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EFFECTS OF BENZENE AND OTHER ORGANIC SOLVENTS ON THE DECARBOXYLATION OF SOME BRAIN AROMATIC-L-AMINOACIDS

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Abstract—The intraperitoneal administration of benzene produced marked increases in mouse striatal concentrations of β-phenylethylamine, *p*-tyramine and, to a lesser extent, *m*-tyramine. Similar increases were observed in rat striatal *p*- and *m*-tyramine. The subcutaneous administration of benzene dissolved in sesame oil increased mouse striatal *p*-tyramine but did not change *m*-tyramine. Benzene administration to mice pretreated with *p*-tyrosine produced marked increases in mouse striatal *p*-tyramine as well as in *m*-tyramine. The statistical analysis of the results indicated that the treatment produced an interaction that led to an increase in the concentration of both the *p*- and *m*-isomers of tyramine. The administration of benzene to *m*-tyrosine-pretreated mice increased striatal *m*-tyramine but *p*-tyramine was not increased. The treatment produced no potentiation in the formation of *p*- or *m*-tyramine. Of the other organic solvents given, pyridine produced the most marked effects. Its administration increased the concentration of both *p*- and *m*-tyramine in the mouse striatum. Treatment with toluene, chloroform, carbon tetrachloride or isoamylalcohol produced moderate increases in mouse striatal *p*-tyramine while toluene, dichloromethane or isobutylalcohol also increased *m*-tyramine. These increases in brain β-phenylethylamine, *p*-tyramine and *m*-tyramine may play a contributory role in the human toxicity of benzene and some of these organic solvents; these toxic effects could be exacerbated after ingestion of foodstuffs containing the aminoacids phenylalanine or *p*-tyrosine or for those under treatment with a monoamine oxidase inhibitor.

The decarboxylation of aromatic-L-aminoacids is an important step in the synthesis of amino neurotransmitters and neuromodulators. Unlike *l*-DOPA and 5-hydroxytryptophan, tyrosine (presumably *p*-tyrosine) and phenylalanine are very poor substrates for aromatic-L-aminoacid decarboxylase, i.e. low V_{max} and high K_m values were observed [1]. Interestingly, the decarboxylase activity towards *p*-tyrosine and phenylalanine can be drastically induced when only a small amount of benzene is added to the incubation mixture of the decarboxylase enzyme obtained from guinea pig kidney [2]. The decarboxylation of *l*-DOPA and 5-hydroxytryptophan, however, is reduced under the same condition. More recently it has been shown that, in addition to benzene, some other solvents can also increase the decarboxylation of *p*-tyrosine by the purified rat brain aromatic-L-aminoacid decarboxylase [3]. It is worthwhile to investigate whether this activation of decarboxylation by organic solvents has actually occurred *in vivo*. To prove this point we have parenterally administered benzene and other solvents to mouse and rat and subsequently analyzed the concentration of β-phenylethylamine, *p*-tyramine, *m*-tyramine and dopamine in the striatum. The activation effect has been further demonstrated following pretreatment of the animals with pharmacological doses of substrates for the decarboxylation, as well as some enzyme inhibitors. Recently it was found that *m*-tyrosine is the precursor of *m*-tyramine [4]. *m*-Tyrosine, in contrast to *p*-tyrosine, appears to be a good substrate for the decarboxylase. The effects of the organic solvents on the decarboxylation of *m*-tyrosine both *in vitro* and *in vivo* have, therefore, also been studied. Some of these results were presented at the Ninth Meeting of the International Society for Neurochemistry [5].

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MATERIALS AND METHODS

Male albino Swiss mice (18–22 g body wt) or male Wistar rats (200–240 g body wt) were killed by decapitation. The brain was removed quickly and the striatum, consisting mainly of the head of the caudate nucleus and including some of the underlying putamen (approximate weights 28–35 mg for the mice and 100–130 mg for the rats), was dissected out.

Striata from three mice or two rats were pooled, immediately frozen in dry ice, weighed, and homogenized in 0.1 N HCl containing disodium edetate (EDTA, 1 mg/ml) and ascorbic acid (5 mg/ml). The amines in the tissue homogenate were derivatized with 5-dimethylamino-L-naphthalene sulphonyl (dansyl) chloride, extracted into toluene–ethylacetate (9:1, v/v), and separated chromatographically in two different monodimensional systems. The first system, toluene–ethylacetate (5:2, v/v), was the same for β-phenylethylamine, *p*-tyramine and *m*-tyramine. The second system was toluene–tri-

ethylamine (5:1, v/v) for β-phenylethylamine, or toluene–triethylamine–methanol (50:5:1, by vol.) for *p*-tyramine and *m*-tyramine. The estimations of β-phenylethylamine, *p*-tyramine or *m*-tyramine were carried out by a high resolution mass spectrometric selected ion monitoring (integrated ion current) technique using the corresponding deuterated amines (which were added to the homogenate at the beginning of the experiment) as internal standards. Complete details concerning this procedure have been described [6–8]. Dopamine was estimated by the fluorimetric method proposed by Laverty and Sharman [9]. The estimations were carried out on the pooled striata of two mice. Dopamine was separated on a Dowex 50 W × 4 ion exchange chromatography column and acetylated, a fluorophore was developed by condensation with 1,2-diaminoethane, and the fluorescence products were extracted into isobutanol and estimated. A check on recovery of 100 ng of added dopamine was carried out in each experiment: the percentage of recovery was 83 ± 4 (4) (mean ± S.E.M., number of experiments in parentheses). The results were corrected for losses.

Aromatic-L-aminoacid decarboxylase was prepared from rat or mouse whole brain. The animals were killed by decapitation and the brain was dissected out and rinsed with cold saline. The brain was homogenized in 0.01 M phosphate buffer (pH 7.2) and centrifuged at 24,000 *g* for 20 min. Ammonium sulfate was added to the supernatant fraction to make up 30% saturation, and the precipitate was separated by centrifugation. More ammonium sulfate was then added to the supernatant fraction until 50% saturation was reached and the resultant precipitate isolated by centrifugation. The pellet was dissolved in 0.01 M phosphate buffer (pH 7.2) and dialyzed. After dialysis, the enzyme was kept frozen at −20° and was stable for at least a month.

The decarboxylation products of phenylalanine, *p*-tyrosine or *m*-tyrosine formed after incubation with rat brain aromatic-L-aminoacid decarboxylase were isolated and separated by thin-layer chromatography as already described for their determinations in tissues, and determined by mass spectrometry [6–8]. The aromatic-L-aminoacid carboxylase activity towards DOPA was determined by the use of a radioenzymatic method and the ^{14}C-labeled amines formed were separated through an ion exchange column (Amberlite GC-50) [10]. The incubation mixture of the enzyme assays contained 2.5×10^{-5} M pyridoxal 5′-phosphate, 1×10^{-5} M pargyline as a monoamine oxidase inhibitor, and 0.3 to 0.5 mg protein of the partially purified aromatic-L-aminoacid decarboxylase from rat or mouse brain. The final volume was 200 μl with 0.1 M phosphate buffer at pH 7.5 for phenylalanine and the *p*- and *m*-isomers of tyrosine and pH 8.5 for DOPA. The incubation was carried out at 37° for 30 min. Blanks were prepared similarly, except that no enzyme or substrates were included.

The chemicals used were: L-phenylalanine, L-tyrosine ethylester hydrochloride (*p*-tyrosine), D,L-*m*-tyrosine, L-β-3,4-dihydroxyphenylalanine methylester hydrochloride (DOPA) and pargyline hydrochloride. *p*-Tyrosine and pargyline were dissolved in saline while D,L-*m*-tyrosine was suspended in saline containing 2.2 mg/ml of Tween 80 and injected subcutaneously. Benzene, toluene, cyclohexane, hexane, dichloromethane, chloroform, carbon tetrachloride, pyridine and 3-methyl-1-butanol (isoamylalcohol) were injected intraperitoneally. In some experiments, benzene was dissolved in sesame oil, as a 5% (v/v) solution, and injected subcutaneously in the required doses. The aminoacids and pargyline were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A., and the organic solvents were Spectranalyzed Grade from Fisher Scientific Ltd., Edmonton, Alberta, Canada.

RESULTS

Effect of various organic solvents on the activity of particularly purified aromatic aminoacid decarboxylase. As can be seen from Table 1, the effect

Table 1. Effects of some organic solvents on the decarboxylation of several aromatic-L-aminoacids by partially purified rat brain aromatic-L-aminoacid decarboxylase (AADC)*

Organic solvent	Relative AADC activity (%)			
	Phenylalanine	*p*-Tyrosine	*m*-Tyrosine	DOPA
Controls	100 ± 20 (4)	100 ± 19 (4)	100 ± 7 (8)	100 ± 18 (8)
Benzene	361 ± 38† (4)	1671 ± 77† (4)	87 ± 2 (5)	49 ± 4‡ (8)
Toluene	225 ± 36‡ (4)	659 ± 75† (4)	94 ± 1 (5)	57 ± 1‡ (4)
Hexane	53, 53 (2)	55 ± 13 (4)	82 ± 9 (4)	106 ± 2 (4)
Cyclohexane	196, 206 (2)	301, 281 (2)	98, 105 (2)	91 ± 2 (4)
Chloroform	88 ± 16 (4)	341 ± 120 (4)	54 ± 7§ (4)	68 ± 3 (4)
Dichloromethane		656, 799 (2)	88, 76 (2)	82 ± 3 (4)
Carbon tetrachloride	309, 326 (2)	720, 687 (2)	102, 96 (2)	75 ± 2 (4)
Pyridine	27, 14 (2)	36, 13 (2)		2 ± 1§ (4)
Isoamylalcohol	0, 0 (2)	19 ± 11§ (4)	6, 6 (2)	0 (4)

* The substrate concentrations used were 1×10^{-3} M for phenylalanine and *p*-tyrosine, 1×10^{-5} M for *m*-tyrosine, and 1×10^{-4} M for DOPA. The specific activities (nmoles·mg^{-1}·h^{-1}) of the untreated enzyme towards phenylalanine, *p*-tyrosine, *m*-tyrosine and DOPA are 0.189 ± 0.037, 0.064 ± 0.012, 2.32 ± 0.16 and 19.89 ± 3.65 respectively. The relative activities are expressed as percentages of these control values. The organic solvents were added so as to comprise 2.5% of the incubation volume (i.e. 3 μl in 200 μl). All values are expressed as means (± S.E.M., number of experiments in parentheses).

†–§ Student's *t*-test: †$P < 0.001$; ‡$P < 0.05$, and §$P < 0.01$ compared with control.

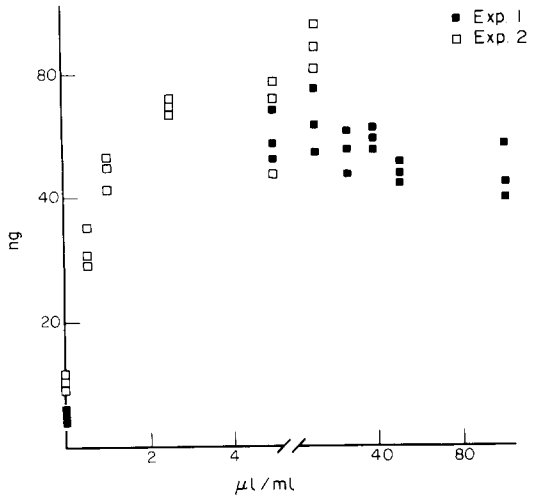

Fig. 1. Effect of benzene on rat brain aromatic-L-aminoacid decarboxylase activity towards *p*-tyrosine. Results from two experiments are presented. In the first experiment (■), benzene (0–100 μl/ml) was directly added to the reaction mixture (200 μl). In the second experiment (□), benzene (0–10 μl/ml) was diluted with ethanol before addition to the reaction mixture. Ethanol concentrations lower than 1% do not effect the enzyme activity.

of different organic solvents on rat brain aromatic-L-aminoacid decarboxylase was related to the substrate used. Aromatic-L-aminoacid decarboxylase exhibited greater specific activity towards DOPA and *m*-tyrosine than *p*-tyrosine and phenylalanine. The effects of the solvents on DOPA and *m*-tyrosine ranged from no effect to marked inhibition. In contrast, the decarboxylation of the poorer substrates, *p*-tyrosine and phenylalanine, was substantially activated by benzene, toluene, cyclohexane, dichloromethane and carbon tetrachloride while chloroform increased the decarboxylation of *p*-tyrosine but not that of phenylalanine. Other solvents, such as isoamylalcohol and pyridine, exhibited strong inhibitory effects on aromatic-L-aminoacid decarboxylase activity towards all four aminoacids.

The activation of benzene on aromatic-L-aminoacid decarboxylase activity towards *p*-tyrosine was proportional to the amount of benzene added and was saturable. As indicated in Fig. 1, the maximal activation was achieved at approximately 0.5% (v/v).

Similar experiments carried out with partially purified mouse brain aromatic-L-aminoacid decarboxylase show that the addition of benzene (0.5 or 2.5%, v/v) produced a 4 to 5-fold increase in the decarboxylation of phenylalanine and 13- to 14-fold increase in that of *p*-tyrosine (Table 2). In contrast, the decarboxylation of *m*-tyrosine was not affected by the addition of benzene (Table 2).

Effect of benzene on the striatal concentrations of some brain monoamines. The intraperitoneal administration of benzene (0.5 ml/kg) significantly increased mouse striatal β-phenylethylamine, as observed during the 2 hr of treatment. Thirty minutes after injection, β-phenylethylamine concentrations had reached a value about eleven times higher than that of the control (Table 3). At 1 or 2 hr after the benzene administration, the striatal concentrations although lower than they were at 30 min were still significantly higher than the control value (594 and 394% respectively).

Mouse striatal concentrations of *p*-tyramine were also increased following intraperitoneal administration of benzene (Table 3). Significant increases could be seen within 0.25 hr of a dose of 0.5 ml/kg (to 153%) or within 1 hr of a dose of 0.25 ml/kg (to 183% of controls). Maximal increases (to 285% of controls) were observed after 1 hr of benzene administration and by 4 hr the concentrations had returned to control levels. The benzene treatment (0.5 ml/kg) also increased mouse striatal *m*-tyramine (Table 3); these effects were observed 30 min after its administration, reached a maximal concentration by 2 hr (to 161% of controls), and lasted for at least 4 hr (Table 3). Benzene administration (0.5 ml/kg intraperitoneally) produced no significant changes in mouse striatal dopamine (Table 3).

To be able to inject lower doses of benzene, a 5% solution of benzene in sesame oil was prepared. The subcutaneous administration of 0.125 ml/kg of benzene produced no significant change in mouse striatal *p*-tyramine (Table 4). At a higher dose of benzene (0.5 ml/kg) significant increases (to 178% of the sesame oil treated controls) were observed

Table 2. Effect of benzene on decarboxylation of some amino acids by partially purified mouse brain aromatic-L-aminoacid decarboxylase (AADC)*

	Relative AADC activity (%)		
	Phenylalanine	*p*-Tyrosine	*m*-Tyrosine
Control	100 ± 1.6	100 ± 0.01	100 ± 1.6
Benzene (0.5%, v/v)	480.5 ± 18.3†	1258.9 ± 37.1†	103.7 ± 0.2
Benzene (2.5%, v/v)	401.4 ± 11.1†	1394.4 ± 55.1†	102.4 ± 2.8

* Experimental conditions are described in Methods and Table 1. The specific activities of the untreated mouse brain AADC towards phenylalanine, *p*-tyrosine and *m*-tyrosine are 0.298 ± 0.048, 0.124 ± 0.001 and 1.60 ± 0.03 respectively. Values are expressed as mean ± S.E.M. from three experiments.
† $P < 0.01$ compared with control.

Table 3. Effect of the intraperitoneal administration of benzene on the striatal concentration of β-phenylethylamine, *p*-tyramine, *m*-tyramine and dopamine in the mouse*

Dose (ml/kg)	Time (hr)	β-Phenylethylamine (ng/g)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)	Dopamine (ng/g)
		1.6 ± 0.4 (7)	24.6 ± 1.3 (15)	6.7 ± 0.4 (15)	10,810 ± 760 (6)
0.25	1		44.9 ± 3.9† (7)	7.0 ± 0.5 (6)	
0.5	0.25		37.6 ± 3.5‡ (6)	8.8 ± 1.2 (6)	
0.5	0.5	17.3 ± 0.5† (3)	52.7 ± 4.0† (6)	9.8 ± 0.8‡ (6)	11,560 ± 430 (6)
0.5	1	9.5 ± 0.5† (6)	70.2 ± 4.0† (9)	10.4 ± 0.9‡ (9)	12,050 ± 720 (6)
0.5	2	6.3 ± 1.6§ (3)	49.6 ± 8.4‡ (5)	10.8 ± 0.9† (5)	10,980 ± 620 (6)
0.5	4		24.8 ± 1.9 (6)	8.6 ± 0.4‡ (6)	10,220 ± 770 (6)

* Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.
†–§ Student's *t*-test: †$P < 0.001$, ‡$P < 0.01$, and §$P < 0.05$ compared with control.

within 30 min, reached maximal values (to 234% of the sesame oil treated controls) at 1 hr, and had returned to control values by 4 hr (Table 4). The subcutaneous administration of the 5% solution of benzene in sesame oil produced no significant changes in mouse striatal *m*-tyramine (Table 4). We also observed that intraperitoneal injections of benzene (0.5 ml/kg) produced marked increases in the concentration of both isomers of tyramine (to 368% for the *para* and 153% for the *meta* of controls, respectively) in the caudate nucleus of the rat at 1 hr after treatment (Table 5).

The administration of benzene to pargyline-treated mice also produced statistically significant increases in mouse striatal *p*-tyramine (to 163% of the pargyline-treated controls) (Table 6); the treatment produced no significant changes in mouse striatal *m*-tyramine concentrations with respect to pargyline-treated controls.

The administration of *p*-tyrosine (400 mg/kg) or *m*-tyrosine (2 mg/kg) produced at 2 hr after treatment marked increases in the respective striatal concentrations of *p*- or *m*-tyramine (to 292 or 972% of their respective controls) (Table 7). The additional administration of benzene (0.5 ml/kg) to *p*-tyrosine-pretreated mice produced a further increase in striatal *p*-tyramine, i.e. with respect to control (986%), benzene-treated (378%) or *p*-tyrosine-treated (338%) mice. Interestingly, a significant potentiation occurred when benzene was administered to *p*-tyrosine-pretreated mice [$F(1,16) = 42.91$, $P < 0.01$] (Table 7). The mouse striatal levels of *m*-tyramine were not changed significantly after *p*-tyrosine alone but were slightly increased by the additional administration of benzene (to 125% of controls) [$F(1,16 = 21.31$, $P < 0.01$] (Table 7). Also, the pretreatment increased the rate of formation of *m*-tyramine [$F(1,16)$] value for the interaction equal to 8.58 ($P < 0.01$). Benzene treatment (0.5 ml/kg) to mice that had already received *m*-tyrosine (2 mg/kg) produced marked increases with respect to controls (1601%), benzene-treated (1278%) or *m*-tyrosine-treated (165%) respectively (Table 7); the combined treatment with the two compounds produced a potentiation of their effects [$F(1,16) = 100.08$, $P < 0.01$]. In contrast, the administration of *m*-tyrosine and benzene produced no potentiation of their effects with respect to *p*-tyramine (Table 7).

Effects of some organic solvents on the striatal concentrations of some monoamines. The intraperitoneal administration of toluene produced a statistically significant increase in the mouse striatal concentration of *p*- and *m*-tyramine (Table 8). The highest increases for *p*-tyramine (to 147% of controls) were observed at 0.5 hr after the administration of 0.5 ml/kg and at 2 hr in the case of *m*-tyramine

Table 4. Effect of the subcutaneous administration of benzene dissolved in sesame oil on the striatal concentration of *p*-tyramine and *m*-tyramine in the mouse*

Dose (ml/kg)	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
		25.3 ± 1.7 (10)	7.4 ± 0.5 (9)
0.125	1	23.7 ± 2.3 (6)	7.2 ± 0.8 (6)
0.5	0.5	45.0 ± 7.0† (6)	7.1 ± 0.4 (6)
0.5	1	59.3 ± 7.7‡ (6)	7.2 ± 0.5 (6)
0.5	2	34.1 ± 4.5 (6)	7.3 ± 0.9 (6)
0.5	4	21.3 ± 2.5 (7)	7.5 ± 0.4 (7)

* Benzene was injected in the indicated doses dissolved in sesame oil as a 5% solution (v/v). The experimental mice were injected with 10 ml/kg of the solution and killed at the times given in the table. Controls were subcutaneously injected with 10 ml/kg of sesame oil and killed at 0.5, 1, 2 or 4 hr after administration and pooled in a single group. Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.
†, ‡ Student's *t*-test: †$P < 0.05$, and ‡$P < 0.001$, compared with control.

Table 5. Effect of the intraperitoneal administration of benzene on the striatal concentration of *p*-tyramine and *m*-tyramine in the rat*

Dose (ml/kg)	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
		9.4 ± 0.6 (4)	3.4 ± 0.2 (4)
0.5	1	34.6 ± 1.8† (4)	5.2 ± 0.2† (4)

* Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.
† Student's *t*-test: $P < 0.001$ compared with control.

Table 6. Effect of the intraperitoneal administration of benzene to mice pretreated with pargyline on the striatal concentration of *p*-tyramine and *m*-tyramine in the mouse*

Treatment	Dose	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
Controls			28.3 ± 2.2 (4)	5.9 ± 1.1 (4)
Benzene	0.5 ml/kg	1	79.7 ± 8.8† (4)	8.1 ± 0.6 (4)
Pargyline	50 mg/kg	4	88.7 ± 14.1† (4)	15.1 ± 1.3† (4)
Pargyline + benzene	50 mg/kg 0.5 ml/kg	4 1	Benz† Parg† 144.4 ± 12.0 (4)	Benz† 18.0 ± 2.8† (4)

* Mice were first subcutaneously injected with pargyline and 3.0 hr later were given benzene; the animals were killed 4 hr after the beginning of the experiment. Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.

† Significances were obtained by multiple comparisons using Newman–Keul's procedure; $P < 0.01$. Benzene and pargyline indicate that the significances were obtained by comparison with the group of mice treated with benzene or pargyline respectively.

(to 129% of controls) (Table 8). Of the other organic solvents tested only dichloromethane or pyridine produced statistically significant increases in *p*-tyramine and *m*-tyramine while chloroform or carbon tetrachloride increased only *p*-tyramine (Table 8); these effects were observed within 1 hr after drug administration. In contrast, cyclohexane or hexane did not change the mouse striatal concentrations of *p*- or *m*-tyramine (Table 8). The administration of pyridine (0.5 ml/kg) produced marked increases in the concentration of mouse striatal *p*-tyramine that were observed at 0.5 or 1 hr after drug administration (Table 8); the concentrations were, respectively, 208% and 260% of controls. The pyridine treatment also increased the concentrations of striatal *m*-tyramine (Table 8) to values around 130% of controls that were observed within 1 hr after the drug was given.

Isoamylalcohol (0.5 ml/kg) produced a moderate increase (to 133% of controls) in mouse striatal *p*-tyramine that was significantly different from control at 15 min after solvent administration (Table 9). Striatal *m*-tyramine was increased at 0.5 hr after the administration of ml/kg of isoamylalcohol (Table 9).

DISCUSSION

These experiments show that benzene can cause a substantial increase in the rate of formation of mouse striatal β-phenylethylamine and *p*-tyramine (Table 3) and of rat striatal *p*-tyramine (Table 5). These results agree well with the *in vitro* activation of the decarboxylation of phenylalanine and *p*-tyrosine by partially purified aromatic-L-aminoacid decarboxylase (Tables 1 and 2) [3] as well as with earlier experiments carried out with rat kidney extracts [2].

Table 7. Effect of the intraperitoneal administration of benzene to mice pretreated with *p*-tyrosine or *m*-tyrosine on the striatal concentration of *p*-tyramine and *m*-tyramine in the mouse*

Treatment	Dose	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
Controls			28.9 ± 1.4 (5)	0.6 ± 0.8 (5)
p-Tyrosine	400 mg/kg	2	87.0 ± 5.8† (5)	5.8 ± 0.7 (5)
m-Tyrosine	2 mg/kg	2	24.0 ± 11.0‡ (5)	69.0 ± 4.6† (5)
Benzene	0.5 ml/kg	1	65.1 ± 0.4† (5)	8.6 ± 0.4† (5)
p-Tyrosine + benzene	400 mg/kg 0.5 ml/kg	2 1	*p*-Tyr† Benz† 299.6 ± 24.9† (5)	*p*-Tyr‡ Benz‡ 14.6 ± 2.1‡ (5)
m-Tyrosine + benzene	2 mg/kg 0.5 ml/kg	2 1	*m*-Tyr‡ 62.9 ± 5.3† (5)	*m*-Tyr† Benz† 108.7 ± 5.6† (5)

* Mice were subcutaneously injected with *p*-tyrosine or *m*-tyrosine, respectively, and 1.0 hr later were given an intraperitoneal injection of benzene; the animals were killed 2 hr after the beginning of the experiment. Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.

†, ‡ ANOVA: †$P < 0.01$, and ‡$P < 0.05$. *p*-Tyr, *m*-Tyr and Benz indicate that the significances were obtained by comparison with the group of mice treated with *p*-tyrosine, *m*-tyrosine or benzene respectively.

Table 8. Effects of the intraperitoneal administration of some organic solvents on the striatal concentration of *p*-tyramine and *m*-tyramine in the mouse*

Treatment	Dose (ml/kg)	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
Controls			23.3 ± 1.2 (19)	6.8 ± 0.3 (19)
Toluene	0.5	0.25	28.1 ± 3.1 (5)	8.8 ± 0.8† (5)
	0.5	0.5	34.2 ± 1.9‡ (5)	8.1 ± 0.5† (5)
	0.5	1	33.3 ± 2.1‡ (5)	8.1 ± 0.2§ (5)
	0.5	2	31.5 ± 3.2† (6)	8.8 ± 0.4‡ (6)
	0.5	4	22.6 ± 3.4 (5)	8.4 ± 0.8 (5)
Cyclohexane	0.5	1	31.7 ± 5.7 (4)	8.0 ± 1.3 (4)
Hexane	0.5	0.5	28.7 ± 2.6 (3)	6.7 ± 1.8 (3)
	0.5	1	23.7 ± 1.4 (5)	6.9 ± 1.2 (5)
Dichloromethane	0.5	0.5	29.7 ± 3.0 (4)	9.5 ± 0.7§ (4)
	0.5	1	35.1 ± 2.9§ (4)	7.8 ± 0.4 (4)
Chloroform	0.5	0.5	26.5 ± 0.8† (4)	7.1 ± 1.0 (4)
	0.5	1	25.6 ± 1.2 (5)	7.7 ± 0.8 (5)
Carbon tetrachloride	0.5	0.5	23.5 ± 3.2 (3)	6.7 ± 0.9 (3)
	0.5	1	32.1 ± 2.0§ (5)	7.8 ± 1.2 (5)
Pyridine	0.5	0.5	48.4 ± 6.6§ (5)	8.9 ± 0.9‡ (5)
	0.5	1	60.5 ± 8.3‡ (5)	9.0 ± 0.2‡ (5)

* Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.
†–§ Student's *t*-test: †$P < 0.05$; ‡$P < 0.001$, and §$P < 0.01$ compared with control.

Benzene reduced the *in vitro* decarboxylation of DOPA by the partially purified brain aromatic-L-aminoacid decarboxylase (Table 1), and the mouse striatal levels of dopamine were not changed after the intraperitoneal injection of benzene (Table 3). In contrast, the *in vitro* decarboxylation of *m*-tyrosine was not altered significantly by benzene addition (Table 1). Unexpectedly, however, the rat or mouse striatal concentrations of *m*-tyramine were both increased after benzene administration (Tables 3 and 5), although the magnitude of the increase was much less than that of the increase of β-phenylethylamine and *p*-tyramine. Moderate increases in mouse striatal *m*-tyramine concentrations were observed after the administration of toluene or dichloromethane (Table 8) that did not change or slightly reduced the *in vitro* decarboxylation of *m*-tyrosine (Table 1).

Pyridine and isoamylalcohol have been shown to inhibit the *in vitro* decarboxylation of phenylalanine, *p*-tyrosine, *m*-tyrosine or DOPA [3] and these findings were confirmed in the present experiments (Table 1). After the intraperitoneal administration of these solvents, however, the mouse striatal concentrations of *p*- and *m*-tyramine were not decreased; on the contrary, pyridine (Table 8) or isoamylalcohol (Table 9) caused significant increases in the concentrations of both amines. It is quite possible that either pyridine or isoamylalcohol may have effects in other enzyme systems that affect the concentration of *p*- or *m*-tyramine, or else it could be that the chemical structures of pyridine or isoamylalcohol are modified *in vivo* and that other related compounds are responsible for these changes.

Table 9. Effect of the intraperitoneal administration of isoamylalcohol on the striatal concentration of *p*-tyramine and *m*-tyramine in the mouse*

Dose (ml/kg)	Time (hr)	*p*-Tyramine (ng/g)	*m*-Tyramine (ng/g)
		24.9 ± 1.9 (7)	7.8 ± 0.7 (7)
0.5	0.25	33.1 ± 1.7† (5)	9.1 ± 1.1 (4)
0.5	0.5	28.1 ± 2.5 (4)	8.5 ± 0.4 (4)
0.5	1	28.6 ± 3.9 (4)	6.7 ± 0.2 (4)
0.5	2	23.2 ± 1.9 (4)	6.9 ± 0.2 (4)
1	0.5	28.9 ± 1.0 (3)	11.0 ± 0.5† (3)

* Values are means (± S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.
† Student's *t*-test: $P < 0.01$ compared with control.

The lack of complete agreement between the *in vitro* and *in vivo* effects of benzene or some other organic solvents on aromatic-L-aminoacid decarboxylase may be due to the differences in the compartmentation of the enzyme under different conditions that may exert subtle control of the access of the solvent to the active sites.

It has been shown recently that after administration of toluene, either intraperitoneally or by inhalation, rat plasma tyrosine (presumably the *p*-isomer) concentrations are reduced [11]. This reduction could be the consequence of increased decarboxylation of tyrosine by toluene administration. In addition, the chronic administration of low doses of toluene reduced the rat striatal dopamine turnover [12]; that could be explained possibly as a result of the inhibitory effect of toluene on the decarboxylation of DOPA as shown in the *in vitro* experiment (Table 1), although the acute administration of benzene did not affect the mouse striatal dopamine concentration (Table 3).

It was found that low doses of central aromatic-L-aminoacid decarboxylase inhibitors block the formation of *p*-tyramine while increasing that of *m*-

tyramine [13, 14], while only the administration of high doses of inhibitors block the formation of both tyramines [15]. The present experiments show that benzene activated the *in vivo* and *in vitro* decarboxylation of phenylalanine and *p*-tyrosine (Tables 1 and 3). Benzene did not affect the *in vitro* decarboxylation of *m*-tyrosine and decreased that of DOPA although its intraperitoneal administration produced some increase in striatal *m*-tyramine and no change in dopamine (Tables 3 and 5). It seems that the activation of aromatic-L-aminoacid decarboxylation does not occur with those substrates that have a *meta*-hydroxy functional group (*m*-tyrosine or DOPA).

The results indicate that benzene and some other organic solvents not only increased the *in vitro* decarboxylation of phenylalanine and *p*-tyrosine by a partially purified brain aromatic-L-aminoacid decarboxylase but also increased the cerebral concentration of β-phenylethylamine, *p*-tyramine and to a lesser extent that of *m*-tyramine, thus suggesting that benzene induced the aromatic-L-aminoacid decarboxylase activity in the brain. Since monoamine oxidase shows similar affinity towards *p*- and *m*-tyramine [16], it seems unlikely that the effect of the organic solvents could occur by *in vivo* inhibition of the enzyme. In addition, benzene administration did not increase the striatal concentrations of dopamine (Table 3) or 5-hydroxytryptamine (A. V. Juorio, unpublished results) as it should occur if monoamine oxidase had been inhibited.

These increases in brain β-phenylethylamine, *p*-tyramine and *m*-tyramine may play a contributory role in the human toxicity of benzene and some of these organic solvents; these toxic effects could be exacerbated after ingestion of foodstuffs containing aminoacids such as phenylalanine or *p*-tyrosine or for those under treatment with a monoamine oxidase inhibitor.

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