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THE ISOLATED PERFUSED RAT BRAIN PREPARATION IN THE STUDY OF MONOAMINE OXIDASE AND BENZYLAMINE OXIDASE

LACK OF SELECTIVE BRAIN PERFUSION*

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Abstract—Monoamine oxidase (MAO) is present in brain blood vessels, and a different amine oxidase, benzylamine oxidase (BzAO), is claimed to exist in porcine cerebral vessels. The object of the present investigation was to evaluate these deaminating activities in the structurally intact brain, utilizing the isolated perfused rat brain preparation (IPRB). [^{14}C]Benzylamine (10 μM), a substrate for both BzAO and MAO, was perfused via the internal carotid arteries for 5 min, and deaminated metabolites were measured. BzAO and MAO activities were distinguished by the use of the selective inhibitors semicarbazide and pargyline. Both BzAO and MAO deaminated benzylamine (10 μM), but over 90 per cent of the total deaminated products resulted from BzAO. This was due to the much lower K_m value of benzylamine for BzAO (2.8 μM) than for MAO (169 μM). *In vitro* assays, however, revealed that the brain contained no measurable BzAO activity, whereas all other head structures (skull, mandible, skin, skeletal muscle, eyes, periocular tissues and tongue) contained BzAO activity. MAO was present both within and outside the brain. These results suggest that the IPRB preparation is not specific for brain perfusion. Experiments with technetium-labeled microspheres showed that, although the brain is preferentially perfused on a per gram basis, 52 per cent of the total perfusate passed through the skull and 13 per cent through extracranial structures. Only 35 per cent was specific for the brain. Other experiments showed that perfusion of the intact rat head produced greater deamination of benzylamine than when the skin and 70 per cent of the muscle were removed. Additionally, perfusion via the pterygopalatine arteries, to bypass the brain, resulted in increased deamination. It is concluded that the IPRB preparation is not specific for brain perfusion and that BzAO activity is present in all head structures other than the brain. The presence of BzAO in bone, skin, and muscle is consistent with suggestions for a physiological function of the enzyme in connective tissue.

The cardiovascular system contains monoamine oxidases (MAO) and other amine oxidases distinct from MAO [1–5]. MAO is predominantly a mitochondrial enzyme utilizing FAD as a cofactor. Two functionally distinct types have been defined, MAO-A and MAO-B [6]. MAO-A is preferentially inhibited by clorgyline and prefers serotonin and norepinephrine as substrates [7]. MAO-B is preferentially inhibited by deprenyl and pargyline and prefers 2-phenylethylamine and benzylamine as substrates [7–9]. All cardiovascular structures contain MAO, including the cerebral microvasculature which shows a very high activity [10–13].

Recently, studies have focused on another amine oxidase in homogenates of heart [2, 3], arteries [1, 3, 5 14], and veins [3, 14]. This enzyme also deaminates monoamines but it is resistant to inhibition by clorgyline, deprenyl, and pargyline. It is sensitive, however, to inhibition by semicarbazide [2, 3, 13] and other hydrazines [3]. The enzyme appears to belong to the diverse group of copper-containing amine oxidases that are presumed to utilize pyridoxal phosphate as a cofactor [15]. The characteristics and properties of this enzymatic activity have not been defined fully, but some evidence exists for heterogeneity [16]. Benzylamine is an excellent substrate for the enzyme which has been termed benzylamine oxidase (BzAO) by some [3, 15] and clorgyline-resistant amine oxidase by others [2]. Histochemically, BzAO has been demonstrated in the medial layer of human placental blood vessels [17], and immunofluorescent studies report BzAO activity in the porcine cerebral vasculature [18]. Thus, BzAO, like MAO [11–13], might serve a physiological function as part of the enzymatic blood–brain barrier.

To test this hypothesis, we utilized the isolated perfused rat brain preparation and studied benzylamine deamination. This approach allows direct

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presentation of substrate to the vasculature of a viable, structurally intact cellular system. The lack of hydrophilic groups in benzylamine suggested that good access to the enzyme would be obtained. Further, benzylamine is structurally similar to 2-phenylethylamine, which is known to penetrate the blood–brain barrier [19].

MATERIALS AND METHODS

Male rats (Sprague–Dawley descendants; Timco Breeding Laboratories, Houston, TX), weighing between 320 and 500 g, were used. The rats received food and water *ad lib.* and were housed in air-conditioned quarters on a 12-hr light–dark cycle.

Isolated perfused rat brain preparation. Rats were anesthetized with urethane (1.5 mg/kg, i.p.) and prepared as described by Andjus *et al.* [20], except that the mandible was not removed. The method involves perfusion of the brain via both internal carotid arteries.

Following tracheotomy, the external carotid arteries below the occipital branches, and the pterygopalatine arteries, were ligated. These arteries serve structures lying outside the brain [21]. After injecting heparin (500 units/rat, i.v.), each common carotid artery was cannulated. Every effort was made to avoid hypoxia. Thus, cannulation was accomplished as fast as possible with the cannulae slowly dripping perfusion medium. Once inserted, the cannulae were advanced to a position just below the bifurcation of the internal and external carotids and were tied securely in place. The spinal cord was ligated high in the cervical region, and the head was removed by cutting below the ligature. The spinal canal was sealed with cotton and bone wax. All of the skin and 70 per cent of the muscle were removed from the head, unless stated otherwise. The preparation was positioned on a collecting funnel and the rate of perfusion was adjusted to 2.5 ml/min. Pulsatile flow was obtained using a peristaltic pump (Holter, model 911, Extracorporeal Med. Spec., Inc., King of Prussia, PA). Perfusion pressure was measured using a transducer (Statham model P23Gb) and was recorded continuously on a polygraph (Narco Biosystems, Houston, TX). Mean basal perfusion pressure was 83 ± 2.5 mm Hg for eighteen experiments. The perfusion medium was saturated with humidified O_2 (95%) and CO_2 (5%), passed through a glass wool filter and warmed to 37°.

For biochemical studies, [^{14}C]benzylamine (10 μCi/μmole) was added to the perfusion medium after a 30-min pre-perfusion, to give a final concentration of 10 μM. Venous effluents from the funnel were collected in 14 × 1 ml fractions, and 100 or 200 μl aliquots of each fraction were taken for the determination of deaminated products and total radioactivity. The effects of semicarbazide and pargyline were studied by perfusing with the drugs for 30 min prior to, and then concomitantly with, the labeled benzylamine. At the termination of the perfusion, the brain was removed, blotted, and homogenized in 10 vol. of 0.4 N perchloric acid to extract the radioactivity for subsequent analysis. Following centrifugation (700 g) the pellet was re-extracted and centrifuged, and the supernatant fractions were analyzed separately. The overall recovery of radioactivity was 80 per cent.

Perfusion medium. Dog erythrocytes were prepared exactly as described by Andjus *et al.* [20]. The final medium was composed of 80 ml washed erythrocytes, 100 ml twice-isotonic Krebs solution and 100 ml polyvinylpyrrolidone (9.27%, pH 7.35). An equivalent molarity of polyvinylpyrrolidone was used in place of bovine serum albumin as used by Andjus *et al.* [20] since commercial samples of albumin (Fraction V) were found to deaminate benzylamine.

Specificity of brain perfusion. Intact heads were perfused for 5 min with either [^{14}C]benzylamine (10 μCi/μmole) or technetium-labeled microspheres (400 mCi, mean diameter 30 μm). All structures of the head were dissected, blotted, and weighed, and the radioactivity was counted. Carbon-14 was counted in a Packard liquid scintillation counter (model 3330) following two perchloric acid extractions. Technetium was counted directly in 2-g portions of the dissected structures using a Searle gamma counter (model 1195).

Viability of the perfused preparation. The following tests were made to assess viability. First, EEG activity was recorded using two screw electrodes placed in the parietal regions of the skull. A large increase in electrical activity followed the bolus injection of pentylenetetrazol (20 mg) into the perfusing fluid. Auditory stimuli also evoked a short burst of activity. Perfusion with Krebs bicarbonate solution only gave a completely flat EEG. Second, dose-related increases in vascular resistance were elicited with norepinephrine (0.01 to 50 μg) and arginine–vasopressin (0.1 to 0.5 U.S.P. units), whereas acetylcholine (0.001 to 0.1 μg) gave dose-related decreases in vascular resistance. Third, the preparation did not gain weight when perfused for 1 hr. Fourth, a bolus injection of [^{3}H]norepinephrine (3.8 mCi/μmole) failed to be taken up into the brain. The above tests demonstrated neurogenic viability, vascular reactivity, and the presence of a functional blood–brain barrier.

Preparation of tissues. All tissues for enzyme assay were rinsed thoroughly with saline (0.9% NaCl, w/v), blotted dry, weighed, and homogenized (1:10) in 1 mM potassium phosphate buffer, pH 7.8. Crude homogenates were centrifuged at 700 *g* for 10 min and supernatant fractions of 1 or 2 ml were frozen at −25° in glass vials for subsequent assay.

Assay of MAO and BzAO. Activities were determined radiochemically by the method of McCaman *et al.* [22] as modified by Callingham and Laverty [23]. The usual reaction mixture contained 50 μl homogenate plus 50 μl benzylamine prepared in 0.2 mM phosphate buffer (pH 7.8). Inhibitors were preincubated with the homogenate for 20 min at 37° prior to the addition of substrate. Quoted concentrations of inhibitors represent final concentrations that were half of the preincubation concentration. Reactions were run in triplicate and stopped by cooling the tubes on ice and acidifying with 10 μl of 3 N HCl. Deaminated products were extracted in 600 μl of toluene–ethyl acetate (1:1, saturated with water), and a 400-μl aliquot was taken for liquid scintilation counting with quench correction. Blank values were obtained by adding 10 μl of 3 N HCl

prior to addition of substrate. None of the drugs used altered extraction efficiency of the deaminated metabolites, which was calculated to be in excess of 98 per cent.

K_m *and* V_{max} *determinations.* The following concentrations of [^{14}C]benzylamine were used for BzAO and MAO respectively: 1.0, 1.25, 1.67, 2.5, 5.0, 10.0 and 20.0 μM and 50, 100, 125, 167, 250, 500 and 800 μM. Initial velocity rates were obtained by incubating homogenates for 5 min. Linearity of the reaction with time and protein concentration was ensured in all assays.

Protein assay. Protein contents were measured by the micro-biuret method of Goa [24].

Statistical tests. The significance of differences between mean values was determined using Student's *t*-test. P values are expressed as two-tailed.

Chemicals. [^{14}C]Benzylamine hydrochloride was purchased from ICN Pharmaceuticals, Irvine, CA, U.S.A. (12.5 mCi/mmole) and [^{14}C]norepinephrine from the New England Nuclear Corp., Boston, MA, U.S.A. (3.8 mCi/μmole).

Benzylamine hydrochloride was purchased from ICN Pharmaceuticals, Plainview, NY, U.S.A., pargyline hydrochloride from the Regis Chemical Co., Morton Grove, IL, U.S.A., and clorgyline was a gift from May & Baker Ltd., Hoddesdon, U.K. Semicarbazide hydrochloride was obtained from the Fisher Scientific Co., Fairlawn, NJ, U.S.A. Urethane was purchased from Merck & Co., Inc. Rahway, NJ, U.S.A., and the polyvinylpyrrolidone used in the perfusion studies from the Arthur Thomas Co., Philadelphia, PA, U.S.A.

RESULTS

Perfusion of the isolated rat brain preparation with medium containing [^{14}C]benzylamine (10 μM) failed to alter the resting perfusion pressure. Deaminated metabolites of benzylamine, however, were detected in the venous effluent (Fig. 1). About 11 per cent of the total benzylamine infused appeared as deaminated products. Deamination was virtually abolished by the BzAO inhibitor semicarbazide (10^{-4} M), whereas the MAO inhibitor pargyline (10^{-4} M) failed to produce significant inhibition. Neither semicarbazide nor pargyline altered the total carbon-14 content of the venous effluent or the brain. No deaminated products were detected in the brain, possibly reflecting a rapid clearance of these metabolites.

Experiments done on brain homogenates *in vitro* produced results (Fig. 2) opposite to those above. Under these conditions, the deamination of [^{14}C]benzylamine (10 μM) was resistant to inhibition by semicarbazide (10^{-11} to 10^{-3} M) but inhibition resulted with the MAO inhibitor clorgyline (10^{-6} to 10^{-3} M). According to the convention established by Johnston [6], benzylamine was deaminated by MAO-B. The greater sensitivity to inhibition by pargyline confirms deamination by the B-form.

The results shown in Fig. 2 were obtained using the 700 *g* supernatant fraction of brain homogenate. Thus, the possibility existed that BzAO activity had sedimented with the pellet. Experiments with clorgyline and semicarbazide made on uncentrifuged homogenates of whole brain, however, gave inhi-

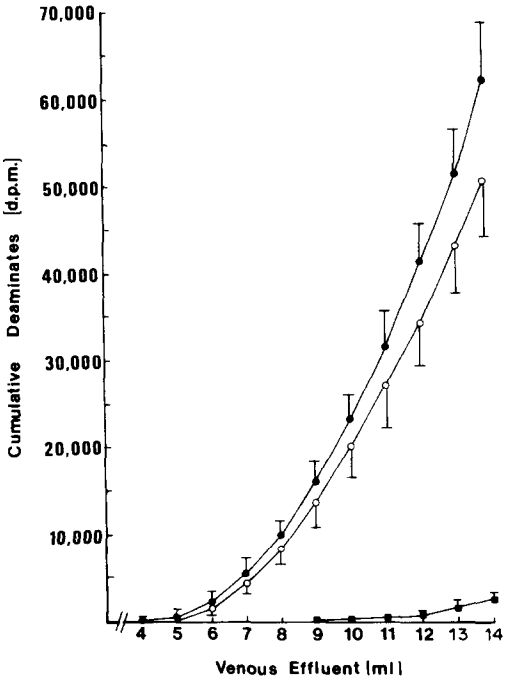

Fig. 1. Cumulative deaminated metabolites in the venous effluent of the isolated rat brain preparation during perfusion with [^{14}C]benzylamine (10 μM). Key: control, no drug (●——●); pargyline (10^{-4} M, ○——○); and semicarbazide (10^{-4} M, ■——■). Shown are the mean values ± S.E. for six experiments (control) and three experiments for each drug treatment. All means for semicarbazide are significantly different from control ($P < 0.001$), whereas those for pargyline are not statistically different.

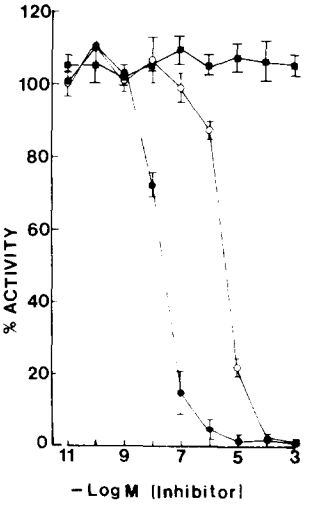

Fig. 2. Effects of semicarbazide (■——■, N = 2), pargyline (●——●, N = 2), and clorgyline (○——○, N = 4) on the deamination of [^{14}C]benzylamine (10 μM) in brain homogenates, shown as percentages (± the S.E. of the ratio).

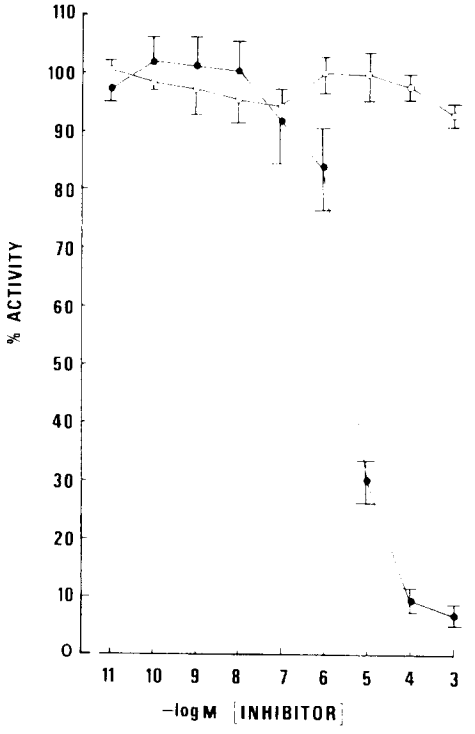

Fig. 3. Effects of semicarbazide (●——●) and pargyline (○——○) on the deamination of [^{14}C]benzylamine (10 μM) in all structures of the rat head minus the brain. Shown are the mean values ± the range for triplicate determinations from a single homogenate.

bition curves identical to those illustrated in Fig. 2. No reliable BzAO activity could be detected (data not shown).

In an attempt to localize BzAO activity, the entire rat head minus the brain was homogenized and the 700 *g* supernatant fraction was assayed. Figure 3 shows that benzylamine deamination was then inhibited by semicarbazide, whereas pargyline produced only slight inhibition.

Table 1 shows the distribution of BzAO and MAO activities in homogenates of tissues dissected from the entire rat head. At 10 μM benzylamine, deamination was greatest in the skull and the brain. All the structures deaminated benzylamine, however, with the periocular tissues (oculomotor muscles and glands) being least active. Over 74 per cent of the deamination in tissues other than the brain was sensitive to inhibition by semicarbazide. Semicarbazide plus pargyline caused almost complete inhibition in all tissues. In most tissues, pargyline alone gave results that were approximately complementary to those with semicarbazide, although activation, rather than inhibition, was measured in the tongue. Clorgyline gave evidence of activation in several tissues, and it is pertinent to note that these tissues contained the highest percentage of BzAO. In confirmation of Fig. 2, semicarbazide failed to affect deamination in the brain whereas pargyline and clorgyline produced almost complete inhibition.

The data in Table 1 show that BzAO and MAO were present in all head structures outside the brain, whereas the brain contained only MAO. In order to explain the results shown in Fig. 1, we assumed that perfusion of structures other than the brain had occurred. Experiments were performed, therefore, to determine the distribution of the perfusate throughout the intact isolated head. Table 2 shows the results using [^{14}C]benzylamine or technetium-labeled microspheres as markers for tissue perfusion. The two left-hand columns of Table 2 show that the skull contained over 50 per cent of the total radioactivity, whereas the brain contained only 34 per cent. The close agreement between the [^{14}C]benzylamine and technetium values indicates that benzylamine was not concentrated selectively by any of the perfused tissues. Table 2 (right-hand columns) presents the same data expressed as perfusion per gram of tissue relative to the brain. It can be seen that the brain received about 2.5 times the perfusate on a per gram basis compared to the skull. The eyes and periocular tissues were the next best perfused.

Table 1. Distribution of benzylamine oxidase (BzAO) and monoamine oxidase (MAO) in the rat head*

Tissue	Specific activity† Control	Percentage inhibition† SC	P	C	SC + P
Skull	2.17 ± 0.05	85.6	11.6	13.5	98.2
Mandible	1.70 ± 0.03	74.0	30.0	26.0	98.7
Skin	1.26 ± 0.02	99.4	3.0	−12.4‡	99.4
Skeletal muscle	1.09 ± 0.04	95.7	4.8	−7.2‡	98.5
Tongue	0.98 ± 0.06	93.0	−12.0‡	−13.0‡	99.5
Eyes	0.49 ± 0.01	84.5	18.0	12.1	99.6
Periocular tissues	0.23 ± 0.01	88.8	21.7	21.0	97.9
Brain	2.20 ± 0.04	0.0	99.0	99.0	99.1

* BzAO was defined by sensitivity to inhibition by semicarbazide (SC, 10^{-3} M) and insensitivity to inhibition by clorgyline (C, 10^{-3} M) and pargyline (P, 10^{-3} M). The reverse inhibitor sensitivities defined MAO. [^{14}C]Benzylamine (10 μM, 10 μCi/μmole) was used as substrate. Specific activity was equal to nmoles benzylamine deaminated·(mg protein)$^{-1}$·15 min^{-1}.

† Each value is the mean of three to six determinations ± S.E.

‡ Equals increase above control values.

Table 2. Distribution of perfusate in tissues dissected from the isolated-perfused rat brain preparation

Tissue	Percentage of total radioactivity		Radioactivity/g tissue relative to brain (brain = 100)	
	[^{14}C]Benzylamine*	Technetium-labeled microspheres†	[^{14}C]Benzylamine*	Technetium-labeled microspheres†
Brain	34.3	34.8	100.0	100.0
Skull	50.8	51.9	39.6	38.9
Skeletal muscle	8.9	6.7	7.3	3.7
Skin	4.2	4.4	2.6	2.1
Mandible	1.2	1.8	4.8	3.4
Eyes	0.3	0.6	7.5	14.1
Tongue	0.2	0.4	1.6	2.0
Periocular tissues	ND‡	0.9	ND	15.4

* Single experiment.
† Mean of three experiments.
‡ ND = not determined.

Table 3. Cumulative deaminated products in the venous effluent of the isolated rat brain preparation at the end of a 5-min perfusion with [^{14}C]benzylamine (10 μM)*

Condition	N	Mean cumulative deaminated products (d.p.m.) ± S.E.	Percentage change from Condition I
I. Skin + 70 per cent muscle removed	6	61,736 ± 6687	
II. Skin + all muscle present (intact head)	3	93,664 ± 9561†	+51.7
III. As for II but with perfusion that bypassed the brain	1	123,060	+99.3

* The preparation was pre-perfused for 30 min without benzylamine.
† Significantly different from condition I, $P < 0.05$.

Confirmation that benzylamine deamination involved extra-brain perfusion was obtained (1), by perfusing the head, with all the skin and skeletal muscle present, and (2) by perfusing the entire head through the pterygopalatine arteries instead of the internal carotid arteries, thereby bypassing the brain. Both procedures increased deaminated products in the venous effluent by 51.7 and 99.3 per cent respectively (Table 3).

The Michaelis–Menten constants for benzylamine deamination by BzAO and MAO-B were measured using skull and brain homogenates as the respective enzyme sources. Skull MAO activity was inhibited with pargyline (10^{-4} M). The resulting Lineweaver–Burk plots were linear, indicating the presence of only one enzymatic activity over the range of concentrations used. Straight lines were also obtained when the data were plotted using Eadie–Scatchard plots. The apparent K_m and V_{max} values are shown in Table 4. Benzylamine deamination was sixty times more sensitive to deamination by BzAO than to MAO-B. Thus, in all the preceding experiments in which 10 μM benzylamine was used, BzAO was measured at saturating concentrations while MAO activity was being measured at subsaturating concentrations. Table 5 shows the relative activities of MAO and BzAO in the skull when both enzymes were saturated (500 μM benzylamine). The

Table 4. Apparent kinetic constants for [^{14}C]benzylamine deamination by benzylamine oxidase (BzAO) and monoamine oxidase (MAO)*

Tissue	K_m (μM ± S.E.)	V_{max} [pmoles deaminated · (mg protein)$^{-1}$ · min^{-1} ± S.E.]
Skull (BzAO)	2.8 ± 0.18	218 ± 0.01
Brain (MAO)	168.7 ± 9.20†	2684 ± 53.5†

* Calculated by the method of Wilkinson [25].
† Significantly different from BzAO, $P < 0.001$.

Table 5. Monoamine oxidase (MAO) and benzylamine oxidase (BzAO) activity in the skull with benzylamine

Benzylamine (μM)	Specific activity [nmoles·(mg protein)$^{-1}$·15 min^{-1}] Total	MAO*	BzAO†
10	2.17 (100%)	0.26 (12%)	1.91 (86%)
500	2.64 (100%)	2.06 (78%)	0.58 (22%)

* Activity resistant to 10^{-4} M semicarbazide.
† Activity resistant to 10^{-4} M pargyline.

relative percentage activities of MAO and BzAO were approximately reversed at the high benzylamine concentration. The modest increase in total specific activity reflects marked substrate inhibition of BzAO. BzAO activity decreased at 500 μM benzylamine, while MAO activity increased by about 8-fold.

DISCUSSION

The isolated perfused rat brain is gaining increasing acceptance as a tool for studying metabolic functions of the brain (for review see Ref. 26). The present investigation, however, demonstrates that the preparation is not specific for brain perfusion and that structures outside the brain, particularly the skull, are also perfused.

A previous study analyzed the specificity of brain perfusion [27]. Using dye and latex injections, the authors claimed no perfusion of extracranial structures and stated that it was no longer necessary to remove the skin, muscle and mandible as first described by Andjus *et al.* [20]. They noted some perfusion of the skull but assumed little significance in terms of metabolic activity. Quantitative assessment now shows that the brain is the best perfused head structure on a per gram basis, but that over 50 per cent of the total perfusate supplies the skull (Table 2). Other structures of the head are also perfused, but to a much smaller extent. These results place severe limitations on the usefulness of the preparation when arterio–venous differences are employed as the measure of a particular brain function. In the vast majority of biochemical experiments it would be mandatory to evaluate the contribution of all head structures relative to that of the brain. In addition, it cannot be concluded that vascular resistance changes to infused compounds reflect selective actions on the brain vasculature. The preparation seems best suited for the measurement of uptake by the brain, where the final analysis is done on removed brain tissue. Even then, metabolism of the particular substance outside the brain, with subsequent uptake into the brain, cannot be excluded entirely.

Lewinsohn *et al.* [3] reported very low BzAO activity [nmoles·(mg protein)$^{-1}$·30 min^{-1}] in rat brain regions, ranging from 0.1 in the cerebellum and forebrain to 0.2 in the brain stem. In the present study, whole brain levels of BzAO activity were not reliably detectable above background values. In some experiments, about 1 per cent of the total activity remained after pargyline or clorgyline, which, if real, would amount to 0.022 nmole deaminated·(mg protein)$^{-1}$·15 min^{-1}. By comparison, abundant activity was found in all the other head structures (Table 1). These results, the non-selectivity of brain perfusion and the data shown in Table 3, leave little doubt that the semicarbazide-sensitive deamination of benzylamine in the perfused system (Fig. 1) originated overwhelmingly from structures outside the brain. The high specific activity of BzAO in the skull (Table 1) and its large mass compared to the brain further support this contention. The relative accessibility of benzylamine to BzAO in the various head structures, however, is another important but unknown factor operating in the perfused system. Immunofluorescent detection of BzAO [18] offers a more direct approach to the question of BzAO in the brain vasculature. The demonstration of BzAO in porcine cerebral vessels by Buffoni *et al.* [18], however, is open to question. The antibodies were prepared from purified pig plasma BzAO and, although their experiments were done on 1-day-old pigs, when plasma BzAO is very low, some assayable plasma BzAO was present (0.25 to 0.5 per cent of adult values).

BzAO activity is generally considered to be resistant to acetylenic MAO inhibitors such as clorgyline, pargyline and deprenyl [2, 9]. Table 1, however, shows some activation by clorgyline (10^{-3} M) in tissues containing over 90 per cent BzAO activity. Al-Naji and Clarke* have observed a marked K_m activation of BzAO with clorgyline (10^{-4} and 10^{-3} M) in commercial samples of bovine serum albumin (see Materials and Methods). Conversely, Houslay and Tipton [28] reported competitive inhibition of purified bovine plasma BzAO with high clorgyline concentrations. Clearly, at concentrations of 10^{-4} M, and above, clorgyline is not selective for MAO and may not clearly differentiate BzAO activity from these enzymes. The other acetylenic inhibitors, deprenyl and pargyline, may be more selective in this respect.

Two previous studies have noted substrate inhibition of tissue BzAO by benzylamine [2, 3]. Table 5 shows the same phenomenon for skull BzAO. Thus, for any given homogenate, a true specific activity ratio for BzAO versus MAO cannot be

* A. S. Al-Naji and D. E. Clarke, unpublished observations.

obtained using "selective" inhibitors and a single benzylamine concentration. According to the respective K_m values (Table 4), BzAO was saturated at 10 μM benzylamine, and kinetic analysis showed that substrate inhibition was not a complicating factor. At this same concentration, however, MAO activity was grossly underestimated (Table 5). At saturating concentrations for MAO (500 μM), BzAO activity was decreased to less than one-third of its true specific activity. Thus, the relative specific activities of BzAO and MAO will depend upon the concentration of benzylamine selected for assay. In this connection, both K_m values and substrate inhibition must be taken into account. A general lack of understanding on these basic points has led to much confusion regarding the classification of amine substrate specificities for MAO-A, MAO-B and BzAO (for further discussion see Refs. 8 and 9).

The presence of BzAO in the diverse tissue types that comprise the rat head confirms the broad tissue distribution reported by Lewinsohn *et al.* [3]. The high specific activity of BzAO in bony structures, however, is an original finding. The presence of BzAO activity in bone, skin and muscle is consistent with the notion of a role for BzAO in connective tissue metabolism [15]. A further association with connective tissue is suggested by observations that human plasma BzAO activity is increased in fibrotic disease states [29–31], whereas aortic activities that deaminate benzylamine are reduced in copper-deficient animals with resulting vessel weakness [32, 33]. BzAO, however, is not a lysyl oxidase, a family of enzymes known to be involved in the cross-linking of collagen (for references, see Ref. 3). According to Shieh *et al.* [5], lysyl oxidase does not deaminate benzylamine. Additionally, lysyl vasopressin (10^{-3} M) is without effect on BzAO from bone and human aorta using benzylamine as substrate (unpublished observations). Clearly, extensive studies are required to elucidate the physiological role of BzAO. In this regard it should not be forgotten that more than one form of BzAO activity is indicated [16, 34, 35] and that functional roles may vary.

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