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Changes in Monoamine Oxidase Activity in Rat Brain During Alloxan Diabetes

C. S. K. Mayanil, S. M. I. Kazmi, and N. Z. Baquer

Hormone and Drug Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Abstract: The effect of alloxan diabetes on the activity of monoamine oxidase was studied in three regions of the rat brain at various time intervals after the onset of diabetes. It was observed that monoamine oxidase activity was decreased at early time intervals after diabetes, followed by a recovery in all three regions of the brain. A reversal of the effect was observed with insulin administration to the diabetic rats. Key Words: Monoamine oxidase—Brain—Diabetes—Catecholamines—Insulin. Mayanil C. S. K. et al. Changes in monoamine oxidase activity in rat brain during alloxan diabetes. J. Neurochem. 38, 179-183 (1982).

MATERIALS AND METHODS

Preparation of homogenates
Albino rats of the Holtzman strain, weighing between 200 and 300 g, were used for all the experiments. A number of rats were made diabetic and were divided into two groups, as described earlier (Murthy and Baquer, 1980). The first group of rats, the diabetic group, was killed after various time intervals. To the second group of rats, protamine-zinc insulin was injected each day (1 unit/day) until the day of sacrifice; these were designated as diabetic rats treated with insulin. Both groups were given food and water ad libitum.

Rats were killed by cervical dislocation, and cerebral hemispheres, cerebellum and brain stem were excised immediately and weighed. Tissue homogenates (1:10) were prepared as described earlier (Murthy and Baquer, 1980). Differential centrifugation was performed essentially according to the method of Hess and Brand (1974). Fractions were washed once with the homogenising medium. The mitochondrial fraction and the supernatant were used for all the assays of monoamine oxidase.

Assay of MAO activity
Monoamine oxidase activity was assayed according to the method of Catravas et al. (1977) with little modification. The assay mixture contained Tris-HCl 0.05 M (pH 7.4), kynuramine dihydrobromide 0.22 mM, MgCl₂ 0.08 mM and the appropriate enzyme preparation containing 0.6 to 1 mg protein in a final volume of 3 ml. The reaction was stopped by the addition of 0.2 ml of 0.5 M-NaOH and 0.4 ml of 10% ZnSO₄. The mixture was heated in a
boiling-water bath for 5 min and centrifuged at 10,000 g for 10 min. The concentration of the reaction product, 4-hydroxyquinoline, was determined spectrophotometrically in the supernatant by measuring the increase in absorbance at 330 nm (Catravas and McHale, 1974). A blank was prepared by replacing kynuramine with water. By measuring the increase in absorbance at 330 nm, instead of the decrease at 360 nm (Weissbach et al., 1960), a three- to fourfold increase in the sensitivity of the reaction can be achieved. Protein was estimated according to the method of Lowry et al. (1951). One unit of enzyme activity is defined as 1 μmol 4-hydroxyquinoline produced per 90 min per g of tissue.

Determination of blood glucose
Blood glucose was determined enzymatically according to the method of Bergmeyer et al. (1974), and is expressed as mmol/litre.

Chemicals
4-hydroxyquinoline and kynuramine dihydrobromide were obtained from Sigma Chemical Co., U.S.A. All the other chemicals were from BDH and were of Analar grade.

RESULTS
The changes in the activity of MAO from different regions of rat brain with alloxan diabetes showed identical patterns. As the time of diabetes progressed, a gradual decrease in the mitochondrial enzyme was observed, up to 8 days in the cerebral hemispheres, cerebellum and brain stem (Fig. 1. A−C). Optimum decrease in all three regions was observed at 8 days after diabetes. At 15 days, the enzyme activity from all three regions increased to

FIG. 1. Percent activity of monoamine oxidase in various regions of rat brain in mitochondrial fraction during alloxan diabetes (taking control as 100%). (A) Cerebral hemisphere; (B) cerebellum; (C) brain stem.

FIG. 2. Percent activity of monoamine oxidase in various regions in rat brain in soluble fraction during alloxan diabetes (taking control as 100%). (A) Cerebral hemisphere; (B) cerebellum; (C) brain stem.
TABLE 1. Activity of monoamine oxidase in cerebral hemisphere

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>Crude mitochondrial fraction</th>
<th>Soluble fraction (microsomal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic</td>
<td>Diabetic + insulin</td>
</tr>
<tr>
<td></td>
<td>Diabetic + insulin</td>
<td>Diabetic + insulin</td>
</tr>
<tr>
<td>3</td>
<td>34.47 ± 5.24</td>
<td>39.31 ± 4.66</td>
</tr>
<tr>
<td>8</td>
<td>30.96 ± 1.53</td>
<td>42.82 ± 3.25</td>
</tr>
<tr>
<td>15</td>
<td>50.40 ± 5.76</td>
<td>39.48 ± 2.32</td>
</tr>
<tr>
<td>22</td>
<td>48.06 ± 6.65</td>
<td>40.50 ± 3.08</td>
</tr>
<tr>
<td>Control</td>
<td>41.61 ± 2.92</td>
<td>33.81 ± 1.71</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol/90 min/g. Each value is a mean ± S.E.M. of four or more experiments.

more than control levels, and thereafter it followed a differential pattern. In the cerebral hemispheres, it remained at this increased level (Fig. 1A), in cerebellum it continued to increase after the 15th day (Fig. 1B), and in the brain stem the enzyme showed the maximum increase at 22 days after the onset of diabetes. Insulin administration brought the mitochondrial activity in the three brain regions to almost control level at all time intervals.

The MAO of the soluble fraction (supernatant) followed a pattern quite similar to that of the mitochondrial enzyme (Fig. 2, A–C). In the cerebral hemispheres and the cerebellum the changes observed in the supernatant enzyme were less than in the mitochondrial fraction of the two regions (Fig. 2, A and B). An almost identical pattern of changes was seen in the brain stem (Fig. 2C). Administration of insulin seemed to bring back the activities nearly to control levels, with the exception of cerebellum where a decrease was still maintained.

The absolute units of enzyme activities, expressed as μmol of 4-hydroxyaminoline formed/90 min/g in both the mitochondrial and the soluble fractions, are presented in Tables 1–3 (diabetic and insulin-treated) and follow a pattern similar to that seen in the percentage changes shown in Figs. 1 and 2.

The severity of diabetes was established by the determination of blood glucose values, which are presented in Table 4. Insulin administration decreased blood glucose levels, but not down to control levels.

DISCUSSION

The observed decrease in the activity of monoamine oxidase at 3 and 8 days after the onset of diabetes may be due to an increase in circulating free fatty acid level, which decreases the total serum tryptophan, with an increase in free tryptophan level (McMenamy and Oncley, 1958; Curzon and Knott, 1974; De Montis et al., 1978), by competing with the same binding site as albumin (McMenamy, 1965). A decrease in the level of the serum fraction of tryptophan would imply its reduced uptake from blood to brain, as the entry of tryptophan from blood to brain in the presence of insulin is controlled by its serum fraction (Knott and Curzon, 1972; Tagliamonte et al., 1973; De Montis et al., 1977).

Moreover, previous reports have shown that administration of insulin increases the concentration of tryptophan (Fernstrom and Wurtman, 1971) and tyrosine (Tagliamonte et al., 1975) in brain, while decreasing that of tyrosine and free tryptophan in serum (Lipsett et al., 1973; Tagliamonte et al., 1975), implying thereby that lack of insulin (diabetes) might decrease the transport of these two amino acids from blood to brain (Tagliamonte et al.,...
Insulin administration reverses the effect of alloxan diabetes. This reversal may be due to a decrease in the concentration of free fatty acids (Lewis et al., 1972; Hall et al., 1979) with a consequent increase in the total serum tryptophan level (De Montis et al., 1978)—hence the entry of aromatic amino acids into the brain from blood, supplementing the precursor pool for catecholamine synthesis and subsequently making available substrates for oxidative deamination. Shohmori et al. (1979) have reported that insulin not only elevates tyrosine hydroxylase activity but also promotes decarboxylation of tyrosine in brain. Elevated tyrosine hydroxylase activity may increase catecholamine synthesis. Since urinary homovanillic acid excretion was also increased, the catecholamine turnover may also be increased following insulin administration, thereby resulting in an increase in monoamine oxidase activity.

**ADDENDUM**

Fowler et al. (1980) have reported the presence of an unsedimentable MAO from sonicates of rat liver homogenates. The properties of this MAO, however, are almost those of the particulate enzyme.

In separate experiments (unpublished) from our laboratory, it was found that the high-speed supernatant (105,000 × g for 60 min) had characteristics that were essentially the same as those of whole homogenate enzyme and the soluble fraction separated at a lower speed, as in the present experiments.

**Acknowledgment:** C. S. K. M. and S. M. I. K. thank the Jawaharlal Nehru University for providing financial help in the form of Junior Research Fellowships.

**REFERENCES**


Baquer N. Z., Hothersall J. S., McLean P., and Greenbaum...


Na⁺,K⁺-ATPase and Mg²⁺-ATPase Activities in Different Regions of Rat Brain During Alloxan Diabetes

C. S. K. Mayanil, S. M. I. Kazmi, and N. Z. Baquer

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Abstract: The effect of alloxan diabetes on the activities of Na⁺,K⁺-ATPase and Mg²⁺-ATPase was studied in three regions of rat brain at various time intervals after the onset of diabetes. It was observed that Na⁺,K⁺-ATPase activity increased at early time intervals after diabetes, followed by a recovery to near control levels in all three regions of the brain. There was an overall increase in Mg²⁺-ATPase activity in all the regions. A reversal of the effect was observed with insulin administration to the diabetic rats. Key Words: Na⁺,K⁺-ATPase—Mg²⁺-ATPase—Dopamine—Norepinephrine—Diabetes—Brain—Insulin. Mayanil C. S. K. et al. Na⁺,K⁺-ATPase and Mg²⁺-ATPase activities in different regions of rat brain during alloxan diabetes. J. Neurochem. 39, 903–908 (1982).

Na,K-ATPase (Na⁺,K⁺-ATPase, Mg²⁺-dependent ATP phosphohydrolase, EC 3.6.1.3) plays a crucial role in neuronal function. The enzyme is thought to be responsible for the maintenance and reestablishment of a cation gradient across the neuronal membrane, which is essential for impulse propagation (Skou, 1965). Nerve terminal Na⁺,K⁺-ATPase may play a role in the release mechanism of transmitters (Vizi, 1977, 1978). The presynaptic inhibitory effect of catecholamines on transmitter release (Gilbert et al., 1975) is also associated with the stimulation of this enzyme (Yoshimura, 1973; Godfraind et al., 1974; Logan and O'Donovan, 1976; Hexum, 1977; Lee and Phillips, 1977; Wu and Phillips, 1978).

Norepinephrine, dopamine, and serotonin have been reported to activate Na⁺,K⁺-ATPase in homogenates of whole mouse brain (Desaiah and Ho, 1976), rat hypothalamus, and rat cerebral cortex (Yoshimura, 1973). No attempt has been made to stress the significance of the ATPase activity as a manifestation of the physiological state, wherein the catecholamine levels in brain might be affected.

The absence of insulin in experimental diabetes may decrease the transport of tyrosine and tryptophan from blood to brain, resulting in the lowering of the activities of brain tyrosine hydroxylase and tyrosine decarboxylase, the enzymes necessary for catecholamine synthesis and hence lowering of the brain catecholamine levels (Tagliamonte et al., 1975; De Montis et al., 1977, 1978; Fernando et al., 1976; Shohmori et al., 1979).

The important role of Na⁺,K⁺-ATPase in the regulation of release and uptake of neurotransmitters (Logan and O'Donovan, 1980a,b), known to have an inhibitory effect on insulin secretion, may indicate the possible inclusion of the catecholamine/ATPase system in the pathophysiology of diabetes.

The present investigation studied the changes in the activity of the enzymes Na⁺,K⁺-ATPase and Mg²⁺-ATPase in various regions of rat brain under conditions of short- and long-term diabetes. To determine whether the changes induced are reversible, insulin was administered to the diabetic animals and the activities of Na⁺,K⁺-ATPase and Mg²⁺-ATPase were assayed.

MATERIALS AND METHODS

Chemicals

Adenosine triphosphate, ouabain, and Tris were obtained from Sigma. Insulin zinc suspension, I.P., LENTE was obtained from the Boots Company (India) Ltd. All
of rats, which were then divided into two subgroups for all the experiments. Diabetes was induced in a group described by Murthy and Baquer (1980).

Rats were sacrificed by cervical dislocation and the cerebral hemispheres, cerebella, and brain stems were excised and weighed immediately. Tissue homogenates were prepared from the disodium salt by passage through a column of Dowex-50 in the H⁺ form, followed by the neutralization of the acid form of the ATP to pH 7.4 with Tris.

For Mg²⁺-ATPase assay, the assay mixture in a final volume of 3 ml contained: 40 mM Tris-HCl buffer, pH 7.4; 1 mM EDTA, pH 7.0; 6 mM MgCl₂, pH 7.0; 100 mM NaCl; 20 mM KCl, and 5 mM ATP. The Tris salt of ATP was prepared from the disodium salt by passage through a column of Dowex-50 in the H⁺ form, followed by the neutralization of the acid form of the ATP to pH 7.4 with Tris.

Preparation of homogenates
Albino Holtzman rats weighing 200–300 g were used for all the experiments. Diabetes was induced in a group of rats, which were then divided into two subgroups as described by Murthy and Baquer (1980).

Rats were sacrificed by cervical dislocation and the cerebral hemispheres, cerebella, and brain stems were excised and weighed immediately. Tissue homogenates (1:10) were prepared as described earlier (Mayanil et al., 1982). Differential centrifugation was performed essentially according to the method of Hess and Brand (1974). Fractions were washed once with the homogenizing medium. The 12,000 × g pellet (excluding the nuclear debris) containing myelin, synaptosomes, and the mitochondria, and the supernatant fraction containing the microsomes, were taken for the enzyme assay. Reiss et al. (1981) reported that the myelin Na⁺,K⁺-ATPase activity was 28% of the microsomal fraction and they assumed that less than half of the Na⁺,K⁺-ATPase in myelin may be due to microsomal contamination. Desaiiah and Ho (1977) had reported earlier a synaptosomal Na⁺,K⁺-ATPase with a specific activity of ~13 μmol/mg protein/h. To ascertain that there was no contamination of the soluble fraction in the 12,000 × g pellet, we also assayed a soluble enzyme, lactate dehydrogenase (Bergmeyer and Bernt, 1974).

### Table 1. Activities of Na⁺,K⁺-ATPase in cerebral hemisphere

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>Diabetic + insulin</th>
<th>Diabetic + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.18 ± 0.09</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.40 ± 0.12</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>0.43 ± 0.10</td>
<td>0.31 ± 0.10</td>
</tr>
<tr>
<td>15</td>
<td>0.36 ± 0.03</td>
<td>0.22 ± 0.10</td>
</tr>
<tr>
<td>22</td>
<td>0.22 ± 0.08</td>
<td>0.34 ± 0.15</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P₃ liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

### Table 2. Activities of Na⁺,K⁺-ATPase in cerebellum

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>Diabetic + insulin</th>
<th>Diabetic + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15 ± 0.02</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.24 ± 0.04</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.33 ± 0.02</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>15</td>
<td>0.31 ± 0.06</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>22</td>
<td>0.17 ± 0.04</td>
<td>0.32 ± 0.09</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P₃ liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

### Assay of ATPase activity
Na⁺,K⁺-ATPase activity was assayed essentially according to the method of Zaheer and Talwar (1968). For the total ATPase assay, the assay mixture in a final volume of 3 ml contained: 40 mM Tris-HCl buffer, pH 7.4; 1 mM EDTA, pH 7.0; 6 mM MgCl₂, pH 7.0; 100 mM NaCl; 20 mM KCl, and 5 mM ATP. The Tris salt of ATP was prepared from the disodium salt by passage through a column of Dowex-50 in the H⁺ form, followed by the neutralization of the acid form of the ATP to pH 7.4 with Tris.

For Mg²⁺-ATPase assay, the assay mixture in a final volume of 3 ml contained: 40 mM Tris-HCl buffer, pH 7.4; 1 mM EDTA, pH 7.0; 6 mM MgCl₂, pH 7.0; 100 mM NaCl; 20 mM KCl. The enzyme was preincubated for 10 min at 37°C in the presence of 1 mM ouabain and the reaction was started by the addition of 5 mM Tris ATP. The reaction was stopped by the addition of 0.6 ml 50% cold TCA.

Na⁺,K⁺-ATPase activity was obtained by subtracting the ouabain-insensitive Mg²⁺-ATPase from the total ATPase activity assayed in the presence of Na⁺,K⁺, and Mg²⁺ and in the absence of ouabain.

The specific activity of the enzyme is expressed as...
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TABLE 3. Activities of Na⁺,K⁺-ATPase in brain stem

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>12,000 x g pellet (myelin, synaptosomes and mitochondria)</th>
<th>Supernatant fraction (microsomal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>Diabetic + insulin</td>
<td>Diabetic + insulin</td>
</tr>
<tr>
<td>Control</td>
<td>0.23 ± 0.07</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.27 ± 0.07</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.88 ± 0.12</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>0.25 ± 0.10</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>22</td>
<td>0.25 ± 0.16</td>
<td>0.15 ± 0.13</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P₂ liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

Determination of blood glucose

Blood glucose was determined enzymatically according to the method of Bergmeyer et al. (1974) and is expressed as mM/L.

RESULTS

Regional changes in the 12,000 x g pellet ATPase activity of the brain with diabetes

The changes in the activity of Na⁺,K⁺-ATPase from the different regions of rat brain with alloxan diabetes showed an almost identical pattern. As the duration of diabetes increased, a gradual increase in the 12,000 x g pellet Na⁺,K⁺-ATPase activity was observed up to 8 days, the changes being more significant in the cerebellum and brain stem (Tables 1-3). This increase was followed by a gradual recovery of the Na⁺,K⁺-ATPase activity nearly to control levels at 22 days after the onset of diabetes.

There were significant increases in the Mg²⁺-ATPase activity in the cerebral hemisphere at 8 and 22 days. The cerebellum and the brain stem, however, did not show any significant change in the Mg²⁺-ATPase activity after the onset of diabetes (Tables 4-6).

Insulin administration to the diabetic rats brought the Na⁺,K⁺- and Mg²⁺-ATPase activities in the 12,000 x g pellet almost to control values in the three regions at each time interval. However, at 22 days the Na⁺,K⁺-ATPase activity in the cerebellum was increased (p < 0.05; Tables 1-3). The Mg²⁺-ATPase activity in the cerebral hemisphere was significantly decreased (p < 0.002) at 15 days of insulin administration (Tables 4-6).

Regional changes in the ATPase activity from supernatant fraction of diabetic rat brain

Na⁺,K⁺-ATPase activities of the supernatant fraction (containing the microsomes) followed a pattern almost similar to that of the 12,000 x g pellet enzyme. As the duration of diabetes increased, a gradual increase in the Na⁺,K⁺-ATPase activity was observed up to 8 days, with more significant changes in the cerebellum and brain stem (p < 0.02) and cerebral hemisphere (p < 0.001) (Tables 1-3). This was followed by a gradual recovery of the Na⁺,K⁺-ATPase activity nearly to control values at 22 days after the

TABLE 4. Activities of Mg²⁺-ATPase in cerebral hemisphere

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>12,000 x g pellet (myelin, synaptosomes and mitochondria)</th>
<th>Supernatant fraction (microsomal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>Diabetic + insulin</td>
<td>Diabetic + insulin</td>
</tr>
<tr>
<td>Control</td>
<td>0.65 ± 0.10</td>
<td>0.53 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>0.50 ± 0.01</td>
<td>0.38 ± 0.21</td>
</tr>
<tr>
<td>8</td>
<td>1.00 ± 0.16</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>0.62 ± 0.21</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>22</td>
<td>1.39 ± 0.21</td>
<td>0.72 ± 0.13</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P₂ liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

* p < 0.001 (Student’s t test).

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onset of diabetes, except in the cerebellum, where a decrease (p < 0.05) was observed.

There was an overall decrease in the Mg\(^{2+}\)-ATPase activity in the three brain regions at 3 days after the onset of diabetes. This was followed by an increase at 8 days, with the most significant change (p < 0.01) in the brain stem. With the progress of diabetes, i.e., at 22 days after its onset, the Mg\(^{2+}\)-ATPase activity increased significantly (p < 0.002) in the cerebral hemisphere and brain stem.

Insulin administration to the diabetic rats brought the Na\(^+,K^+\)- and Mg\(^{2+}\)-ATPase activities in the supernatant fraction to levels that were not significantly different from the control values in the three brain regions at each time interval. However, the Na\(^+,K^+\)-ATPase activity in the cerebral hemisphere decreased significantly at 8 (p < 0.002), 15 (p < 0.01), and 22 days (p < 0.02) after insulin administration (Tables 1–3). The Mg\(^{2+}\)-ATPase activity fell (p < 0.02) only in the brain stem at 22 days after insulin administration (Tables 4–6).

The severity of diabetes was established by the determination of blood glucose levels, which are presented in Table 7. Insulin administration decreased the blood glucose levels but did not bring them back to the control values.

In the three brain regions, <10% of lactate dehydrogenase was found in the pellet (Table 8).

## DISCUSSION

Previous reports have demonstrated that dopamine and norepinephrine influence the Na\(^+,K^+\)-ATPase activity in mouse brain. The response is biphasic, i.e., stimulation at low concentration and inhibition at higher concentration (Desai and Ho, 1976, 1977). During alloxan diabetes, the lack of insulin may decrease the transport of tyrosine and tryptophan from blood to brain (Tagliamonte et al., 1975, De Montis et al., 1977, 1978; Fernando et al., 1976), which might result in the lowering of the activities of brain tyrosine hydroxylase and tyrosine decarboxylase (Shohmori et al., 1979), the enzymes necessary for catecholamine synthesis, thereby decreasing the catecholamine levels in brain. A 4- to 6-fold increase in the 12,000 × g pellet Na\(^+,K^+\)-ATPase activity (Tables 2 and 3) on day 8 after the onset of diabetes was found in the cerebellum and brain stem and in microsomal Na\(^+,K^+\)-ATPase from the brain stem only. These changes may be a consequence of the decreased catecholamine levels. The monoamine oxidase activity of rat

### Table 5. Activities of Mg\(^{2+}\)-ATPase in cerebellum

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>12,000 × g pellet (myelin, synaptosomes and mitochondria)</th>
<th>Supernatant fraction (microsomal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Diabetic Diabetic + insulin</td>
<td>Diabetic Diabetic + insulin</td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ± 0.20</td>
<td>0.70 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>0.14 ± 0.04</td>
<td>0.37 ± 0.10</td>
</tr>
<tr>
<td>8</td>
<td>0.64 ± 0.09</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>15</td>
<td>0.50 ± 0.14</td>
<td>0.40 ± 0.20</td>
</tr>
<tr>
<td>22</td>
<td>1.17 ± 0.30</td>
<td>0.80 ± 0.35</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P\(_i\) liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

* p < 0.05 (Student's t test).

### Table 6. Activities of Mg\(^{2+}\)-ATPase in brain stem

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>12,000 × g pellet (myelin, synaptosomes and mitochondria)</th>
<th>Supernatant fraction (microsomal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Diabetic Diabetic + insulin</td>
<td>Diabetic Diabetic + insulin</td>
</tr>
<tr>
<td>Control</td>
<td>0.52 ± 0.21</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>3</td>
<td>0.10 ± 0.03</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>0.53 ± 0.10</td>
<td>0.27 ± 0.07</td>
</tr>
<tr>
<td>15</td>
<td>0.50 ± 0.20</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>22</td>
<td>0.13 ± 0.10</td>
<td>0.66 ± 0.03</td>
</tr>
</tbody>
</table>

Activities are expressed as μmol P\(_i\) liberated/mg protein/min. Each value is the mean ± SEM of four or more experiments.

* p < 0.002; *p < 0.01; *p < 0.02 (Student’s t test).
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TABLE 7. Blood glucose values of control and diabetic rats

<table>
<thead>
<tr>
<th>Days after insulin withdrawal</th>
<th>Diabetic</th>
<th>Diabetic + insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.70 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28.5 ± 1.9</td>
<td>16.3 ± 1.3</td>
</tr>
<tr>
<td>8</td>
<td>26.6 ± 1.0</td>
<td>18.8 ± 1.3</td>
</tr>
<tr>
<td>15</td>
<td>28.7 ± 1.5</td>
<td>18.0 ± 1.1</td>
</tr>
<tr>
<td>22</td>
<td>18.7 ± 2.2</td>
<td>17.7 ± 1.2</td>
</tr>
</tbody>
</table>

Blood glucose values are expressed as mmol/L. Each value is a mean ± SEM of four or more experiments.

brain during alloxan diabetes and insulin deficiency was influenced by a decreased level of catecholamines (Mayanil et al., 1982). Another possible explanation for such an observation is that ATP content of the brain increases, thus conferring to an increase in the substrate availability during alloxan diabetes, when the blood glucose level is higher (Lewis et al., 1974).

The decrease in the Na⁺,K⁺-ATPase activity with the progress of diabetes cannot be attributed to the decrease in the substrate availability, because the ATP levels cannot fall until the blood glucose concentration falls to less than 1 μmol/g (Hinzen and Muller, 1971). Thus the plausible explanation for the observed decrease in the Na⁺,K⁺-ATPase with the progress of diabetes in the present experiment may be attributed to (a) increased availability of extraneuronally synthesized catecholamines, the levels of which are higher in long-term diabetic subjects (Christensen, 1979); (b) the increase in the catecholamine levels because of a reduced uptake of catecholamines into the presynaptic terminals (Logan and O’Donovan, 1980a).

Physiological concentrations of norepinephrine do not affect the activity of ouabain-insensitive Mg²⁺-dependent ATPase (Jariashvilli and Kometiani, 1975; Kometiani and Jariashvilli, 1975). However, Mg²⁺-ATPase plays an important role in the release of norepinephrine (Wyllie and Gilbert, 1979). The observed increase in the Mg²⁺-ATPase activity in our present experiment may be due to the lowering of catecholamine levels (Yoshimura, 1973; Schaefer et al., 1974; Godfraind et al., 1974; Desai and Ho, 1976). The decrease in Mg²⁺-ATPase activity with duration may be due to the inhibition of the enzyme by an increase in the release of norepinephrine (Wyllie and Gilbert, 1979), the levels of which are higher in long-term diabetes (Christensen, 1979).

Insulin administration seems to reverse the effect of alloxan diabetes on the activities of the enzymes Na⁺,K⁺-ATPase and Mg²⁺-ATPase, bringing them to near control values. Changes in ATP levels cannot be attributed to the effect, because only pronounced hypoglycemia causes a decrease in ATP levels (Goldberg et al., 1966; Hinzen and Muller, 1971; Lewis et al., 1974). However, we have shown that insulin-induced pronounced hypoglycemia decreases the ATPase activity (Mayanil et al., unpublished data).

Thus, the possible explanation, in light of our present experimental findings, can be attributed to a decrease in the concentration of free fatty acids (Lewis et al., 1973; Hall et al., 1979) with a consequent increase in total serum tryptophan levels (De Montis et al., 1978), hence the entry of aromatic amino acids into the brain from blood (Fernando et al., 1976), supplementing the precursor pool for catecholamine synthesis. Shohmori et al. (1979) have reported that insulin not only elevates tyrosine hydroxylase activity but also promotes decarboxylation of tyrosine in brain. Elevated tyrosine hydroxylase may increase catecholamine synthesis, thereby adjusting the catecholamine levels to the physiological concentrations and also the Na⁺,K⁺-ATPase activity to near control levels. This reversal of the Na⁺,K⁺-ATPase and Mg²⁺-ATPase activities with insulin administration is in good agreement with the finding of Kometiani et al. (1978), wherein the effect of norepinephrine on Na⁺,K⁺-ATPase is reversible.

Acknowledgments: C.S.K.M. and S.M.I.K. thank the Jawaharlal Nehru University for providing financial help in the form of Junior Research Fellowships. N.Z.B. is a Homi Bhabha Fellow.

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SHORT COMMUNICATIONS

Clorgyline and deprenyl insensitive monoamine oxidase in rat brain soluble fraction

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Monoamine oxidase, MAO (monoamine:O2 oxidoreductase, EC 1.4.3.4) has been widely studied and characterized in different tissues from several species and the results obtained have been interpreted as indices for the existence of multiple forms of the enzyme [1, 2]. The original report by Johnston [3], demonstrated that the two forms of MAO could be differentiated on the basis of their sensitivity to inhibition by clorgyline.

The mitochondrial sonicate was prepared according to the method of Fowler et al. [6] and the 'high speed supernatant' fraction was further purified by gel filtration on Sephadex G-25 to yield an enzyme preparation of activity 2.30 units/mg protein. The supernatant fraction S2 (1.08 units/mg protein), microsomal fraction P3 (0.75 units/mg protein), partially purified cell soluble fraction S1 (0.88 units/mg protein), the mitochondrial membrane vesicles M (1.17 units/mg protein) and the 'high speed supernatant' (2.30 units/mg protein) were used for MAO assay according to the method of Catravas et al. [7].

For in vitro studies, clorgyline was added to the assay mixture at the final concns of 10⁻⁸ to 10⁻¹⁰ M and was preincubated with the enzyme preparation for 30 min before the addition of substrate kynuramine to allow for the irreversible inhibition of MAO-A [8]. For in vivo studies, rats were given an intramuscular injection of both clorgyline and deprenyl (12 mg/kg body wt) 2 hr before sacrifice to achieve maximum inhibition of MAO-A and MAO-B. The control rats were injected with 0.9% saline. Subcellular fractionation of the brains was then carried out as described above and assayed for MAO activity.

One unit of enzyme activity is defined as 1 μmole 4-hydroxyquinoline formed/90 min/g of tissue. Protein was estimated by the method of Lowry et al. [9].

Clorgyline, (1-methyl-N-propargyl-5-[2,4-dichlorophenoxyl]propylamine hydrochloride, M and B 9302) was kindly provided by Dr. R. A. Robinson of May & Baker, Dagenham, U.K. (−)-Deprinyl (phenyl-isopropyymethyl propionylamine hydrochloride, E-250) was a gift from Dr.

Fig. 1. (a) Percentage inhibition of MAO activity towards kynuramine by various concns of clorgyline. (C) The pure mitochondrial and (Δ) 'high speed supernatant' fractions obtained from the sonicated mitochondrial fraction were preincubated at 37° for 30 min in the presence of different concns of clorgyline in a total vol. of 3 ml prior to addition of kynuramine. MAO activity is expressed as μmole/g/90 min. Each point is the mean ± S.D. of three determinations. (b) Percentage inhibition of MAO activity towards kynuramine by various concns of clorgyline. (C) The supernatant fraction, S2, (Δ) the soluble fraction, S3, and (□) the microsomal fraction, P3, were preincubated at 37° for 30 min in the presence of different concns of clorgyline in a total vol. of 3 ml prior to addition of kynuramine. MAO activity is expressed as μmole/g/90 min. Each point is the mean ± S.D. of three determinations.
The inhibition of MAO by clorgyline in the mitochondrial and supernatant fraction, $S_2$, was biphasic when kynuramine was used as a substrate (Figs. 1a and 1b). The MAO activity from the mitochondrial fraction, $M$, was inhibited by 98%. If the supernatant fraction, $S_2$, were to have both A and B forms of MAO then inhibition similar to that in the mitochondrial preparation would be expected, but the inhibition was only 22%. This observation leads us to believe that the $S_2$ fraction, apart from having the clorgyline-sensitive MAO activity, which shows the usual biphasic dose-response to increasing concen of the inhibitor, may also contain a clorgyline-insensitive form of MAO.

The presence of the form of MAO in the supernatant fraction, $S_2$, which is sensitive to clorgyline inhibition was earlier reported by Student and Edwards [5], who in their in vitro studies showed that MAO-A and MAO-B activities were 3% and 3.5%, respectively, of the total MAO activity in $S_2$. The recovery of the total MAO activity in our experiments from the mitochondrial fraction, $M$, is found to be approx. 35%, which is almost consistent with the earlier reports of approx. 30.5% [5]. The figures for the supernatant fraction, $S_2$, reported by Student and Edwards, including forms A and B was 6.5%, compared with the recovery of supernatant MAO activity of 24–30% in our studies (including forms A and B and an inhibitor-insensitive enzyme).

To find out the nature of this MAO activity present in the supernatant fraction, $S_2$, which is insensitive to clorgyline inhibition, the in vitro inhibition by clorgyline studied in the 'high speed supernatant' obtained from the sonicated mitochondrial fraction gave the same biphasic dose-response kinetics as the non-sonicated mitochondrial fraction. However, the soluble fraction, $S_3$, did not show the same biphasic curve as that of the parent supernatant fraction, $S_2$. The activity was found to be localized in the microsomal pellet fraction, $P_3$, which showed the usual biphasic patterns (Figs. 1a and 1b). The inhibition was nearly 100% in the microsomal fraction and no inhibition was observed in the soluble fraction, $S_3$. These results, therefore, show the presence of a new form of MAO localized in the soluble fraction, which is insensitive to clorgyline inhibition.

The results from the in vivo studies showed that the MAO activity in the mitochondrial preparation, $M$, which is believed to have A and B forms of MAO, was inhibited by 91% while the inhibition was about 30% in the supernatant fraction, $S_2$, by clorgyline. This confirms our in vitro finding, where the usual biphasic dose-response curve was obtained, but the inhibition was not 100% (Table 1). The MAO activity was inhibited 98% in the 'high speed supernatant' obtained from the mitochondrial fraction, while it remained unaffected in the soluble fraction, $S_3$. The enzyme activity in the microsomal fraction, however, showed an inhibition of 94% as observed during our in vitro experiments.

It may be speculated that the ineffectiveness of some of the antidepressant drugs, which act by inhibiting the monoamine oxidase, may be attributed to the fact that these inhibitors of MAO-A and MAO-B may not be inhibiting the enzyme totally, i.e. both the mitochondrial and the soluble forms, thereby causing a partial effect by inhibiting only the mitochondrial forms; or, that the process of inhibition may be mediated through specific drug receptors present on the particulate fractions of the cell, the drug being unable to inhibit the enzyme in the soluble fraction.

Evidence is presented to show that the soluble fraction of the rat brain contains a MAO form which is different from the mitochondrial and the soluble forms, thereby causing a partial effect by inhibiting only the mitochondrial forms; or, that the process of inhibition may be mediated through specific drug receptors present on the particulate fractions of the cell, the drug being unable to inhibit the enzyme in the soluble fraction.

In summary, a substantial percentage of monoamine oxidase activity was found in the soluble fraction of rat brain homogenates. The enzyme was not inhibited in vivo by high doses of clorgyline and deprenyl (12 mg/kg for 2 hr). Monoamine oxidase activity associated with the soluble fraction did not show the usual biphasic dose-response kinetics with clorgyline when kynuramine was used as the substrate. The present data show that there may be a new form of monoamine oxidase associated with the soluble fraction of the cell which has properties different from the two well-known monoamine oxidases A and B.
Regulation of brain and hepatic glutathione-S-transferase by sex hormones in rats

(Received 23 February 1982; accepted 7 July 1982)

The presence of glutathione-S-transferase (GST)* activity in mammalian and avian brains and its characterization in rat brain has been reported by us recently [1, 2]. Brain and hepatic GSTs were found to catalyze the conjugation of acrylamide with GSH [3] and to bind this potent neurotoxin [4]. The enzyme may therefore play a biological role in removal and expression of the toxicity of certain neurotoxic xenobiotics.

The brain regulates the secretion of hormones in pre- and post-pubertal animals. Recent studies by Lamartiniere [5] have shown that the hypothalamic–hypophyseal–gonadal axis plays an important role in the sexual differentiation and regulates the hepatic GST activity in the rat. Evidence has been presented to show that the hypothalamic nerve endings of female rats exposed to androgen during the critical period of early development result in the programming of a male type of metabolism that is expressed post-pubertally via the hypothalamic–hypophyseal–gonadal axis [6–10]. Therefore it would be of potential interest to investigate whether brain GST is regulated by these sex hormones. The present study deals with some of our observations on the regulation of brain and hepatic GSTs by sex hormones.

Material and methods

The experiments were performed on young and post-pubertal Wistar albino rats derived from the ITRC animal breeding colony. The animals were raised on a commercial pellet diet (Hindustan Lever, Bombay, India) and allowed free access to water. The litters were kept with their mothers until weaning, i.e. until 3 weeks of age (five pups with one mother per cage). After weaning five animals were housed in a cage.

Post-pubertal male rats (12 weeks old) were castrated by removing both the testicles after anaesthetising the animals with ether. Anaesthetic ether was found to produce no significant change in GST activity of the brain and liver.

Castration or sham operations in neonatal (1-day-old) male rats were performed by placing them in an ice-water bath prior to surgery. TP and DES [each from Steroid Inc. (Wilton, NH)] were dissolved in peanut oil and injected at a dose of 2.5 mg/kg body weight subcutaneously in the adult rats daily for 7 days beginning 1 week after castration. The control rats received the same volume of vehicle. All the animals were killed 7 days after the last treatment. In the case of neonatal rats the pups were given TP and/or DES from day 7 to 13 of their life and killed by decapitation at 3 and 9 weeks of age.

The brain and liver homogenates were centrifuged at 14,000 and 9000 g for 15 min respectively to obtain postmitochondrial fractions which were used for the measurement of GST activity by the method of Habig et al. [11]. GSH and protein contents were estimated according to the methods of Ellman [12] and Lowry et al. [13] respectively.

Results

Effect of neonatal castration and influence of sex hormones on brain and hepatic GST activity in pre- and post-pubertal rats

At prepubertal (3 weeks) age, brain and hepatic GST activity towards CDNB did not show any significant difference between male and female rats (Fig. 1 and Table 1). The GST activity in both tissues remained unaffected on neonatal castration at prepubertal age but administration of TP and DES to castrated pups induced enzyme activity significant in comparison to uncastrated male or female animals (Fig. 1 and Table 1). No change in brain or hepatic GSH content was observed on castration or treatment of castrated rats with TP or DES (data not shown).

Fig. 2 demonstrates that brain GST activity towards CDNB of male rats at post-pubertal age (9 weeks) was significantly lower than that of females. Neonatal castration

* Abbreviations: GST, glutathione-S-transferase; GSH, glutathione; TP, testosterone propionate; DES, diethylstilbestrol; CDNB, 1-chloro-2,4-dinitrobenzene.
MECHANISM OF THE INVOLVEMENT OF MONOAMINE OXIDASE IN THE REGULATION OF (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase IN RAT BRAIN

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Key words: Monoamine oxidase; (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase; Dopamine; (Rat Brain)

Increasing concentrations of dopamine fail to give a biphasic response to (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity in various subcellular fractions of rat brain preincubated with monoamine oxidase inhibitors, viz. \(1 \times 10^{-4}\) M \textit{p}-chloroglyline and \(1 \times 10^{-4}\) M deprenyl. The product of the monoamine-oxidase-catalysed reaction with dopamine as substrate is 3-methoxy-4-hydroxyphenylacetaldehyde. An analogue of this product is 3-methoxy-4-hydroxybenzaldehyde. This analogue, when incubated with the subcellular fractions which had been preincubated with monoamine oxidase inhibitors and dopamine, gave a more pronounced biphasic response to (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity than that observed in the fractions incubated with dopamine alone.

Introduction

Monoamine oxidase (amine: oxygen oxidoreductase (deaminating), EC 1.4.3.4) is a mitochondrial enzyme responsible for the oxidative deamination of a variety of biogenic amines [1]. (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase (Mg\textsuperscript{2+} dependent ATP phosphohydrolase, EC 3.6.1.3) plays a crucial role in the neuronal function. The enzyme is thought to be responsible for the maintenance and re-establishment of a cation gradient across the neuronal membrane, which is essential for the impulse propagation [2]. Nerve-terminal (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase is important in the release or uptake mechanism of neurotransmitters [3–6]. There is evidence that changes in (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity will affect several physiological processes and that a cellular mechanism must exist which can closely regulate (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity [7].

Catecholamines are reported to influence (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity in brain in a biphasic manner, i.e., stimulation at low concentrations and inhibition at high concentrations [8,9]. Since catecholamines are direct substrates of monoamine oxidase [10], the role of this enzyme in the regulation of (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase has to be considered. Moreover, inhibition of monoamine oxidases \(A\) and \(B\) by \textit{p}-chloroglyline and deprenyl also affect the uptake of dopamine, noradrenaline and serotonin by rat-brain synaptosomal fraction [11]. Hence this study was initiated to find out whether the catecholamine-dependent (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity was regulated by catecholamine directly or by catecholamine-derived aldehydes prior to monoamine oxidase activity.

Materials and Methods

4-Hydroxyquinoline, kynuramine dihydrobromide, ATP, ouabain, Tris base, dopamine and EGTA were from Sigma, U.S.A. \textit{p}-Chloroglyline, \(9302\) were kindly provided by Dr R.A. Robin...
Deprenyl was a gift from Dr K. Magyar, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary. 3-Methoxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde were gifts from the organic Chemistry Laboratory, Indian Institute of Technology, New Delhi, India. All other chemicals were from BDH and were of analytical grade.

Wistar strain Albino rats weighing between 250 and 300 g were used for all the experiments. Whole-brain homogenates (1:10) were prepared as described earlier [12]. The subcellular fractionation was performed essentially according to the method of Student and Edwards [13]. The specific activities (mol P$_i$ formed/mg protein per min) in various subcellular fractions are shown in Table III.

Monoamine oxidase was assayed according to the method of Catravas et al. [14]. For in vitro studies, 1·10$^{-4}$ M chloroglyline and 1·10$^{-4}$ M deprenyl were preincubated with the enzyme preparations prior to addition of kynuramine to allow for the irreversible inhibition of monoamine oxidase [10]. One unit of enzyme activity is defined as 1 μmol 4-hydroxyquinoline produced per 90 min per g tissue at 37°C. (Na$^+$ + K$^+$)-ATPase activity was assayed according to the method of Zaheer et al., as described earlier [15,16]. One unit of enzyme activity is expressed as 1 μmol inorganic phosphate liberated per min per g tissue at 37°C. Inorganic phosphate was estimated according to the method of Fiske and SubbaRow [17]. Protein estimation was performed according to the method of Lowry et al. [18], taking bovine serum albumin as standard.

Results

Whole-brain homogenates, when incubated with increasing concentrations of dopamine, gave a biphasic response to (Na$^+$ + K$^+$)-ATPase activity (Fig. 1), which is in good agreement with previous reports [8]. To ascertain whether the (Na$^+$ + K$^+$)-ATPase activity is influenced directly by dopamine or is mediated via monoamine oxidase activity, the (Na$^+$ + K$^+$)-ATPase was assayed in the whole homogenate and in the 12000 × g pellet, which were preincubated with chloroglyline and deprenyl (monoamine oxidase A and B inhibitors, respectively), this being followed by dopamine incubation. The magnitude of the biphasic response was found to be decreased in the whole homogenate, which may be attributed to the chloroglyline.
TABLE I
EFFECT OF DOPAMINE ON (Na⁺ + K⁺)-ATPase ACTIVITY OF VARIOUS SUBCELLULAR FRACTIONS OF RAT BRAIN

(A) Subcellular fractions were incubated with 10⁻⁴ M dopamine; (B) subcellular fractions were first incubated with 1·10⁻⁴ M clorgyline + 1·10⁻⁴ M deprenyl and then with 1·10⁻⁴ M dopamine, and (Na⁺ + K⁺)-ATPase activity was assayed [16]. All incubations were carried out at 4°C for 30 min. Each value is a percentage (mean ± S.E.) of three determinations taking control as 100%. P (probability) was calculated for treatment A from Student’s t-test for paired variables. (n.s., not significant). Values for treatment B were not significantly different from control values.

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>(Na⁺ + K⁺)-ATPase activity</th>
<th>A</th>
<th>P (t)</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>12000 × g pellet</td>
<td>166.7 ± 35.1</td>
<td>&lt; 0.01</td>
<td>106.7 ± 24.9</td>
<td></td>
</tr>
<tr>
<td>12000 × g supernatant</td>
<td>181.3 ± 40.9</td>
<td>&lt; 0.02</td>
<td>114.0 ± 26.3</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>117.0 ± 24.9</td>
<td>n.s.</td>
<td>86.3 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>Synaptosomes</td>
<td>171.1 ± 20.5</td>
<td>&lt; 0.05</td>
<td>106.7 ± 14.6</td>
<td></td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>140.4 ± 29.2</td>
<td>&lt; 0.05</td>
<td>100.9 ± 20.5</td>
<td></td>
</tr>
<tr>
<td>Cytosol</td>
<td>119.9 ± 55.6</td>
<td>n.s.</td>
<td>109.6 ± 10.2</td>
<td></td>
</tr>
</tbody>
</table>

clorgyline- and deprenyl-insensitive monoamine oxidase in the soluble fraction [19]. As the particulate monoamine oxidase activity was completely inhibited, the increasing concentrations of dopamine did not elicit the same response in the 12000 × g pellet preincubated with the monoamine oxidase inhibitors. These results led us to believe that dopamine may elicit its response on (Na⁺ + K⁺)-ATPase not directly, but via monoamine oxidase activity, and that the modulator might be either the substrate or the product of the monoamine oxidase-catalysed reaction.

3-Methoxy-4-hydroxybenzaldehyde, a structural analogue of 3-methoxy-4-hydroxyphenylacetaldehyde (the product of the monoamine oxidase-catalysed reaction), was incubated with the 12000 × g pellet. It was observed that methoxyhydroxybenzaldehyde gave a strikingly pronounced biphasic response as compared to that obtained when dopamine was incubated (Fig. 2).

TABLE II
EFFECT OF 3-METHOXY-4-HYDROXYBENZALDEHYDE ON (Na⁺ + K⁺)-ATPase ACTIVITY OF VARIOUS SUBCELLULAR FRACTIONS OF RAT BRAIN

Fractions were incubated with 2.6 μM (treatment A) or 26 μM (treatment B) methoxyhydroxybenzaldehyde at 4°C for 30 min and then (Na⁺ + K⁺)-ATPase activity was assayed [16]. The probability of differences between means and controls being significant was assessed by Student’s t-test for paired variables. Each value is a percentage (mean ± S.E.) of three determinations, taking control as 100%. n.s., not significant.

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>(Na⁺ + K⁺)-ATPase activity</th>
<th>A</th>
<th>P</th>
<th>B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>12000 × g pellet</td>
<td>200 ± 20.2</td>
<td>&lt; 0.01</td>
<td>84.1 ± 10.1</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>12000 × g supernatant</td>
<td>175.4 ± 14.5</td>
<td>&lt; 0.02</td>
<td>60.9 ± 10.1</td>
<td>&lt; 0.002</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>95.7 ± 9.3</td>
<td>n.s.</td>
<td>81.2 ± 10.1</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Synaptosomes</td>
<td>124.6 ± 4.3</td>
<td>&lt; 0.05</td>
<td>66.7 ± 8.7</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>120.1 ± 10.1</td>
<td>&lt; 0.05</td>
<td>50.1 ± 10.1</td>
<td>&lt; 0.002</td>
<td></td>
</tr>
<tr>
<td>Cytosol</td>
<td>102.0 ± 8.1</td>
<td>n.s.</td>
<td>81.7 ± 5.8</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>
(Na⁺ + K⁺)-ATPase and Monoamine Oxidase Activities in Various Subcellular Fractions of Rat Brain

(Na⁺ + K⁺)-ATPase was assayed as described earlier [16]. Monoamine oxidase was assayed as described earlier [14]. In the case of monoamine oxidase, subcellular fractions were incubated with 1.10⁻⁴ M deprenyl at 4°C for 30 min and then the monoamine oxidase activities were assayed using kynurenine as substrate. Each value is a mean ± S.E. of six determinations.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>(Na⁺ + K⁺)-ATPase</th>
<th>Monoamine oxidase (μmol/g per 90 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol P_i/g per min</td>
<td>μmol P_i/mg protein per min</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>-</td>
<td>Control 10⁻⁴ M chloroglyline / deprenyl</td>
</tr>
<tr>
<td>12000 × g pellet</td>
<td>4.70 ± 0.14</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>12000 × g supernatant</td>
<td>1.61 ± 0.40</td>
<td>0.03 ± 0.001</td>
</tr>
<tr>
<td>Mitochondrial fraction</td>
<td>0.61 ± 0.18</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Synaptosomes</td>
<td>3.60 ± 0.04</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>2.95 ± 0.05</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Cell-soluble fraction</td>
<td>1.63 ± 0.06</td>
<td>0.04 ± 0.005</td>
</tr>
</tbody>
</table>

been incubated with increasing concentrations of aldehyde alone (no preincubation with monoamine oxidase inhibitors). Similar effects were obtained with different subcellular fractions (Tables I and II). Control monoamine oxidase activities and the activities in the various subcellular fractions after chloroglyline and deprenyl incubation are presented in Table III. These findings confirm that dopamine elicits its response on (Na⁺ + K⁺)-ATPase activity via a monoamine oxidase-catalysed reaction.

To elucidate whether the hydroxyl group at position 4 of 3-methoxy-4-hydroxybenzaldehyde contributes at all to the biphasic response of (Na⁺ + K⁺)-ATPase activity, an aldehyde without -OH at position 4, i.e., methoxybenzaldehyde, was incubated with the 12000 × g pellet. It was observed that 3-methoxybenzaldehyde did not give a biphasic response to (Na⁺ + K⁺)-ATPase activity. Similarly, when monoamine oxidase activity was inhibited, 3-methoxybenzaldehyde did not give a biphasic response as had been seen in the previous case (Fig. 2). It was thus concluded that the hydroxyl group at position 4 of the aromatic ring of the aldehyde is necessary for the biphasic response; (ii) that dopamine cannot directly affect the (Na⁺ + K⁺)-ATPase; (iii) that the dopamine-derived aldehyde is responsible for the biphasic response of (Na⁺ + K⁺)-ATPase activity.

The possibility of the acid and the alcohol metabolites of the aldehyde participating in the biphasic response is ruled out, as there was no detectable aldehyde dehydrogenase, oxidase or reductase activity when monitored with aldehydes, viz. methoxyhydroxybenzaldehyde and methoxybenzaldehyde as substrate and without the coenzymes.

Previous reports [11] have shown that chloroglyline and deprenyl inhibit the uptake of catecholamine in the synaptosomes, which can be compared to the high-affinity uptake of catecholamine involving the synaptosomal (Na⁺ + K⁺)-ATPase and ATP breakdown [6]. These observations lead to the assumption that the uptake might be dependent on the oxidatively deaminated product of catecholamine, which in turn has a modulatory effect on (Na⁺ + K⁺)-ATPase. Moreover, monoamine oxidase is not an uptake enzyme and hence should not cause an inhibition in the uptake of catecholamine after its inhibition by chloroglyline and deprenyl.

Thus, one of the roles of monoamine oxidase in

**Discussion**

In the light of our experimental findings, three important observations emerge: (i) that the presence of hydroxyl group at position 4 of the aromatic ring of the aldehyde is necessary for the biphasic response; (ii) that dopamine cannot directly affect the (Na⁺ + K⁺)-ATPase; (iii) that the dopamine-derived aldehyde is responsible for the biphasic response of (Na⁺ + K⁺)-ATPase activity.
the uptake of catecholamine may be that the en-
zyme interacts with the amine to form its aldehyde
derivative, which in turn causes an activation of the (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity resulting in the amine uptake. That very low aldehyde concentrations in vitro are required to increase the (Na<sup>+</sup> + K<sup>+</sup>)-ATPase may affirm that at the initial stages of the amine incubation, monoamine oxidase oxidatively deaminates a few molecules of amine to form aldehyde, which increases the (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity and the consequent uptake of the catecholamine.

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References

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