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Effects of benzene and pyridine on the concentration of mouse striatal tryptamine and 5-hydroxytryptamine

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We have shown recently that the parenteral administration of benzene or some other organic solvents produces significant increases in the striatal concentrations of β-phenylethylamine, *p*-tyramine and *m*-tyramine [1]. These findings are in good agreement with early reports that the addition of benzene to guinea pig kidney aromatic-L-aminoacid decarboxylase preparations produces marked increases in the *in vitro* (decarboxylation of phenylalanine and tyrosine (presumably the *p*-isomer) [2]. The increase in the decarboxylation of *p*-tyrosine has also been observed with rat [3] or mouse brain [1] extracts. In addition, an increase in the decarboxylation of tryptophan by guinea pig kidney aromatic-L-aminoacid decarboxylase preparations [2] was observed in the presence of benzene.

In this communication, we report the effects of benzene and some other organic solvents on the decarboxylation of tryptophan and 5-hydroxytryptophan by partially purified brain aromatic-L-aminoacid decarboxylase and the effect of the parenteral administration of benzene or pyridine on the striatal concentration of tryptamine, 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA).

Methods

Male albino Swiss mice (18–22 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) was dissected out. Tryptamine concentrations were determined in the pooled tissues of three mice that were homogenized in 1 ml of 0.1 HCl containing ethylene diamine tetracetic acid disodium salt (EDTA, 1 mg/ml) and ascorbic acid (5 mg/ml), and 50 ng of tetradeutero tryptamine internal standard was added. The amines in the tissue homogenate were derivatized with 5-dimethylamino-1-naphthalene sulfonyl (dansyl) chloride, and the resultant derivatives were extracted into toluene–ethylacetate (9:1, v/v), evaporated to a small volume, and separated chromatographically in two different unidimensional systems [4]. Tryptamine was estimated by the high resolution mass spectrometric selected ion monitoring (integrated ion current) technique using deuterated tryptamine as an internal standard. Blanks of 0.1 N HCl, to which was added 50 ng of deuterated tryptamine, were carried out throughout the procedure. These gave blank values of 200 pg and thus enabled quantification of as little as 200 pg of tryptamine in the tissue samples. Complete details concerning these procedures have been described [5].

Brain regional concentrations of 5-HT and 5-HIAA were determined by high performance liquid chromatography with electrochemical detection [6]. The apparatus consisted of a solvent delivery system (model M45, Waters Associates, Inc., Mississauga, Ontario, Canada) equipped with a WISP automatic injector set with a fixed 20 μl loop (model 710B, Waters Associates, Inc., Mississauga, Ontario, Canada). The separations were achieved in a column (Altex Ultrasphere ODS) with the following characteristics: length, 250 mm; internal diameter, 4.6 mm; and particle size, 5 μm. Also, a 170 mm long precolumn (Whatman Inc., Clifton, NJ, U.S.A) was used. The 5-hydroxyindole compounds were detected on a carbon paste electrode (model TL-3, Bioanalytical Systems, West Lafayette, IN, U.S.A.) set at 0.75 V versus a Ag/AgCl reference electrode. Standards and test curves were displayed on a dual channel recorder (BD41, Kipp & Zonen, Holland). The mobile phase, consisting of 0.1 M NaH_2PO_4, 1 mM sodium octyl sulfate, 1 mM disodium EDTA and 12% acetonitrile adjusted to pH 3.3 with phosphoric acid, was filtered through a Buchner funnel (fritted glass pore, diameter 10–15 μM) and degassed by vacuum. Tissues obtained from one rat were homogenized in 0.1 M $HClO_4$ containing 0.67 mM EDTA and 100 ng/ml of isoproterenol as internal standard. Following centrifugation (Eppendorf centrifuge, model 5412), the supernatant fraction was injected directly into the system. The corresponding calibration curves were prepared daily and the correction factor for 5-HT and 5-HIAA with respect to the internal standard (2 ng isoproterenol) was determined. The minimum detectable amount was 100 pg for both 5-HT and 5-HIAA, with a signal to noise ratio <3.

Aromatic-L-aminoacid decarboxylase was prepared from rat 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 it was again centrifuged and to the supernatant more ammonium sulfate was then added 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 aromatic-L-aminoacid decarboxylase activity towards 5-hydroxytryptophan was determined by the use of a radioenzymatic method with ^{14}C-labeled substrate and amberlite CG-50 ion exchange resin to separate the labeled product as previously described [1, 7]. The tryptamine formed *in vitro* by tryptophan decarboxylation was determined by a mass spectrometric method as indicated above. The incubation mixtures contained 0.1 M phosphate buffer (pH 7.5), 2.5×10^{-5} M pyridoxal-5′-phosphate,

1×10^{-5} M pargyline as a monoamine oxidase inhibitor and 1×10^{-4} M 5-hydroxytryptophan and 1×10^{-3} M tryptophan as substrate.

In the treatment of the animals, benzene and pyridine were injected intraperitoneally and pargyline hydrochloride by subcutaneous injection. The organic solvents were Spectranalyzed Grade from Fisher Scientific Ltd., Edmonton, Alberta, Canada, and pargyline hydrochloride was obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A.

Results

The specific activity of the partially purified rat brain aromatic-L-aminoacid decarboxylase is much higher towards 5-hydroxytryptophan than towards tryptophan. Organic solvents such as benzene, toluene, chloroform and pyridine significantly inhibited the decarboxylation of 5-hydroxytryptophan (Table 1). In contrast, the decarboxylation of tryptophan was increased significantly (to about three or four times its control level) after addition of benzene or toluene (Table 1). No significant changes were observed with chloroform, while hexane and pyridine inhibited the enzyme activity (Table 1).

The intraperitoneal administration of 0.5 ml/kg of benzene or pyridine produced a significant increase (to 181 and 213% of controls respectively) in the concentration of mouse striatal tryptamine (Table 2). Benzene administration moderately increased the striatal concentrations of 5-HT as well as those of its acid metabolite 5-HIAA (to 117 and 121% of control respectively), while pyridine treatment only increased 5-HIAA (to 131% of controls) (Table 2). The administration of benzene (0.5 ml/kg) to mice pretreated with pargyline produced a marked increase in the concentration of striatal tryptamine (to 223% of its respective pargyline-treated control) (Table 3); even larger increases (to 540% of its respective pargyline-treated control) were observed after pyridine (0.5 ml/kg) (Table 3).

Discussion

The present experiments show that the parenteral administration of benzene causes a marked increase in the rate of formation of tryptamine in the mouse striatum (Table 2). This effect was further substantiated after the animals were pretreated with a monoamine oxidase inhibitor (Table 3). These results agree well with the observation that the decarboxylation of tryptophan by the partially purified rat brain aromatic-L-aminoacid decarboxylase was activated by benzene (Table 1), as well as with earlier experiments carried out with guinea pig kidney extracts [2]. Earlier work has shown that the activation of *p*-tyrosine decarboxylation by benzene is a saturable reaction that follows a linear relationship [1]; these findings strongly suggest that the activation of tryptophan decarboxylation by benzene should possess similar characteristics.

Pyridine appeared to inhibit the decarboxylation of both tryptophan and 5-hydroxytryptophan by the partially purified enzyme (Table 1). Similar observations of the pyridine *in vitro* inhibitory effects [3] and *in vivo* activation on the decarboxylation of the *p*- and *m*-isomers of tyrosine have been observed [1]. It is interesting, but not clear, why pyridine exhibits different effects *in vitro* and *in vivo*.

Table 1. Effects of some organic solvents on the decarboxylation of tryptophan and 5-hydroxytryptophan by partially purified rat brain aromatic-L-aminoacid decarboxylase (AADC)*

Organic solvent	Relative AADC activity (%) Tryptophan	5-Hydroxytryptophan
Controls	100 ± 9 (8)	100 ± 12 (8)
Benzene	398 ± 34† (4)	48 ± 2† (4)
Toluene	283 ± 31† (4)	57 ± 3† (4)
Hexane	50, 58 (2)	101 ± 3 (4)
Chloroform	127 ± 22 (4)	65 ± 4‡ (4)
Pyridine	14, 14 (2)	34 ± 3† (4)

* The substrate concentrations used were 1×10^{-4} M and 1×10^{-3} M with respect to 5-hydroxytryptophan and tryptophan. The specific activities (nmoles·mg^{-1}·hr^{-1}) of the untreated enzyme towards tryptophan and 5-hydroxytryptophan were 0.42 ± 0.1 and 21.2 ± 6.8 respectively. The organic solvents were added so as to comprise 2.5% of the incubation volume (i.e. 5 μl in 200 μl). All values are expressed as means (±S.E.M., number of experiments in parentheses).

†,‡ Significance was calculated by Student's *t*-test: † $P < 0.01$, and ‡ $P < 0.05$.

Table 3. Effect of the intraperitoneal administration of benzene or pyridine to mice pretreated with pargyline on the striatal concentration of tryptamine*

	Dose (ml/kg)	Time (hr)	Tryptamine (ng/g)
Pargyline controls			9.3 ± 1.9 (7)
Benzene	0.5	1	20.8 ± 1.9† (7)
Pargyline controls			8.2 ± 0.7 (5)
Pyridine	0.5	1	44.3 ± 5.7† (5)

* The mice were first injected with pargyline (50 mg/kg) and 3 hr later given benzene or pyridine; 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. Comparisons were made with their respective pargyline-treated controls.

† Significance was calculated by Student's *t*-test: $P < 0.005$.

Table 2. Effect of the intraperitoneal administration of benzene or pyridine on the striatal concentrations of tryptamine, 5-HT and 5-HIAA in the mouse*

	Dose (ml/kg)	Time (hr)	Tryptamine (ng/g)	5-HT (ng/g)	5-HIAA (ng/g)
Controls			1.6 ± 0.4 (19)	850 ± 20 (11)	620 ± 20 (11)
Benzene	0.5	1	2.9 ± 0.4† (13)	990 ± 60‡ (9)	750 ± 40‡ (9)
Pyridine	0.5	1	3.4 ± 0.9† (6)	910 ± 40 (12)	810 ± 50‡ (12)

* Values are means (±S.E.M., number of experiments in parentheses) in ng/g of fresh tissue.

†,‡ Significance was calculated by Student's *t*-test: † $P < 0.05$, and ‡ $P < 0.01$.

The increases in mouse striatal tryptamine produced by parenteral administration of benzene or pyridine (Table 2) suggest that the treatment produces an induction of aromatic-L-aminoacid decarboxylase. The fact that the administration of benzene or pyridine increased the concentration of striatal 5-HIAA (Table 2) rules out the possibility that the organic solvents act by inhibition of monoamine oxidase but suggest that the treatment increases 5-HT turnover.

In conclusion, some organic solvents such as benzene or pyridine can selectively affect aromatic-L-aminoacid decarboxylase *in vitro*, as well as *in vivo* after parenteral administration; the effect *in vivo*, however, may not be the same as that *in vitro*.

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Lack of effect of several barbiturates on liver blood flow

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Liver blood flow is an important determinant of the pharmacokinetics of drugs with a high hepatic intrinsic clearance such as lignocaine and propranolol [1]. For such compounds hepatic enzyme activity is not the major determinant of the rate of hepatic elimination. It is important, therefore, to have a knowledge of the effects of drugs on liver blood flow in order to anticipate interactions with drugs whose elimination is significantly dependent on liver blood flow.

Phenobarbitone treatment has been shown not only to increase hepatic microsomal enzyme activity but also to increase liver blood flow in the rat [2–5], in the monkey [6] and in man [7]. Furthermore, this increase in hepatosplanchnic blood flow has also been shown to be responsible for that part of the increase in the hepatic elimination of indocyanine green [8] and propranolol [6] not accounted for by enzyme induction.

Amylobarbitone treatment in the rat does not increase liver blood flow despite significant enzyme induction [5]. However, the effect of other barbiturates on liver blood flow has not been reported. Therefore, we have investigated the effect of several 5-alkyl and 5-allyl barbiturates on hepatosplanchnic blood flow in the rat in order to determine whether molecular structure, the degree of enzyme induction or hepatomegaly can be correlated with the effects on blood flow.

Methods

Animals and pretreatment. Male Wistar rats weighing 220–250 g (Bantin & Kingman Ltd., Hull, U.K.) were fed on standard laboratory diet (Labsure, C. Hill Ltd., Poole, Dorset, U.K.) in drop-through cages on a 12-hr light/dark cycle. In the blood flow studies two groups of experiments were performed. In one the treatment rats received the sodium salts of either barbitone (Sigma, St. Louis, MO), amylobarbitone (Lilly, Basingstoke, Hants., U.K.) or quinalbarbitone (Sigma) dissolved in saline (0.9% NaCl) and the control animals received saline. In the other the treatment rats received either butabarbitone (Sigma), mephobarbitone (Sigma), allobarbitone (Sigma) or aprobarbitone (Sigma) dissolved in an equivalent vol. of 1 M NaOH and diluted in saline. For these animals the control group received 0.1 M NaOH in saline. All treated animals were given 120 mg/kg/day of the barbiturate intraperitoneally (i.p.) in a vol. of 4 ml/kg/day in divided doses for 5 days and the control rats were given the same vol. of vehicle (2 ml/kg) i.p. twice daily for 5 days. In the sleeping time study only one, saline, control group was used. All animals were used on the 6th day after the start of treatment and were starved for 15–20 hr before use.

Determination of liver blood flow. The rats were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.; Sagatal, May & Baker, Dagenham, Essex, U.K.) and a tracheal cannula inserted. The left femoral artery was cannulated and connected to a Bell & Howell type 4-422-0001 transducer to measure systemic arterial blood pressure which was recorded on a Grass 79D polygraph. The left femoral artery was also cannulated and connected to a Braun Perfusor IV pump for the withdrawal of blood. With the aid of pressure monitoring, a cannula was passed down the right common carotid artery into the left ventricle. 60,000–80,000 ^{113}Sn labelled microspheres (15 ± 3 μm; NEN), suspended in saline containing 0.01% Tween 80, were injected into the ventricle over 20 sec. During and for 70 sec after the microsphere injection blood was withdrawn from the left femoral artery at a rate of 0.5 ml/min. Cardiac output and liver blood flow were determined as described by Nies *et al.* [4].

Pentobarbitone sleeping time. The animals were given sodium pentobarbitone (40 mg/kg, i.p.) and the time