEAL TRYPTAMINE

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Abstract—To determine whether α,α-dideutero substitution in the side chain of the tryptamine molecule can exert primary isotope effects and enhance its bioavailability, equimolar mixtures of tryptamine (T) and either α,α-dideutero-tryptamine (α,α-[$^2H_2$]T) or β,β-dideutero-tryptamine (β,β-[$^2H_2$]T) were injected i.p. into rats. The amounts of these amines in the brain, liver and plasma were then measured at various times following the injection, and ratios between the deuterated T and T were computed. The ratio remained close to unity in plasma, but exceeded unity in the liver and brain when α,α-[$^2H_2$]T and T were injected; however, when β,β-[$^2H_2$]T and T were injected, the ratios were unity in all cases at all times. In the presence of a monoamine oxidase inhibitor, the relative enrichment of α,α-[$^2H_2$]T compared to T was reduced. It is concluded that α,α-dideutero substitution exerts a primary isotope effect during oxidative deamination so that much more of this amine penetrates into, and persists in, the brain.

Tryptamine elicits very little or no behavioural effect in rodents, unless it is injected after pretreatment with a monoamine oxidase inhibitor (MAOI) or unless it is injected in very high, and thus lethal, amounts [1–4]. In the absence of an MAOI, therefore, it seems that insufficient amounts of tryptamine escape deamination to reach the CNS, or other organs, to cause behavioural activation [3]; or if sufficient amounts do reach the CNS, it is metabolised too quickly. It is known that tryptamine exhibits a very fast turnover rate and a very short half-life in the brain [5, 6]. To date the amounts of tryptamine reaching the rodent brain following its systemic administration have been measured by relatively nonspecific, insensitive fluorimetric methods [2–4, 7]. Such methods cannot measure endogenous levels of tryptamine reliably, so that it is impossible to compare exogenous tryptamine (i.e. the amount reaching and persisting in the brain after injection) to endogenous tryptamine levels. Using a specific and sensitive mass spectrometric procedure, we have been able to quantitate both exogenous and endogenous tryptamine at various times after i.p. injection of an equimolar mixture of tryptamine and deuterated tryptamine in rat plasma, liver and brain, both in the presence and absence of an MAOI.

In 1960, Belleau *et al.* [8] reported that intravenous α,α-dideutero-tryptamine (α,α-[$^2H_2$]T) was more potent than tryptamine in its ability to increase arterial pressure and nictitating membrane contraction in the cat. Recently, interest in the concept of stereospecific deuterium substitution to enhance the effects of drugs has been rekindled [9–11]. We have shown, for example, that the behavioural effects of $\alpha,\alpha,\beta,\beta$-tetradeuterophenylethylamine are more potent than those of phenylethylamine itself [12]. The basis for this potentiation lies in the resistance of the deuterated amine to deamination by MAO [13], thus allowing much larger quantities of the deuterated phenylethylamine to reach the brain [14]. As yet, the magnitude of protection from MAO conferred on tryptamine by α,α-deuteration has not been assessed; however, other amines, such as *m*- and *p*-tyramine, dopamine and 5-hydroxytryptamine, are deaminated 2- to 4-fold less easily by MAO if they are suitably deuterated [13, 15]. To assess the proportions of tryptamine and deuterated tryptamine reaching and persisting in the brain and certain peripheral organs, we injected equimolar mixtures of tryptamine and α,α-deutero-tryptamine intraperitoneally into rats. In addition, several experiments were carried out to substantiate that the basis for the enhanced effects of α,α-deutero-tryptamine was due to a primary isotope effect on the deamination of tryptamine by MAO *in vivo*.

MATERIALS AND METHODS

Chemicals. Iproniazed phosphate (Ipron) and tryptamine hydrochloride (T) were purchased from the Sigma Chemical Co. (St. Louis, MO). The hydrochloride salts of α,α-[$^2H_2$]tryptamine, β,β-[$^2H_2$]tryptamine and $\alpha,\alpha,\beta,\beta$-[$^2H_4$]tryptamine were purchased from Merck, Sharp & Dohme (Dorval, Quebec).

Drug treatments. Male albino Wistar rats (200–250 g, Charles River Laboratories, Canada, Montreal, Quebec) were maintained in hanging wire cages with free access to food and water. Drugs were dissolved in isotonic saline and injected intraperitoneally. In the first series of experiments, 160 μmoles/kg each of T and α,α-[$^2H_2$]T were

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injected, and the rats were killed 5, 10, 30, 45 or 60 min later. In the next series of experiments, 40 mg/kg Ipron was injected 4 hr and 10 min before sacrifice, and 16 μmoles/kg T and α,α-[$^2H_2$]T were injected 10 min before sacrifice (smaller amounts of the amines were injected in this case to prevent lethality). Finally, in other experiments, 160 μmoles/kg each of T and either α,α-[$^2H_2$]T or β,β-[$^2H_2$]T were injected 10 min before sacrifice.

Amine analyses. The rats were killed by cervical dislocation, and the brain and part of the liver were dissected out, rinsed with ice-chilled saline, blotted, weighed, and frozen on solid CO_2. The hepatic vein was cut, and the blood in the abdominal cavity was collected into heparinized tubes. After centrifugation, 1-ml portions of plasma were removed and kept cold in an ice bath. Known amounts of the internal standard (α,α,β,β-[$^2H_2$]T) were added to homogenates of the tissue samples and to the plasma. The amines therein were converted to their dansyl derivatives and successfully separated unidimensionally on two silica gel thin-layer plates as described previously [16]. The amines were quantitated mass spectrometrically using a low resolution technique so that T, [$^2H_2$]T and [$^2H_4$]T (the internal standard) could be analysed simultaneously, as described [17].

RESULTS

The amounts of α,α-[$^2H_2$]T and T present in liver and plasma obtained from rats injected with 160 μmoles/kg each of these amines were fairly substantial (about 100 nmoles/g) for 5–10 min after injection (Tables 1 and 2). After this time, the amine concentrations decreased rapidly so that only approximately 1–2 nmoles/g of each amine was present 1 hr after injection. In the brain, however, the amounts of α,α-[$^2H_2$]T and T were much lower; at the most only 1 nmole/g of the amines were present 5–10 min after injection of 160 μmoles/kg of each amine (Table 3). Thereafter, the brain amine concentrations decreased so as to reach the endogenous level after 45 min. It is interesting to note that, in plasma, the ratio between [$^2H_2$]T and T was approximately unity; the amounts of [$^2H_2$]T and T were not significantly different at any of the times studied. In the liver, significantly more [$^2H_2$]T than T was present from 30 to 60 min after injection; a maximum ratio of 3.7 was reached after 30 min. In the brain, the ratio reached 5.4 at 30 min, and with the exception of the 5- and 45-min time periods, significantly more [$^2H_2$]T than T was present.

Pretreatment of the rats with Iproniazid antagonized the ability of α,α-deuterium substitution to increase the amounts of amine reaching the brain and liver (Table 4). In the control brain, the amount of [$^2H_2$]T was 3.3 times greater than the amount of T 10 min after injection of a 1:1 mixture of amines, but when the rats were pretreated with Ipron, the ratio of [$^2H_2$]T to T decreased to 1.1. Similarly, in the livers obtained from the Ipron-treated rats, the ratio of [$^2H_2$]T to T decreased to 1.0 (the ratio of amines injected) from 1.6 in the control.

In the final series of experiments, the ratios of the amounts of [$^2H_2$]T and T in brain, liver and plasma after injection of 160 μmoles/kg of α,α[$^2H_2$]T and T were compared to the corresponding ratios after injection of 160 μmoles/kg of β,β-[$^2H_2$]T and T. Ten minutes after injection, the [$^2H_2$]T to T ratio was greater than 1 in the brain and liver of rats that had

Table 1. Comparison of the amounts of α,α-[$^2H_2$]T and T in plasma obtained from rats injected with an equimolar mixture of α,α-[$^2H_2$]T and T

Time (min)	[$^2H_2$]T (nmoles/ml)	T (nmoles/ml)	[$^2H_2$]T:T*
5	104 ± 35	107 ± 38	1.1 ± 0.1
10	71 ± 21	66 ± 20	1.1 ± 0.03
30	15 ± 2.9	11 ± 2.1	1.4 ± 0.04
45	2.5 ± 0.94	1.80 ± 0.75	1.4 ± 0.1
60	1.2 ± 0.37	0.75 ± 0.25	1.7 ± 0.1

Values are mean ± S.E.M., N = 6; control values for T were 0.072 ± 0.029 nmoles/ml. Rats were injected with 160 μmoles/kg each of α,α-[$^2H_2$]T and T.

* Each value is the mean of the individual [$^2H_2$]T to T ratios, and thus is not necessarily equal to the ratio of means of the [$^2H_2$]T and T values, especially if the variance is large.

Table 2. Comparison of the amounts of α,α-[$^2H_2$]T and T in the liver of rats injected with a equimolar mixture of α,α-[$^2H_2$]T and T

Time (min)	[$^2H_2$]T (nmoles/g)	T (nmoles/g)	[$^2H_2$]T:T*
5	117 ± 35	84 ± 26	1.6 ± 0.3
10	72 ± 22	42 ± 14	1.6 ± 0.1
30	35 ± 8.1	9.5 ± 2.1†	3.7 ± 0.2
45	4.9 ± 0.9	2.1 ± 0.9†	3.0 ± 0.4
60	1.6 ± 0.2	0.5 ± 0.06†	3.5 ± 0.2

Values are mean ± S.E.M., N = 6; control values for T were 0.09 ± 0.03 nmoles/g. Rats were injected with 160 μmoles/kg each of α,α-[$^2H_2$]T and T.

*Each value is the mean of the individual [$^2H_2$]T and T ratios, and thus, is not necessarily equal to the ratio of the mean [$^2H_2$]T and T values.

† $P < 0.05$ comparing [$^2H_2$]T and T using Student's *t*-test (one-tailed).

Table 3. Comparison of the amounts of α,α-[$^2H_2$]T and T in whole brain of rats injected with an equimolar mixture of α,α-[$^2H_2$]T and T

Time (min)	[$^2H_2$]T (nmoles/g)	T (nmoles/g)	[$^2H_2$]T:T*
5	0.86 ± 0.29	0.34 ± 0.14	2.6 ± 0.2
10	0.90 ± 0.22	0.20 ± 0.06†	3.3 ± 0.1
30	0.33 ± 0.08	0.05 ± 0.01†	5.4 ± 1.2
45	0.08 ± 0.03	0.03 ± 0.01	4.0 ± 1.9
60	0.02 ± 0.005	0.004 ± 0.003†	6.3 ± 4.0

Values are mean ± S.E.M., N = 6; control values for T were 0.0037 ± 0.0012 nmoles/g. Rats were injected i.p. with 160 μmoles/kg each of α,α[$^2H_2$]T and T.

* Each value is the mean of the individual [$^2H_2$]T and T values, and thus is not necessarily equal to the ratio of the mean [$^2H_2$]T and T values.

† $P < 0.05$ comparing [$^2H_2$]T and T using Student's *t*-test (one-tailed).

Table 4. Effect of Iproniazid on the ratios of the amounts of α,α-[$^2H_2$]T and T in whole brain, liver and plasm of rats previously injected with an equimolar mixture of these two amines

		[$^2H_2$]T (nmoles/g)	T (nmoles/g)	[$^2H_2$]T:T*
Brain	Control	0.90 ± 0.22	0.20 ± 0.06	3.3 ± 0.1
	Ipron	0.79 ± 0.17	0.46 ± 0.07	1.1 ± 0.06†
Plasma	Control	71 ± 21	66 ± 20	1.1 ± 0.03
	Ipron	9.4 ± 3.7	9.1 ± 3.6	1.0 ± 0.01
Liver	Control	72 ± 22	42 ± 14	1.6 ± 0.1
	Ipron	47 ± 9.7	47 ± 9.7	1.0 ± 0.01†

Values are mean ± S.E.M., N = 6. Control rats received 160 μmoles/kg each of α,α-[$^2H_2$]T and T 10 min before being killed. Iproniazid (40 mg/kg, 4 hr 10 min) treated rats received 16 μmoles/kg each of α,α-[$^2H_2$]T and T 10 min before being killed.

* Each value is the mean of the individual [$^2H_2$]T and T values, and thus is not necessarily equal to the ratio of the mean [$^2H_2$]T and T values.

† $P < 0.05$ comparing the means of the ratios of the [$^2H_2$]T and T values of the control and Iproniazid-treated groups.

received the α,α[$^2H_2$]T (Table 5), but in no case was the ratio of [$^2H_2$]T to T greater than 1 in those rats that had been injected with an equimolar mixture of β,β-[$^2H_2$]T and T. The [$^2H_2$]T to T ratios in the brain and the liver from rats injected with α,α[$^2H_2$]T were greater than the corresponding ratios from rats injected with β,β-[$^2H_2$]T. The amounts of [$^2H_2$]T and T present are shown in Table 6. The amounts of α,α-[$^2H_2$]T remaining 10 min after injection were clearly greater than the amounts of β,β-[$^2H_2$]T remaining. Interestingly, the amounts of T remaining in the brain and plasma appeared to be greater when T was injected concomitantly with α,α-[$^2H_2$]T than when it was injected with β,β-[$^2H_2$]T.

DISCUSSION

The data presented in Tables 2 and 3 show that, when equimolar amounts of α,α-[$^2H_2$]T and T were injected i.p. simultaneously, the amounts of α,α-[$^2H_2$]T present in brain and liver were greater than the amounts of T at various times after the injection. It is clear that the α,α-[$^2H_2$]T persisted longer than T; the reason for this appears to be its greater resistance to oxidative deamination by MAO. This effect was not seen in the plasma (Table 1); the amounts of α,α-[$^2H_2$]T were not significantly greater than the amounts of T at any of the times analysed. In a previous comparable study, α,α-[$^2H_2$]-phenylethylamine (PE) persisted much longer than PE in brain, liver and plasma [14]. In addition, the ratios of [$^2H_2$]PE to PE were much greater than the ratios seen here for [$^2H_2$]T to T. The differences in the isotope effects on PE and T turnover may perhaps be a reflection of difference in the substrate selectivity of type A and type B MAO. PE is believed to be a type B MAO substrate, while T is thought to be a mixed substrate for both type A and type B MAO. The greatest isotope effects were seen with PE, a type B substrate. If deuterium substitution had little effect on deamination by type A MAO, then this might account for its smaller effect on tryptamine. Alternatively, it is possible that deuterium substitution caused a metabolic shift in the metabolism of α,α-[$^2H_2$]T, so that it was metabolised to a greater extent than normal by some enzyme other than MAO.

Since pretreatment of rats with the MAOI, iproniazid, reduced the ratio of the amounts of

Table 5. Effect of α,α- versus β,β-deuterium substitution on the ratios of the amounts of [$^2H_2$]T and T in whole brain, liver and plasma of rats injected with an equimolar mixture of [$^2H_2$]T and T

	α,α-[$^2H_2$]T:T	β,β-[$^2H_2$]T:T
Brain	3.31 ± 0.11	1.1 ± 0.06*
Liver	1.63 ± 0.05	1.0 ± 0.01*
Plasma	1.06 ± 0.03	1.0 ± 0.01

Values are mean ± S.E.M., N = 6. Rats were injected i.p. with 160 μmoles/kg each of T and [$^2H_2$]T (either α,α or β,β) 10 min before being killed.

* Comparing the two ratios, $P < 0.05$ (Student's *t*-test).

Table 6. Effect of α,α- versus β,β-deuterium substitution on the concentrations of [$^2H_2$]T and T in whole brain, liver and plasma of rats injected with an equimolar mixture of [$^2H_2$]T and T

		[$^2H_2$]T (nmoles/g)	T (nmoles/g)
Brain	α,α	1.29 ± 0.15	0.39 ± 0.06*
	β,β	0.16 ± 0.06	0.15 ± 0.05
Liver	α,α	96.5 ± 7.7	59.5 ± 3.0*
	β,β	50.8 ± 11.6	58.2 ± 11.9
Plasma	α,α	76.7 ± 8.7	73.5 ± 9.3
	β,β	40.7 ± 12.1	41.4 ± 12.3

Values are mean ± S.E.M., N = 6. Rats were injected i.p. with 160 μmoles/g of T and either α,α- or β,β-[$^2H_2$]T 10 min before being killed.

* $P < 0.05$ comparing [$^2H_2$]T to T (Student's *t*-test).

α,α-[$^2H_2$]T to T in the brain and liver of rats injected with an equimolar mixture of these amines, it can be concluded that the basis for the increased amounts of the deuterated amine to the non-deuterated amine was due to the decreased rate of deamination by MAO of α,α-[$^2H_2$]T compared to T. Further evidence to support this conclusion was obtained in the comparison of α,α- versus β,β-[$^2H_2$]T. When tryptamine is deaminated to produce indoleacetic acid, the α,α-hydrogen (or deuterium) atoms are removed; thus, only α,α-deuterium substitution and not β,β-deuterium substitution decreased the rate of oxidative deamination of tryptamine (see Table 5). The amounts of α,α-[$^2H_2$]T were greater than the amounts of T in the brain and in the liver of rats injected with an equimolar mixture of these two amines. This indicates that the deuterated T was deaminated more slowly in the brain and in the liver. In contrast, the amounts of β,β-[$^2H_2$]T were equal to the amounts of T in the brain, liver and plasma of rats injected with an equimolar mixture of these amines. Thus, in this case, the β-deuterated T was deaminated at the same rate as T itself. In the brain and in the liver, where an isotope effect of α-deuterium substitution was observed (Table 5), we can expect to find more α,α- than β,β-[$^2H_2$]T. Such was the case (Table 6). In the plasma, where no deuterium isotope effect was observed, the amounts of α-α- and β,β-[$^2H_2$]T should be equal. We found, however, that more α,α- than β,β-[$^2H_2$]T was present in plasma. The difference was fairly small (about 2-fold compared to 10-fold in the brain) and may have been produced artifactually by the high degree of variance in the samples.

Though intraperitoneally administered tryptamine can cross the blood–brain barrier [18], the data presented here show that very little actually does so. In the comparable study with PE and deuterated PE, the amounts of these amines entering the brain were about 100-fold higher than the amounts of T and [$^2H_2$]T entering [14]. These low brain levels of tryptamine are consistent with previous findings that rodents do not respond behaviourally to i.p. tryptamine unless the animals are also pretreated with an MAOI [1–4]. Since none of the animals in this study exhibited behavioural changes compared to controls, it appears that the brain tryptamine levels required for behavioural effects must be higher than the levels observed here.

In conclusion, this study shows that appropriate deuterium substitution can protect tryptamine from oxidative deamination *in vivo*. Thirty minutes after injecting a 1:1 mixture of α,α[$^2H_2$]T and T, the α,α-[$^2H_2$]T levels in the liver were 3.7 times greater than the T levels, while the α,α-[$^2H_2$]T levels in the brain were 5.4 times greater than T. These findings are consistent with previous findings regarding the effect of α,α-deuterium substitution on oxidative deamination of arylalkyl amines by MAO [13]; in addition, this paper demonstrates the magnitude of this isotope effect on the degradation of T *in vivo* after i.p. injection.

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