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# "BIOGENIC" ALDEHYDE METABOLISM*

# RELATION TO PENTOSE SHUNT ACTIVITY IN BRAIN

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**Abstract**—Biogenic amines, added to brain homogenates, were demonstrated to stimulate oxidative decarboxylation of glucose isotopically labeled at C-1. This effect was ascribed to the stimulation of the pentose phosphate shunt in brain and was found to depend on the monoamine oxidase (MAO)-catalyzed production of the aldehyde derivatives (biogenic aldehydes) of the biogenic amines. The stimulation produced by the amines and the aldehydes was shown to be inhibited by barbiturates, and the enzymes responsible for the stimulated metabolism of glucose were found to be present in the cytosol. Evidence is presented indicating that the stimulation produced by biogenic aldehydes depends on the oxidation of NADPH to NADP by aldehyde reductase present in brain cytosol. Acid derivatives of the biogenic amines [i.e. 5-hydroxyindoleacetic acid (5-HIAA)] were found to inhibit aldehyde-stimulated metabolism of glucose by the pentose phosphate shunt.

THE PENTOSE shunt for the metabolism of glucose has been thought to be inoperative *in vitro* in central nervous system (CNS) tissue.[1] However, McGuire and Pesh[2] indicated that the pentose shunt was the major pathway for glucose oxidation in the anterior pituitary if NADP was available to the system. NADP levels are the limiting factor for the oxidation of glucose via the pentose shunt.[3] Subsequently, enzymes of the pentose phosphate pathway have been shown to be present in various regions of rat brain[4] and several metabolites of this pathway in brain, as well as changes in the concentrations of these metabolites after drug administration, have been demonstrated.[5] These data indicate that the pentose shunt is functional *in vivo* in brain. In addition, Kaufman *et al.*[5] indicated that the two initial NADP-dependent dehydrogenases regulate the pentose phosphate pathway.

Recently, Appel and Parrot[6] demonstrated, using glucose-1-$^{14}$C, substantial amounts of pentose shunt activity *in vitro* in both brain mince and synaptosomes prepared from rat cerebral cortex. This demonstration was possible by measuring $^{14}CO_2$ evolution during short incubation periods (15–30 min), since longer incubation periods[7] may result in a decrease in NADP levels and randomization of the carbon atoms of glucose. The activity of the pentose shunt in synaptosomes was shown to be stimulated by norepinephrine and serotonin, as well as by acetylcholine.[6] The

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ability of norepinephrine and serotonin to stimulate the pentose shunt has been previously demonstrated in the anterior pituitary.[8,9] This effect was ascribed to the metabolism of the aldehyde derivatives of these biogenic amines by an alcohol dehydrogenase utilizing NADPH as a cofactor.[9] Others,[2] however, have indicated that this effect is mediated through activation of a transhydrogenase by serotonin and norepinephrine, while Pastan *et al.*[10] concluded that the stimulation of the pentose shunt by epinephrine in the thyroid was mediated by its conversion to adrenochrome, which would act as an electron acceptor. The above mechanisms were all invoked to provide a means of generating NADP for the two initial reactions of the pentose shunt. We have recently isolated[11] and characterized[12] an NADPH-dependent aldehyde reductase from brain, which catalyses the conversion of certain aldehydes to alcohols with a concomitant oxidation of NADPH to $NADP^+$. In the light of our studies on the specificity of this enzyme for various biogenic aldehydes[12] and the inhibition of the enzyme by barbiturates,[13] we decided to examine whether this enzyme system may provide a source of NADP to control the metabolism of glucose via the pentose shunt in brain.

## MATERIALS AND METHODS

D-Glucose-6-$^{14}$C (sp. act., 5 mCi/m-mole), D-glucose-1-$^{14}$C (sp. act., 5 mCi/m-mole) and D-glucose-6-phosphate-1-$^{14}$C (sp. act., 10 mCi/m-mole) were purchased from New England Nuclear Corp. D-Glucose, D-glucose-6-phosphate, 6-phosphogluconate, NAD, NADH, NADP, NADPH, serotonin creatinine sulfate, tyramine HCl, octopamine HCl, norepinephrine HCl and indoleacetaldehyde sodium bisulfite were purchased from Sigma Chemical Co. Glucose-6-phosphate dehydrogenase (yeast) was purchased from Mannheim Corp. and 6-phosphogluconate dehydrogenase (yeast, type V) was purchased from Sigma. Pargyline was kindly donated by Abbott Pharmaceuticals. *p*-Nitrobenzaldehyde was obtained from Aldrich and acetaldehyde was obtained from Eastman Kodak. Acetaldehyde was redistilled before being used in our studies. 5-Hydroxyindoleacetic acid (5-HIAA) was obtained from Regis Chemical Co. All other reagents were of the highest available commercial quality.

Adult male Sprague–Dawley rats were decapitated. Blood was collected in a heparinized syringe and glucose-6-phosphate dehydrogenase (D-glucose 6-phosphate: NADP oxido-reductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate: NADP oxido-reductase (decarboxylating), EC 1.1.1.44] activity was obtained from hemolyzed red blood cells as described by Teitz.[14] Brains were quickly removed, weighed and homogenized. A 10% homogenate was prepared with isolation medium (0·32 M sucrose containing 0·05 mM sodium EDTA and 5 mM sodium phosphate, pH 7·0) using a glass homogenizer with a tight-fitting Teflon pestle. In order to obtain the cytosol, the homogenate was centrifuged at 27,000 *g* for 30 min. The supernatant fluid was recovered and again centrifuged for 60 min at 126,000 *g*. The supernatant fluid recovered after this centrifugation (cytosol) was used to assay for enzymatic activity. Subcellular fractionation was accomplished by a modification[15] of the method of Whittaker.[16] Various subcellular fractions obtained in pellet form were resuspended by gentle homogenization with the isolation medium. Protein was measured by the method of Lowry *et al.*[17] or by a modified biuret reaction.[18]

*Incubation and assay procedures.* Incubation was performed in rubber-stoppered 50-ml reaction flasks (Kontes Glass Co.) with scintillation vials attached to a side arm. Hydroxide of Hyamine (Packard), 0·4 ml, was added to the scintillation vials to trap the evolved $^{14}CO_2$. Control reaction mixtures contained nicotinamide (5 mM), magnesium chloride (1 mM), manganese chloride (1 mM), calcium chloride (0·5 mM), Tris HCl (25 mM, pH 7·0) and glucose-1-$^{14}$C (5 mM; sp. act., 0·02 $\mu$Ci/$\mu$mole) or glucose-6-$^{14}$C (5 mM; sp. act., 0·02 $\mu$Ci/$\mu$mole) or glucose-6-phosphate-1-$^{14}$C (5 mM; sp. act., 0·0166 $\mu$Ci/$\mu$mole). Certain of these reaction mixtures also contained NADP ($5{\cdot}7 \times 10^{-5}$ M), NADPH ($5{\cdot}7 \times 10^{-5}$ M), NAD ($5{\cdot}7 \times 10^{-5}$ M) or NADH ($5{\cdot}7 \times 10^{-5}$ M). All other additions (see tables) were made, and the solutions were preincubated for 5 min at 37°, before the reaction was started by injecting 0·5 ml enzyme through the rubber stopper. The total volume of the incubation mixtures was 3·5 ml. The incubation took place in a metabolic shaker for 20 min at 37°. The reaction was stopped by an injection of 0·2 ml of 4 N perchloric acid and the incubation was continued for another 60 min. Control incubation mixtures containing either: (1) enzyme, labeled glucose or glucose-6-phosphate and buffer solution; or (2) enzyme, labeled glucose or glucose-6-phosphate, nucleotide cofactor and buffer solution, were monitored with each experiment. After the incubation, another 1 ml Hyamine was added to the scintillation vials followed by 15 ml of scintillation fluid (5 *g* PPO and 0·3 *g* POPOP per liter of toluene) and the resultant solution was counted in a Packard Tri-Carb scintillation counter. Aldehyde reductase activity was assayed spectrophotometrically as described by Tabakoff and Erwin.[11] Reaction mixtures contained enzyme (0·1 to 0·4 mg protein) NADPH ($1 \times 10^{-4}$ M), *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) and sodium phosphate (0·1 M, pH 7·0) in a total volume of 1 ml. Oxidation of NADPH was monitored at 340 nm using a Beckman Acta III recording spectrophotometer. The activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase prepared from rat blood and yeast glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were also measured spectrophotometrically. Assay mixtures contained enzyme; yeast glucose-6-phosphate dehydrogenase (1·25 $\mu$g protein), or yeast-6-phosphogluconate dehydrogenase (1·6 $\mu$g protein), or rat blood hemolysate (0·675 mg protein), NADP ($1 \times 10^{-4}$ M), glucose-6-phosphate ($1 \times 10^{-4}$ M) or 6-phosphogluconate ($1 \times 10^{-4}$ M); calcium chloride, magnesium chloride, and manganese chloride were all present at a concentration of 1 mM and Tris HCl was present at 25 mM (pH 7·0). Total volume was 1 ml. Activity was monitored by an increase in absorbance at 340 nm.

Monoamine oxidase (MAO) activity was assayed as described by Tabakoff and Alivisatos[19] or by a modification of the radioisotopic method of McCaman *et al.*[20] using serotonin-$^{14}$C as substrate.

The aldehyde derivatives of the various biogenic amines (i.e. serotonin, octopamine and tyramine) were prepared enzymatically as previously described.[12] Indoleacetaldehyde was prepared free of the bisulfite[13] and the concentration of all aldehydes was ascertained using rat liver aldehyde dehydrogenase.[12]

## RESULTS

Rat brain homogenates were found to produce $^{14}CO_2$ when glucose-1-$^{14}$C, glucose 6-$^{14}$C, and glucose-6-phosphate-1-$^{14}$C were used as substrates. The C-1 labeled substrates were found to produce significantly greater amounts of $^{14}CO_2$ during the

TABLE 1. EFFECT OF BIOGENIC AMINES AND ALDEHYDES ON GLUCOSE-6-PHOSPHATE-1-$^{14}$C METABOLISM BY BRAIN HOMOGENATE

| Addition (M) | Pargyline* | Δ (nmoles) $^{14}CO_2$† | Per cent increase |
|---|---|---|---|
| Serotonin | | | |
| $1 \times 10^{-3}$ | − | 7·9 | 16·4 |
| | + | 0·3 | 0·6 |
| $5 \times 10^{-4}$ | − | 3·2 | 6·6 |
| | + | 0 | 0 |
| Norepinephrine | | | |
| $1 \times 10^{-3}$ | − | 27·8 | 57·6 |
| | + | 0 | 0 |
| $5 \times 10^{-4}$ | − | 17·5 | 36·2 |
| | + | 0 | 0 |
| Octopamine | | | |
| $1 \times 10^{-3}$ | − | 8·4 | 17·4 |
| | + | 0 | 0 |
| $5 \times 10^{-4}$ | − | 6·4 | 13·3 |
| | + | 0 | 0 |
| 5-Hydroxyindole-acetaldehyde | | | |
| $3 \times 10^{-5}$ | − | 6·3 | 13·0 |
| | + | 5·9 | 12·2 |
| Indoleacetaldehyde | | | |
| $3 \times 10^{-5}$ | − | 11·2 | 23·2 |
| | + | 12·4 | 25·7 |
| *p*-Nitrobenzaldehyde | | | |
| $1{\cdot}7 \times 10^{-4}$ | − | 35·4 | 73·3 |
| | + | 35·3 | 73·1 |

* Rat-brain homogenate was preincubated for 15 min in ice with pargyline ($1 \times 10^{-3}$ M) before being added to incubation mixtures.

† Values are the means of two separate experiments, each done in duplicate, and denote the increase over control values of $^{14}CO_2$ released from glucose-6-phosphate-1-$^{14}$C due to the addition. Control incubations contained 10% rat brain homogenate (50 mg brain, wet wt) and NADPH ($5{\cdot}7 \times 10^{-5}$ M) in incubation buffer described in the text. Approximately $48 \pm 2{\cdot}6$ nmoles $^{14}CO_2$ was produced by control incubation mixtures during the 20-min incubation.

short incubation period compared to the C-6 labeled substrate. Approximately 12–15 nmoles $^{14}CO_2$ was produced during the 20-min incubation when glucose-6-phosphate-1-$^{14}$C was incubated with brain homogenate (50 mg brain, wet wt) under the assay conditions described above. The inclusion of NADPH ($5{\cdot}7 \times 10^{-5}$ M) in these reaction mixtures increased the production of $^{14}CO_2$ to approximately $48 \pm 2{\cdot}6$ nmoles. We had previously found[11] that NADPH is oxidized to NADP in the presence of brain homogenates. Table 1 indicates that the presence of various biogenic amines further increased $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}$C in the presence of NADPH and brain homogenate. Little or no effect was observed with biogenic amines without added NADPH. The greatest stimulation of $^{14}CO_2$ production was obtained by the addition of norepinephrine in the presence of NADPH. However, if brain homogenate was preincubated for 15 min with pargyline ($1 \times 10^{-3}$ M) before being added to incubation mixtures, the stimulation of $^{14}CO_2$ production by the amines even with NADPH present was no longer evident. Monoamine oxidase activity in the homogenates was found to be inhibited 98–100 per cent by preincubation with pargyline. Aldehyde derivatives of the biogenic amines, and the aro-

matic aldehyde, *p*-nitrobenzaldehyde, were also shown to stimulate $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}$C (Table 1). Preincubation of the homogenate with pargyline, however, had no effect on the stimulated $^{14}CO_2$ production due to the addition of the aldehydes. We had previously isolated an NADPH-dependent aldehyde reductase [alcohol:NADP oxido-reductase (EC 1.1.1.2)] from brain which reduced "biogenic" and aromatic aldehydes to the corresponding alcohols with a concomitant oxidation of NADPH to NADP.[11,12] This enzyme was found to be inhibited by barbiturates.[11,13] Table 2 demonstrates the effect of inclusion of pentobarbital sodium in incubation mixtures. Sodium pentobarbital ($8{\cdot}5 \times 10^{-4}$ M) not only diminished the stimulation produced by the various amines, but also diminished the stimulation of $^{14}CO_2$ production by aldehydes.

The subcellular location of the enzymatic activity, which would be stimulated by aldehydes to produce $^{14}CO_2$ from glucose-6-phosphate-1-$^{14}$C, was investigated. After removal of nuclei and cellular debris, the major portion of the activity was recovered with the brain cytosol (Table 3). Although glucose-1-$^{14}$C was still metabolized to $^{14}CO_2$ by brain fractions after the removal of mitochondria, no metabolism of glucose-6-$^{14}$C to $^{14}CO_2$ occurred with such fractions during the 20-min incubation. The addition of NADPH or various aldehydes did not stimulate $^{14}CO_2$ production from glucose-6-$^{14}$C. Approximately 40 nmoles $^{14}CO_2$ was produced from glucose-1-$^{14}$C when incubated with supernatant fluid (2 mg protein) recovered after centrifugation at 27,000 *g*. When the crude mitochondrial fraction was subfractionated

TABLE 2. EFFECT OF PENTOBARBITAL ON THE STIMULATION OF GLUCOSE 6-PHOSPHATE-1-$^{14}$C METABOLISM BY ALDEHYDES AND AMINES IN BRAIN HOMOGENATE*

| Addition (M) | Pentobarbital† | Δ (nmoles) $^{14}CO_2$‡ | Per cent inhibition§ |
|---|---|---|---|
| Serotonin | | | |
| $1 \times 10^{-3}$ | – | 7·1 | |
| | + | 3·4 | 52·2 |
| Octopamine | | | |
| $1 \times 10^{-3}$ | – | 8·4 | |
| | + | 0 | 100 |
| Norepinephrine | | | |
| $1 \times 10^{-3}$ | – | 27·8 | |
| | + | 18·5 | 33·3 |
| Indoleacetaldehyde | | | |
| $3 \times 10^{-5}$ | – | 11·2 | |
| | + | 1·2 | 89·3 |
| *p*-Nitrobenzaldehyde | | | |
| $1{\cdot}7 \times 10^{-4}$ | – | 35·4 | |
| | + | 17·2 | 51·5 |

* Production of $^{14}CO_2$ was compared between incubation mixtures containing amines or aldehydes and control incubation mixtures. Control incubations contained 10% rat brain homogenate (50 mg brain, wet wt) and NADPH ($5{\cdot}7 \times 10^{-5}$ M) in incubation buffer described in the text.

† The plus symbol indicates that sodium pentobarbital ($8{\cdot}5 \times 10^{-4}$ M) was added to incubation mixtures just prior to the addition of brain homogenate.

‡ Values are the means of two separate experiments done in duplicate and indicate the increased $^{14}CO_2$ production in the presence of amine or aldehyde compared to control incubations.

§ Per cent inhibition indicates the diminution in $^{14}CO_2$ production produced by pentobarbital in assay mixtures stimulated by the addition of amine or aldehyde.

TABLE 3. SUBCELLULAR LOCALIZATION OF ALDEHYDE-DEPENDENT STIMULATION OF $^{14}CO_2$ PRODUCTION FROM GLUCOSE-6-PHOSPHATE-1-$^{14}C$

| Fraction* | Total protein† (mg) | $^{14}CO_2$ released due to addition of aldehyde‡ (nmoles/g brain wet wt) | Per cent recovery† |
|---|---|---|---|
| Homogenate | 153·0 | 3290 | 100 |
| 1000 *g* Supernatant fluid | 58·6 | 1440 | 43·8 |
| 27,000 *g* Supernatant fluid | 22·4 | 970 | 29·5 |
| 27,000 *g* Pellet ("crude" mitochondria) | 20·5 | 414 | 12·6 |
| 126,000 *g* Supernatant fluid (cytosol) | 20·9 | 742 | 22·6 |
| 126,000 *g* Pellet (microsomes) | 3·2 | 229 | 6·9 |
| Whittaker A layer (myelin) | 4·4 | 27 | 0·8 |
| Whittaker B layer (synaptosomes) | 4·7 | 71 | 2·2 |
| Whittaker C layer (mitochondria) | 4·9 | 60 | 1·8 |

* Subcellular fractions were prepared by modification[15] of the method of Whittaker[16].

† Total protein is based on 1 g brain wet wt.

‡ Values are the means of two separate experiments and indicate the increase in $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}C$ during a 20-min incubation due to the inclusion of *p*-nitrobenzaldehyde ($1·7 \times 10^{-4}$ M) in the incubation mixture compared to control incubations containing no aldehyde. All incubations contained enzyme (1·0 to 2·0 mg protein), and NADPH ($5·7 \times 10^{-5}$ M) in the incubation buffer described in the text.

§ Per cent recovery is based on aldehyde-stimulated metabolism of glucose-6-phosphate-1-$^{14}C$ to $^{14}CO_2$.

on a discontinuous sucrose gradient,[16] the highest amount of activity was recovered with synaptosomes (Table 3). This activity was found to be easily solubilized by mild sonication.

The activity found in the cytosol was further examined. Approximately 27 nmoles $^{14}CO_2$ was produced during a 20-min incubation from glucose-6-phosphate-1-$^{14}C$ when brain cytosol (approximately 1·5 mg protein) was incubated with NADPH ($5·7 \times 10^{-5}$ M) without aldehyde. The addition of NAD or NADH ($5·7 \times 10^{-5}$ M) to rat brain cytosol did not stimulate $^{14}CO_2$ production in incubation mixtures containing glucose-6-phosphate-1-$^{14}C$ with or without *p*-nitrobenzaldehyde. The addition of biogenic aldehydes to incubation mixtures containing NAD or NADH also did not increase $^{14}CO_2$ production over those values obtained by the addition of aldehyde alone. The addition of biogenic amines to incubation mixtures containing brain cytosol also produced no stimulation in the rate of $^{14}CO_2$ production (Table 4). No MAO activity could be demonstrated in the cytosol by either the spectrophotometric[19] or the radioisotopic[20] assays for this enzymatic activity. On the other hand, certain aldehydes were still found to stimulate the metabolism of glucose-6-phosphate-1-$^{14}C$. In contrast to the other aldehydes tested, acetaldehyde ($2 \times 10^{-4}$ M) produced no stimulation of the metabolism of glucose-6-phosphate-1-$^{14}C$ (Table 4). The greatest amount of stimulation on a molar basis was obtained by inclusion of *p*-nitrobenzaldehyde in incubation mixtures. Of the biogenic aldehydes, the α-hydroxy-substituted, *p*-hydroxyphenylglycolaldehyde, was found to increase $^{14}CO_2$ pro-

duction to the greatest extent (Table 4). The stimulation by indole-substituted aldehydes was low and did not increase with increasing concentration.

Pentobarbital, at several concentrations, was found to inhibit the stimulation of the metabolism of glucose-6-phosphate-1-$^{14}C$ by the various aldehydes in brain cytosol (Table 5). The one exception was that pentobarbital had no effect on the low stimulation produced by 5-hydroxyindoleacetaldehyde. In fact, a greater amount of $^{14}CO_2$ was produced when pentobarbital was present in incubation mixtures with 5-hydroxyindoleacetaldehyde (Table 5). Two other barbiturates as well as pentobarbital were demonstrated to be potent inhibitors of aldehyde-induced stimulation of glucose-6-phosphate-1-$^{14}C$ metabolism (Table 6) with the other aldehydes tested. *p*-Nitrobenzaldehyde stimulated $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}C$ in the presence of NADP as well as NADPH (Table 7). This stimulation by the aldehyde was inhibited in both cases by pentobarbital. Pentobarbital also inhibited the

TABLE 4. EFFECT OF BIOGENIC AMINES AND ALDEHYDES ON GLUCOSE-6-PHOSPHATE-1-$^{14}C$ METABOLISM IN THE CYTOSOL OF BRAIN*

| Addition (M) | $^{14}CO_2$ produced due to addition† (nmoles) | Increase over control (%) |
|---|---|---|
| Serotonin | | |
| $1 \times 10^{-3}$ | 0 | 0 |
| Norepinephrine | | |
| $1 \times 10^{-3}$ | 0 | 0 |
| Octopamine | | |
| $1 \times 10^{-3}$ | 0 | 0 |
| Dopamine | | |
| $1 \times 10^{-3}$ | 0 | 0 |
| 5-Hydroxyindole acetaldehyde | | |
| $6{\cdot}6 \times 10^{-5}$ | $4{\cdot}4 \pm 1{\cdot}2$ | 16·1 |
| $3{\cdot}7 \times 10^{-5}$ | $5{\cdot}1 \pm 1{\cdot}6$ | 18·6 |
| $1{\cdot}7 \times 10^{-5}$ | $3{\cdot}7 \pm 1{\cdot}1$ | 13·5 |
| Indoleacetaldehyde | | |
| $1{\cdot}3 \times 10^{-4}$ | $4{\cdot}3 \pm 0{\cdot}6$ | 15·7 |
| $6{\cdot}5 \times 10^{-5}$ | $5{\cdot}9 \pm 1{\cdot}8$ | 21·5 |
| $3{\cdot}2 \times 10^{-5}$ | $2{\cdot}1 \pm 0{\cdot}4$ | 7·7 |
| *p*-Hydroxyphenylglycolaldehyde | | |
| $1{\cdot}3 \times 10^{-4}$ | $17{\cdot}4 \pm 4{\cdot}2$ | 63·5 |
| $6{\cdot}4 \times 10^{-5}$ | $14{\cdot}6 \pm 2{\cdot}1$ | 53·3 |
| $3{\cdot}2 \times 10^{-5}$ | $5{\cdot}2 \pm 1{\cdot}1$ | 19·0 |
| *p*-Hydroxyphenylacetaldehyde | | |
| $3{\cdot}2 \times 10^{-4}$ | $15{\cdot}3 \pm 3{\cdot}6$ | 55·8 |
| $1{\cdot}3 \times 10^{-4}$ | $6{\cdot}9 \pm 2{\cdot}1$ | 25·2 |
| $6{\cdot}5 \times 10^{-5}$ | $0{\cdot}7 \pm 0{\cdot}5$ | 2·6 |
| *p*-Nitrobenzaldehyde | | |
| $1{\cdot}7 \times 10^{-4}$ | $29{\cdot}9 \pm 5{\cdot}6$ | 101·0 |
| $3{\cdot}4 \times 10^{-5}$ | $19{\cdot}3 \pm 3{\cdot}2$ | 70·4 |
| Acetaldehyde | | |
| $2 \times 10^{-4}$ | 0 | 0 |

* Control incubation mixtures contained brain cytosol (approximately 1·5 mg protein) and NADPH ($5{\cdot}7 \times 10^{-5}$ M) in incubation medium described in the text. Approximately $27{\cdot}4 \pm 2{\cdot}5$ nmoles $^{14}CO_2$ was produced in control mixture from glucose 6-phosphate-1-$^{14}C$ during the 20-min incubation.

† Values are the means of three to four separate experiments and indicate the increase in $^{14}CO_2$ production over control values.

TABLE 5. EFFECT OF PENTOBARBITAL ON ALDEHYDE-STIMULATED METABOLISM OF GLUCOSE-6-PHOSPHATE-1-$^{14}C$ BY BRAIN CYTOSOL

| Pentobarbital (M) | | Aldehyde (M) | $\Delta\ ^{14}CO_2$ due to addition of aldehyde* (nmoles) | Per cent inhibition† |
|---|---|---|---|---|
| | | 5-Hydroxyindoleacetaldehyde | | |
| $1{\cdot}7 \times 10^{-3}$ | − | $3{\cdot}7 \times 10^{-5}$ | $4{\cdot}8 \pm 1{\cdot}3$ | |
| | + | | $7{\cdot}4 \pm 2{\cdot}6$ | 0‡ |
| $8{\cdot}5 \times 10^{-4}$ | − | $3{\cdot}7 \times 10^{-5}$ | $4{\cdot}8 \pm 1{\cdot}3$ | |
| | + | | $5{\cdot}3 \pm 1{\cdot}5$ | 0‡ |
| | | Indoleacetaldehyde | | |
| $1{\cdot}7 \times 10^{-3}$ | − | $6{\cdot}5 \times 10^{-5}$ | 6·7 | |
| | + | | 0 | 100 |
| $8{\cdot}5 \times 10^{-4}$ | − | $6{\cdot}5 \times 10^{-5}$ | 6·7 | |
| | + | | 2·2 | 67·2 |
| | | *p*-Hydroxyphenylglycolaldehyde | | |
| $1{\cdot}7 \times 10^{-3}$ | − | $1{\cdot}3 \times 10^{-4}$ | 17·5 | |
| | + | | 0·5 | 97·2 |
| $8{\cdot}5 \times 10^{-4}$ | − | $1{\cdot}3 \times 10^{-4}$ | 17·5 | |
| | + | | 4·1 | 76·6 |
| | | *p*-Nitrobenzaldehyde | | |
| $1{\cdot}7 \times 10^{-3}$ | − | $1{\cdot}7 \times 10^{-4}$ | 28·9 | |
| | + | | 7·9 | 72·8 |
| $8{\cdot}5 \times 10^{-4}$ | − | | 28·9 | |
| | + | | 8·1 | 72·1 |
| $3{\cdot}4 \times 10^{-4}$ | − | | 28·9 | |
| | + | | 12·5 | 56·8 |
| $1{\cdot}7 \times 10^{-4}$ | − | | 28·9 | |
| | + | | 16·4 | 43·2 |

* Values indicate the difference in $^{14}CO_2$ production in incubations containing aldehyde compared to control incubation mixtures. Control incubation contained NADPH and brain cytosol as described in Table 4. Values for 5-hydroxyindoleacetaldehyde were derived from three separate experiments; all other values are the means of two separate experiments.

† Per cent inhibition, indicates the reduction in $^{14}CO_2$ production by sodium pentobarbital in aldehyde-stimulated incubation mixtures.

‡ A slight increase in $^{14}CO_2$ production was noted in incubation mixtures containing both pentobarbital and 5-hydroxyindoleacetaldehyde compared to mixtures with only aldehyde added.

stimulation of $^{14}CO_2$ production by NADPH in the absence of aldehyde. However, pentobarbital did not significantly inhibit the production of $^{14}CO_2$ in the presence of NADP when aldehyde was not added to incubation mixtures. Pentobarbital ($1 \times 10^{-3}$ M) was shown to inhibit aldehyde reductase activity in the brain cytosol. Reductase activity, measured spectrophotometrically, in the presence of *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) and NADPH ($1 \times 10^{-4}$ M) in the cytosol, was found to be 7·6 nmoles NADPH oxidized/min/mg of protein. This activity was inhibited 75–80 per cent by inclusion of pentobarbital ($1 \times 10^{-3}$ M) in the reaction mixtures. Pentobarbital, however, had no effect on glucose-6-phosphate dehydrogenase activity. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activity, measured spectrophotometrically or by the production of $^{14}CO_2$ in standard incubation mixtures, was not diminished by pentobarbital when either hemolysate of rat red blood cells or the commercial yeast glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase was used as the enzyme source.

Ris and von Wartburg[21] have indicated that biogenic acids (i.e. acid derivatives of biogenic amines) inhibit aldehyde reductase activity. We have substantiated these

TABLE 6. EFFECT OF VARIOUS BARBITURATES ON ALDEHYDE-ACTIVATED GLUCOSE-6-PHOSPHATE-1-$^{14}$C METABOLISM WITH BRAIN CYTOSOL

| Barbiturate* (M) | Δ $^{14}CO_2$ produced due to addition of aldehyde (nmoles) | Per cent inhibition |
|---|---|---|
| 0 | 33·6† | |
| Phenobarbital | | |
| $5 \times 10^{-4}$ | 14·5 | 56·7 |
| $2 \times 10^{-4}$ | 20·9 | 37·8 |
| Barbital | | |
| $5 \times 10^{-4}$ | 16·2 | 51·9 |
| $2 \times 10^{-4}$ | 23·3 | 30·8 |
| Pentobarbital | | |
| $5 \times 10^{-4}$ | 14·6 | 56·5 |
| $2 \times 10^{-4}$ | 20·1 | 40·1 |

* Barbiturates were added to incubation mixtures containing *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M), NADPH ($5{\cdot}7 \times 10^{-5}$ M) and brain cytosol (1·5 mg protein) under standard assay conditions.

† Value, derived from two separate experiments, indicates the increase in $^{14}CO_2$ production due to the addition of *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) over control values (see Table 4). Other values indicate the increase in $^{14}CO_2$ production in the presence of both *p*-nitrobenzaldehyde and barbiturates.

reports using 5-hydroxyindoleacetic acid (5-HIAA), the acid derivative of serotonin (Table 8). The kinetic parameters of this inhibition of aldehyde reductase are the subject of another report. 5-HIAA was also found to inhibit the aldehyde-stimulated production of $^{14}CO_2$ from glucose-6-phosphate-1-$^{14}$C in the presence of NADPH (Table 9). 5-HIAA ($1 \times 10^{-3}$ M) had little effect when NADP was used in place of NADPH.

TABLE 7. EFFECT OF NADP, NADPH, PENTOBARBITAL AND ALDEHYDE ON GLUCOSE 6-PHOSPHATE-1-$^{14}$C METABOLISM WITH BRAIN CYTOSOL*

| *p*-Nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) | Cofactor ($5{\cdot}7 \times 10^{-5}$ M) | Pentobarbital ($8{\cdot}5 \times 10^{-4}$ M) | $^{14}CO_2$ produced† (nmoles) |
|---|---|---|---|
| − | | − | 4·0 |
| + | | − | 5·8 |
| − | NADPH | − | 29·0 |
| − | NADPH | + | 25·5 |
| + | NADPH | − | 53·8 |
| + | NADPH | + | 32·8 |
| − | NADP | − | 94·9 |
| + | NADP | − | 148·0 |
| + | NADP | + | 104·2 |
| − | NADP | + | 93·8 |
| − | NADP | (Pargyline)† − | 92·6 |
| − | NADP | (Pargyline)† + | 93·4 |

* Values are the means of two separate experiments and indicate the $^{14}CO_2$ production due to various additions to control mixtures containing only brain cytosol (≈ 1·5 mg protein) in the presence of glucose-6-phosphate-1-$^{14}$C under standard assay conditions described in the text.

† Indicates that brain cytosol was preincubated with pargyline ($1 \times 10^{-3}$ M) before being added to incubation mixtures.

TABLE 8. INHIBITION OF ALDEHYDE REDUCTASE BY 5-HYDROXYINDOLEACETIC ACID (5-HIAA)*

| 5-HIAA (M) | Aldehyde reductase activity† | Per cent inhibition |
|---|---|---|
| 0 | 7·6 | |
| $1 \times 10^{-4}$ | 6·7 | 12·3 |
| $5 \times 10^{-4}$ | 5·6 | 25·7 |
| $1 \times 10^{-3}$ | 4·2 | 45·3 |

* Aldehyde reductase activity was measured in brain cytosol, spectrophotometrically, as described in the text with and without added 5-HIAA. The pH of reaction mixtures was determined to be 7·0 by the use of a Corning model 10 pH meter.

† NADPH, nmoles oxidized/min/mg of protein using *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) as substrate.

TABLE 9. EFFECT OF 5-HIAA ON ALDEHYDE-DEPENDENT STIMULATION OF GLUCOSE-6-PHOSPHATE-1-$^{14}$C METABOLISM WITH BRAIN CYTOSOL*

| 5-HIAA (M) | $\Delta\ ^{14}CO_2$ produced in the presence of aldehyde* (nmoles) | Per cent inhibition |
|---|---|---|
| 0 | 31·7 ± 1·6 | |
| $1 \times 10^{-3}$ | 25·0 ± 1·5 | 21·1 |

* Values are the mean of three experiments and indicate the increase in $^{14}CO_2$ production due to the addition of *p*-nitrobenzaldehyde ($1{\cdot}7 \times 10^{-4}$ M) over control values (see Table 4). Addition of 5-HIAA inhibits $^{14}CO_2$ production

## DISCUSSION

Hostetler *et al.*[22] have demonstrated that 5–8 per cent of the glucose metabolized by brain is metabolized by the pentose phosphate shunt. The stimulation of this activity by various neurohumors such as norepinephrine and serotonin, particularly in synaptosomes,[6] indicates that this pathway may prove to be important in the normal function of the CNS. The results of Appel and Parrot[6] also indicate that the evolution of $^{14}CO_2$ from C-1 labeled glucose under our assay conditions may be taken as evidence for the activity of the pentose phosphate shunt. In our studies, with rat brain homogenates, little metabolism of glucose-6-phosphate-1-$^{14}$C to $^{14}CO_2$ was noted, even in the presence of various amines, until NADPH was added to the incubation mixtures. In the presence of NADPH, several of the amines produced a further stimulation of $^{14}CO_2$ production (Table 1). The stimulation by the amines in these assays seemed to be dependent on a functional MAO. The inhibition of MAO by preincubation of the homogenate with pargyline eliminated the stimulatory effect of the amines. Pargyline did not affect the stimulation of $^{14}CO_2$ evolution produced by biogenic aldehydes or by an aromatic aldehyde. Pargyline also has no effect on pentose shunt activity when NADP was provided as a cofactor (Table 7). Similar results were obtained by Barondes[8] with beef anterior pituitary. In the studies of Barondes and Field,[8,9] however, norepinephrine produced no more stimulation than did serotonin. Indoleacetaldehyde, when compared to the other aldehydes tested, was found to stimulate the production of $^{14}CO_2$ to the greatest extent.[8] Our results with whole brain homogenate and NADPH indicate that norepinephrine produces

a greater stimulation of $^{14}CO_2$ production compared to serotonin, while the substituted aromatic aldehyde, *p*-nitrobenzaldehyde, was the best aldehyde tested (Table 1).

Since NADP is the obligatory cofactor for both D-glucose-6-phosphate: NADP oxido-reductase (EC 1.1.1.49) and 6-phospho-D-gluconate: NADP oxido-reductase (decarboxylating; EC 1.1.1.44) it seemed that the biogenic amines, through their aldehyde derivatives, may be influencing the conversion of added NADPH to NADP. We had demonstrated the presence of an aldehyde reductase in the cytosol of brain which catalyzed the following reaction:[11]

$$\text{aldehyde} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{alcohol} + \text{NADP}^+.$$

Pentobarbital was shown to be a potent noncompetitive inhibitor of this enzyme.[13] The inclusion of pentobarbital with brain homogenates diminished the stimulation of the metabolism of glucose-6-phosphate-1-$^{14}$C produced by both the amines and the aldehydes (Table 2). It is interesting to note that Carl and King[23] demonstrated that the decrease in brain glucose-6-phosphate and xylulose-5-phosphate produced by electrically induced convulsions in mice could be blocked by pretreating the animals with phenobarbital. It is not surprising that the major portion of aldehyde-stimulated metabolism of glucose-6-phosphate-1-$^{14}$C is located in the cytosol (Table 3), since this is where both aldehyde reductase and glucose-6-phosphate and 6-phosphogluconate dehydrogenases are primarily found. When the crude mitochondrial fraction was further separated on a discontinuous sucrose gradient,[16] the peak in aldehyde-stimulated decarboxylation occurred in the fraction containing synaptosomes (Table 3).

The results in Table 4 provide further evidence that the biogenic amines have to be converted to their aldehyde derivatives before the stimulation of the pentose shunt is evident. Thus, when the amines were added to the cytosol from which MAO activity was removed by centrifugation, no stimulation was observed, whereas the aldehydes did stimulate glucose-6-phosphate-1-$^{14}$C metabolism. Of all the biogenic aldehydes, *p*-hydroxyphenylglycolaldehyde (HPGA) provided the greatest stimulation when added to brain cytosol in the presence of NADPH. In low concentrations (approximately $6{\cdot}4 \times 10^{-5}$ M) HPGA produced an increase in $^{14}CO_2$ evolution 2- to 20-fold greater than that produced by the other biogenic aldehydes tested (Table 4). *p*-Nitrobenzaldehyde, previously shown to be a good substrate for brain aldehyde reductase,[11] produced the greatest stimulation. Similarly, HPGA was found to be the best biogenic aldehyde substrate of nine aldehydes derived from biogenic amines for which kinetic constants were determined by Tabakoff *et al.*[12] In addition to HPGA, other α-hydroxy-substituted aldehydes, such as those derived from norepinephrine, are excellent substrates for aldehyde reductase.[12] The relatively large stimulatory effect of norepinephrine in brain homogenates (Table 1) may be related to this observation and not to norepinephrine being a better substrate for MAO. MAO metabolizes norepinephrine at a slower rate than serotonin.[15] Acetaldehyde produced no stimulation of glucose-6-phosphate-1-$^{14}$C metabolism to $^{14}CO_2$. Although NADPH-dependent metabolism of acetaldehyde by aldehyde reductase has been reported,[24] this metabolism proceeds at a rate 2–5 per cent of that obtained with *p*-nitrobenzaldehyde. Thus, the oxidation of NADPH in the presence of acetaldehyde may not be of sufficient magnitude to induce a stimulation of $^{14}CO_2$ production via

the pentose shunt. The stimulation produced by indole-substituted aldehydes did not increase with increasing concentrations of these aldehydes (Table 4). In addition, pentobarbital had little effect on the small amount of stimulation produced by 5-hydroxyindoleacetaldehyde with brain cytosol (Table 5). These results may reflect the presence of isozymes of aldehyde reductase which have recently been reported in rat brain.[21] One of these isozymes is much less sensitive to inhibition by barbiturates.[21] However, pentobarbital (Table 5) and other barbiturates (Table 6) were found to be potent inhibitors of the stimulation of glucose-6-phosphate-1-$^{14}$C metabolism produced by other biogenic aldehydes and *p*-nitrobenzaldehyde.

Pentobarbital was also found to inhibit the stimulation of the pentose shunt when no aldehyde was added but NADPH was present in incubation mixtures (Table 7). However, the inhibition by pentobarbital was more pronounced, on a percentage basis, in the presence of both NADPH and aldehyde. The addition of *p*-nitrobenzaldehyde also increased $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}$C in the presence of NADP over those values obtained by adding NADP alone to incubation mixtures containing brain cytosol. This stimulation by *p*-nitrobenzaldehyde was inhibited by pentobarbital, but pentobarbital had little effect on the NADP-stimulated $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}$C in brain cytosol (Table 7). In addition, pentobarbital did not inhibit the activity of glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase in blood hemolysate or the yeast enzymes. This indicates that the inhibition by pentobarbital occurs, not on the dehydrogenase systems, but on the system responsible for the conversion of NADPH to NADP, the necessary cofactor for the dehydrogenases. The data also indicate that in brain cytosol, the glucose-6-phosphate and 6-phosphogluconate dehydrogenases may be coupled to the aldehyde reductase, since aldehydes seem to increase $^{14}CO_2$ production even in the presence of high concentrations of NADP. The cycling of NADPH to NADP by aldehyde reductase within the enzyme complex may bypass a rate-limiting step in the reaction sequence of glucose-6-phosphate dehydrogenase.[25] A coupling of reactions has been shown to bypass such a rate-limiting step in the reaction sequence of alcohol dehydrogenase.[26]

Ris and von Wartburg[21] noted that biogenic acids (e.g. 5-HIAA) inhibited aldehyde reductase activity. We have confirmed these observations (Table 8) and demonstrated that the presence of the acid derivative of serotonin inhibits $^{14}CO_2$ production from glucose-6-phosphate-1-$^{14}$C (Table 9). Thus, the end product of amine metabolism derived through another metabolic pathway in brain (i.e. aldehyde dehydrogenase)[27] may control the stimulation of glucose metabolism produced by the biogenic aldehydes.

The levels of NADP in brain[28] are below the $K_m$ values reported for glucose-6-phosphate dehydrogenase isolated from blood.[25] If one assumes the $K_m$ values for the coenzyme to be similar for the brain enzyme, the availability of NADP in brain might severely limit the activity of this initial enzyme of the pentose shunt. In addition, NADPH has been shown to inhibit[25] glucose 6-phosphate dehydrogenase activity at concentrations which may be found in brain.[28] Therefore, the oxidation of NADPH to NADP by aldehyde reductase may be an important mechanism for the activation of pentose shunt activity. The metabolism of biogenic amines to their aldehyde derivatives and the further metabolism of the aldehydes by aldehyde reductase would be most prominent during nervous activity. Thus, the pentose shunt could be

activated during this period. Our data indicate that the aldehyde derived from norepinephrine would be most active in stimulating shunt activity. The inhibition by barbiturates of this aldehyde-mediated metabolism of glucose-6-phosphate via the pentose shunt may have physiologic implications.

Appel and Parrot[6] have speculated on the importance of the pentose shunt in brain. Little evidence was obtained to relate increases in pentose shunt activity in brain to an enhanced incorporation of sugars into macromolecules. In addition, Kaufman[4] has indicated that NADPH production by the pentose shunt is not necessarily earmarked for lipid metabolism in brain. Therefore, in light of the present demonstration of the relationship between the metabolism of the biogenic amines and the pentose shunt, one may advance the hypothesis that the pentose shunt may be involved in maintaining high NADPH levels for the metabolism of the reactive aldehyde derivatives of the biogenic amines. These biogenic aldehydes have been shown to be physiologically active[29–31] and the control of their metabolism may be dependent on pentose shunt activity.

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## REFERENCES

1. J. J. O'Neill, S. H. Simon and W. W. Shreeve, *J. Neurochem.* **12,** 797 (1965).
2. J. McGuire and L. R. Pesh, *Proc. natn. Acad. Sci. U.S.A.* **48,** 2157 (1962).
3. C. E. Wenner and S. Weinhouse, *J. biol. Chem.* **219,** 691 (1956).
4. F. C. Kaufman, *J. Neurochem.* **19,** 1 (1972).
5. F. C. Kaufman, J. G. Brown, J. V. Passonneau and O. H. Lowry, *J. biol. Chem.* **244,** 3647 (1969).
6. S. H. Appel and B. L. Parrot, *J. Neurochem.* **17,** 1619 (1970).
7. D. DiPietro and A. Weinhouse, *Archs Biochem. Biophys.* **80,** 268 (1959).
8. S. H. Barondes, *J. biol. Chem.* **237,** 204 (1962).
9. S. H. Barondes and J. B. Field, *Biochim. biophys. Acta* **75,** 324 (1963).
10. I. Pastan, B. Herring, P. Johnson and J. B. Field, *J. biol. Chem.* **237,** 287 (1962).
11. B. Tabakoff and V. G. Erwin, *J. biol. Chem.* **245,** 3263 (1970).
12. B. Tabakoff, R. Anderson and S. G. A. Alivisatos, *Molec. Pharmac.* **9,** 428 (1973).
13. V. G. Erwin, B. Tabakoff and R. Bronaugh, *Molec. Pharmac.* **7,** 169 (1971).
14. N. W. Tietz, *Fundamentals of Clinical Chemistry*, p. 457. Saunders, Philadelphia (1970).
15. B. Tabakoff, L. Meyerson and S. G. A. Alivisatos, *Brain Res.* **66,** 491 (1974).
16. V. P. Whittaker, *Biochem. J.* **72,** 694 (1959).
17. O. H. Lowry, N. J. Rosenbrough, A. J. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
18. K. F. Tipton, *Biochim. biophys. Acta* **135,** 910 (1967).
19. B. Tabakoff and S. G. A. Alivisatos, *Analyt. Chem.* **44,** 427 (1972).
20. R. E. McCaman, M. W. McCaman, J. M. Hunt and M. S. Smith, *J. Neurochem.* **12,** 15 (1965).
21. M. M. Ris and J-P. von Wartburg, *Eur. J. Biochem.* **37,** 69 (1973).
22. K. Y. Hostetler, B. R. Landau, R. J. White, M. S. Albin and D. Yashan, *J. Neurochem.* **17,** 33 (1970).
23. J. L. Carl and L. J. King, *J. Neurochem.* **17,** 293 (1970).
24. B. Tabakoff, C. Vugrincic, R. Anderson and S. G. A. Alivisatos, *Biochem. Pharmac.* **23,** 455 (1974).
25. A. Yoshida, *Science, N.Y.* **179,** 532 (1973).
26. N. H. Raskin and L. Sokoloff, *J. Neurochem.* **17,** 1677 (1970).
27. R. A. Deitrich, *Biochem. Pharmac.* **15,** 1911 (1966).
28. H. B. Burch, M. E. Bradley and O. H. Lowry, *J. biol. Chem.* **242,** 4546 (1967).
29. H. C. Sabelli, W. J. Giardina, S. G. A. Alivisatos, P. K. Seth and F. Ungar, *Nature, Lond.* **223,** 73 (1969).
30. B. Tabakoff, F. Ungar and S. G. A. Alivisatos, *Adv. Exp. Med. Biol.* **35,** 45 (1973).
31. P. J. G. Mann and J. H. Quastel, *Biochem. J.* **34,** 414 (1940).