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Identification and distribution of benzylamine in tissue extracts isolated from rats pretreated with pargyline

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In the recent analyses of some biogenic amines in mammalian tissues [1–4], the monoamine oxidase inhibitor pargyline [*N*-methyl-*N*-(2-propynyl)benzylamine] has been used to increase amine levels to facilitate their identification. Chromatographic separation of the dansyl derivatives of amine extracts obtained from rats treated with pargyline revealed the presence of significant amounts of benzylamine. Since benzylamine is the shortest homologue of a series of phenylalkylamines which includes the sympathomimetic amines β-phenylethylamine and amphetamine, and may itself possess excitant properties, we have attempted to determine whether benzylamine occurs endogenously in the rat and is elevated by pargyline, or whether it originates *in vivo* as a metabolite of pargyline.

Tissues were obtained from male Wistar rats (150–200 g), either untreated, or treated with pargyline hydrochloride (i.p., 75 mg/kg body weight) or iproniazid phosphate (i.p., 100 mg/kg). Four hr after administration of the drug, the animals were stunned and decapitated, and the brain, heart, kidneys, liver, lungs and spleen were removed, weighed and homogenized in 0·4 N perchloric acid. Blood was collected in a beaker containing 0·1 ml of 1% sodium heparin solution. In those cases in which the concentration of benzylamine was found to be very low, tissues from several animals (up to eight) were pooled during subsequent experiments. Deuterated benzylamine (1,1-dideutero-1-phenylmethylamine, 25 ng of free base in the case of tissues obtained from untreated and iproniazid-treated animals, and 250–2500 ng of free base, depending on the tissue, in tissues obtained from pargyline-treated animals) was added. The suspension was mixed, and then centrifuged at 12,000 *g* for 10 min. The supernatant was decanted, an amine fraction obtained by percolating the extract through a column of Biorad AG 50W-X2[H^+], and the dansyl amines were prepared as previously reported [5]. Dansyl benzylamine was separated from the reaction mixture by successive unidimensional chromatography on 20 × 20 cm glass plates coated with Silica gel [Brinkmann Instruments (Canada) Ltd., Rexdale, Ont.] in the solvent systems chloroform–butylacetate, 4:1 (v/v); benzene–triethylamine, 8:1 (v/v); and carbon tetrachloride–triethylamine, 5:1 (v/v). The dansyl benzylamine zone was removed from the plate, and the dansyl amine eluted with 30 μl of Fisher Spectranalyzed grade ethyl acetate [2]. Dansyl benzylamine was identified by its mass spectrum and quantitated mass spectrometrically using the integrated ion current procedure [5].

To determine which, if any, tissues were metabolizing pargyline to benzylamine, several tissues were removed from untreated animals and minced by slicing into approximately 1-mm cubes. The minces were suspended in 5 ml of an isotonic solution (pH 7·2) containing NaCl (120 mM), KCl (4·8 mM), $CaCl_2$ (2·6 mM), $MgSO_4$ (1·2 mM), Na_2HPO_4 (15 mM) and glucose (10 mM), then preincubated for 10 min at 37° in a shaking water bath. Pargyline hydrochloride solution (125 μg/50 μl, final concentration $1·3 \times 10^{-4}$ M) was added, and the incubation continued for 60 min. Control samples, containing tissue minces heated for 5 min in a boiling water bath, as well as samples containing only pargyline in buffer solution, were also incubated. The incubations were terminated by adding 0·5 ml of 4 N perchloric acid to each vessel. The tissues were homogenized, deuterated benzylamine (100 ng free base) was added, and benzylamine isolated and quantitated as described.

In some cases, tissue minces were incubated with pargyline [7-^{14}C] hydrochloride (7·03 μCi/mg, Abbot Laboratories, North Chicago, Ill.). The minces were suspended in incubation medium containing unlabeled pargyline hydrochloride ($1·3 \times 10^{-4}$ M) and preincubated for 10 min at 37° in a shaking water bath. Pargyline[^{14}C] hydrochloride solution (375,000 dis./min/50 μl) was then added and the incubation continued for 90 min. Boiled tissue samples and samples containing only pargyline[^{14}C] hydrochloride were incubated in an identical manner. Incubations were ended by homogenizing the minced tissue in 0·4 N perchloric acid; unlabeled benzylamine (25 ng) was added as carrier and benzylamine isolated as above. Radioactivity was measured in a Nuclear Chicago Isocap 300 liquid scintillation counter.

Benzylamine has not been previously reported as a normal tissue constituent. Edwards and Blau [6, 7] detected the amine in tissues of rats pretreated with pargyline, but claimed that it arose as an extraction artefact of the drug; they were unable to determine whether it occurred endogenously.

We have attempted to measure benzylamine in tissues of both untreated rats and rats treated with iproniazid. Although most tissue samples containing 25 ng deuterated internal standard indicated the presence of benzylamine in amounts greater than that found in the reagent blanks, the differences were not statistically significant. Benzylamine can be oxidized by mitochondrial monoamine oxidase and the plasma amine oxidases of a number of species, including the pig [8, 9], man [10] and the rabbit [11]. In the rat, a soluble plasma monoamine oxidase has not been detected [12]. Thus, it appears likely that any benzylamine would have to be oxidized by mitochondrial monoamine oxidase. The type B monoamine oxidase enzyme, which preferentially oxidizes benzylamine, is known to be inhibited by iproniazid [13, 14]. The concentration of β-phenylethylamine, another substrate for the type B enzyme [15], is increased dramatically in the tissues of rats treated with iproniazid [1]. The fact that benzylamine cannot be detected in amounts significantly greater than blank values, even after blockade of the monoamine oxidase with iproniazid, leads us to believe that the amine is not a normal constituent of tissue in the rat.

Pargyline also inhibits the type B enzyme [15] and, like iproniazid, results in a marked increase in the tissue concentration of phenylethylamine [1]. Unlike iproniazid, however, pargyline treatment leads to tissue levels of benzylamine approximating those of phenylethylamine. Four hr after an intraperitoneal injection of pargyline hydrochloride, tissue levels of benzylamine in the rat were [mean $\pm$ standard deviation (ng/g), n = 6]: blood 51 $\pm$ 36, brain 136 $\pm$ 83, heart 168 $\pm$ 109, kidney 1172 $\pm$ 915, liver 726 $\pm$ 243, lung 426 $\pm$ 253, and spleen 366 $\pm$ 259. It seemed likely that benzylamine was arising as a result of metabolic conversion of pargyline, rather than endogenously as a result of monoamine oxidase inhibition. This was confirmed by studies *in vitro* in which unlabeled and ^{14}C-labeled pargyline were metabolized to benzylamine and [^{14}C]benzylamine, respectively, by liver, and to a much smaller extent, by lung (Table 1).

Table 1. Conversion *in vitro* of pargyline to benzylamine*

Tissue	Benzylamine† (ng/g tissue)	Benzylamine[^{14}C]† (dis./min/g tissue)
Brain	2·3 $\pm$ 4·7 (4)	0 (4)
Heart	0·8 $\pm$ 1·7 (4)	0 (4)
Kidney	0·2 $\pm$ 0·3 (3)	3 $\pm$ 4 (4)
Liver	91 $\pm$ 49 (10)	750 $\pm$ 492 (4)
Lung	6·6 $\pm$ 3·6 (4)	6 $\pm$ 4 (4)
Spleen	0·4 $\pm$ 0·4 (4)	0 (4)

* Tissue minces were incubated with pargyline ($1{\cdot}3 \times 10^{-4}$ M) for 1 hr, or with pargyline ($1{\cdot}3 \times 10^{-4}$ M) plus pargyline[^{14}C] (375,000 dis./min) for 90 min in isotonic phosphate buffer, pH 7·2, and benzylamine was isolated. Benzylamine was measured mass spectrometrically, using deuterated benzylamine as internal standard, and benzylamine[^{14}C] was measured by liquid scintillation counting.

† Mean $\pm$ standard deviation; number of experiments is in parentheses. Boiled tissue values have been subtracted.

Although the rate of conversion of pargyline to benzylamine *in vitro* was very low (in liver, approximately 0·04 per cent of unlabeled pargyline was converted to benzylamine in 1 hr, and 0·09 per cent of pargyline[^{14}C] was converted to benzylamine[^{14}C] in 90 min), the concentration of benzylamine in tissues became substantial 4 hr after administration of the drug. It is probable that most benzylamine found in the body is produced in the liver and distributed by the blood to other tissues.

The mechanism by which benzylamine is formed from pargyline is not known. Pargyline was not converted to benzylamine in any part of our analytical procedure, however, as incubation of the drug in buffer solution, to which 25 ng of deuterated benzylamine was subsequently added, yielded non-deuterated benzylamine in amounts no greater than that found in blank solutions containing only 25 ng of deuterated benzylamine. It may be that benzylamine is produced via the hydroxylamine metabolic route, either directly or through chemical conversion of an intermediate hydroxylamine [16], (also R. T. Coutts, personal communication). These possible mechanisms have yet to be investigated.

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