# Evaluation of Carbonic Anhydrase (CA) and Monoamine Oxidase (MAO) Enzyme Activations in Organs and Blood of Goat *(Capra aegagrus hircus)*

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**Summary:** The purpose of the present study was to compare values of activation and inhibition monoamine oxidase (MO) and carbonic anhydrase (CA) in some organs and blood of male goat (*Capra aegagrus hircus).* MAO and CA activations were determined in cerebrum, cerebellum, medulla spinalis, heart, liver, eye homogenised and blood by chemical methods. MAO A and B levels were found highest in thalamus and hypothalamus than other tissues of cerebrum ($P<0.01$). CA concentrations were found less than that of MAO A and B acivations ($P< 0.01$). Inhibitor pargyline was significantly decreased both activations MAO A and B fractions in cerebrum (thalamus, hypothalamus), cerebellum, medulla spinalis, heart (atriculus-MAO B, ventriculus-MAO A, mitral valvula-MAO B), liver, eye (humor vitreus, humor aqueous, retina, nervous opticus) and blood ($P<0.01$). The results support previous evidence suggesting that platelet MAO activity is a useful biochemical measure for the cerebrum, medulla spinalis, heart, liver, eye and blood.

## Introduction

Mammalian monoamine oxidase (MAO, EC 1.4.3.4) is an integral flavin-containing enzyme of the outer mitochondrial membranes of neuronal, glial, and other cells, which is responsible for regulation and metabolism of major monoamine neurotransmitters such as serotonin (5-OH tryptamine), adrenaline, *nor*-adrenaline and dopamine. Monoamine oxidase (MAO) is an enzyme that oxidizes various physiologically and pathologically important monoamine neurotransmitters and hormones such as dopamine, *nor*-adrenaline, adrenaline, and serotonin. Two types of MAO, *i.e.* type A (MAO-A) and type B (MAO-B) were first discovered pharmacologically. MAO-A is inhibited by clorgyline and MAO-B by deprenyl. cDNAs MAO-A and MAO-B were cloned for structure determination. MAO-A and MAO-B are made of similar but different polypeptides and encoded by different nuclear genes located on the X chromosome (Xp11.23). MAO-A and MAO-B genes consist of 15 exons with identical intron-exon organization, suggesting that they were derived from a common ancestral gene. Both enzymes require a flavin cofactor, flavin adenine dinucleotide (FAD), which binds to the cysteine residue of a pentapeptide sequence (Ser-Gly-Gly-Cys-Tyr). Both enzymes exist on the outer membrane of mitochondria of various types of cells in various tissues including the brain. In humans, MAO-A is abundant in the brain and liver, whereas the liver, lungs and intestine are rich in MAO-B. MAO-A oxidizes nor-adrenaline and serotonin and MAO-B mainly β-phenylethylamine. In the human brain, MAO-A exists in catecholaminergic neurons but MAO-B is found in serotonergic neurons and glial cells. MAO-A knockout mice exhibit increased serotonin levels and aggressive behavior, whereas MAO-B knockout mice show little behavioral change[1-16]. The gene knockout mice of MAO-A or MAO-B together with the observation that some humans lack MAO-A and MAO-B or both have contributed to our understanding of the function of MAO-A and MAO-B in health and disease. MAO-A and MAO-B may be closely related to various neuropsychiatric disorders such as depression and Parkinson's disease, and inhibitors of them are the subject of drug development for such diseases. It is found in two different forms designated as MAO-A and MAO-B (with molecular weights of 59,700 and 58,800, respectively) which are encoded by two different genes and distinguished by different substrate specificities and sensivities to the selective inhibitors. MAO-A was suggested to be inhibited by *e.g.* clorgyline and prefers serotonin and *nor*-epinephrine as substrates, whereas MAO-B is inhibited by *e.g.*

pargyline and prefers dopamine and benzylamine as substrates. Although both forms of the enzyme are present in the liver and brain of mammals; it was suggested that only the B-form is present in human platelets and only the A-form is present in placenta. It is now well established that oxidation of substrates by monoamine oxidases is coupled to the reduction of flavin adenine dinucleotide (FAD) cofactor and gives an imine as the product which is hydrolysed spontaneously to yield the corresponding aldehyde and ammonia. The reaction catalyzed by monoamine oxidase enzymes for most substrates can be summarized as follows [1,5,8,9,10,14,15]:

$$RCH_2NR_1R_2 + H_2O \longrightarrow RCHO + NHR_1R_2 + H_2O_2$$

Monoamine oxidases (MAO B and MAO A) are well-known targets for antidepressant drugs and for drugs used to treat neurological disorders and diseases of aging, such as Parkinson's disease and Alzheimers disease. MAO A and MAO B are attached to the outer membrane of the mitochondria and function to oxidize amine neurotransmitters such as dopamine and serotonin[5,8,13].

Pharmacologists have designed a number of drugs, both reversible and irreversible, that inhibit MAO B and are used to treat neurological disorders. Recent studies have also demonstrated that MAO B is inhibited by compounds present in tobacco smoke, which may contribute to the addictive properties of tobacco use. Scientific and clinical interest in these enzymes has been ongoing for more that 40 years and has resulted in more than 15,000 papers published on their biological properties. MAO B has been shown to be elevated more than three fold in the brain tissue of elderly individuals. Recent studies have shown that elevated levels of MAO B in neurons and kidney cells can lead to cell death (apoptosis). Clinical trials currently are underway in several centers to target increased levels of MAO B that have been identified in astrocytes (a type of brain cell) in Alzheimer's patients. Since understanding of the interactions of the two MAO isoforms with their preferred substrates and inhibitors will help to supply some basic information related to the substrate binding and inhibitory properties of the isoforms.In the present study the activations and inhibition of MAO isoforms purified from rat barain, medulla spinalis an deye tissues mitochondria and rat platelets were investigated [3,5,9,10,11,12, 13,14].

Among the 14 human isozymes of carbonic anhydrase (CA, EC 4.2.1.1) presently known, the cytosolic hCA II is the most active and plays a host of physiological functions, whereas the mitochondrial hCA V is unique due to its role in several biosynthetic reactions. An inhibition study of these isozymes with a series of sulfonamides is reported here, with the scope to detect lead molecules for the design of isozyme-specific CA inhibitors (CAIs) targeting the mitochondrial isoform. Indeed, recently it has been shown that CA V is a novel target for the drug design of anti-obesity agents among others. Carbonic anhydrases are widespread in nature, being found in animals, plants, and certain bacteria. In animals they play an important role in respiration by facilitating transport of $CO_2$ and are involved in the transfer and accumulation of $H^+$ and $HCO^{3-}$. Mammalian carbonic anhydrases are of several forms differing in enzymatic properties, amino acid sequences and inhibitor binding. In erythrocytes there is usually present both a high activity and a low activity form. The pH-rate profiles and pH-binding curves indicate that the same group with pKa of approximately seven is involved in both forms. All the isoenzymes have a molecular weight of approximately 30,000 a.m.u and contain one zinc atom per molecule. Although some authors report six carbonic anhydrases in horse red blood cells, most interest has been focused on high and low activity types. Bovine erythrocytes contain two electrophoretically separable forms designated A and B in order of mobility [17-51]. Both have a high order of activity similar to the human variant "C".

Carbonic anhydrases catalyze the reaction (33,45,49,50).

$$CO_2 + H_2O \rightarrow H_2CO_3$$

The outstanding characteristic of carbonic anhydrase is its very high turnover number. Zinc may be replaced by cobalt, In a study found that the cobalt-substituted enzyme is dependent upon the state of oxidation of the metal and indicate the activity is related to the ionization of a group close to the zinc. Carbonic anhydrase (CA; EC 4.2.1.1) is a zinc enzyme that is widely distributed in the living world and is involved in many biochemical processes that depend on the hydration/dehydration of carbon dioxide/bicarbonate [21-37].

This study was carried out for determination of MAO A and B and CA activations in goat

**Results and Discussion**

Values of MAO A and B and CA enzymes were given in Table 1. Quantities of these enzymes in

Table 1. Activations and inhibitions values of MAO and CA in some tissues.

| Organ | Tissue | Activity of MAO- A nmol/ mg protein for tissue homogenates | Inhibition (%) of MAO- A by Pargyline | Activity of MAO- B nmol/ mg protein for tissue homogenates | Inhibition (%) of MAO- B by Pargyline | Activity of C A (Unit(s) /day) | Inhibition (%) of CA by acetazolamide |
|---|---|---|---|---|---|---|---|
| | Control* | 62±1.19[a]** | - | 59±3.32[a] | - | 58±0.91[d] | - |
| **Cerebellum** | Lobus frontalis | 157±1.02[b] | 31±2.18[a] | 191±0.18[c] | 42±4.03[a] | 25±3.01[b] | 46±3.76[c] |
| | Lobus temporalis | 152±3.27[b] | 38±3.16[b] | 193±3.27[c] | 41±2.01[a] | 28±1.00[b] | 56±4.59[c] |
| | Lobus parietalis | 165±2.26[b] | 43±0.92[b] | 206±4.37[c] | 55±2.87[a] | 19±2.87[a] | 42±3.08[c] |
| | Lobus occipitalis | 285±4.21[c] | 56±5.01[b] | 334±5.20[e] | 61±3.28[a] | 22±2.44[b] | 63±5.34[c] |
| | Thalamus | 402±5.01[e] | 63±2.00[b] | 402±6.25[f] | 72±1.56[b] | 62±4.76[d] | 28±0.27[b] |
| | Hypothalamus | 389±1.04[d] | 71±2.62[d] | 411±2.76[f] | 85±5.01[b] | 73±5.27[d] | 32±2.55[b] |
| **Cerebellum** | Control | 54±0.09[a] | - | 186±4.52[b] | - | 28±2.54[b] | - |
| | Cerebellum | 217±0.18[c] | 66±1.10[c] | 335±1.17[e] | 78±3.35[b] | 43±1.20[c] | 48±5.28[b] |
| Medulla | Control | 88±2.53[b] | | 55±3.57[a] | | 9±0.93[a] | |
| Spinalis | Nervus cervicalis | 399±3.05[e] | 72±2.55[c] | 465±2.85[f] | 73± 2.01 | 11±0.24[a] | 29±1.19[b] |
| | Nervus thoracicus | 201±5.28[c] | 47±1.84[b] | 296±6.54[d] | 55±3.28[a] | 10±2.74[a] | 11±3.20[a] |
| | Nervus lumbalis | 309±2.98[c] | 65±2.60[c] | 341±5.66[e] | 75±3.65[b] | 28±3.56[b] | 17±2.11[a] |
| | Nervus sacralis | 152±5.52[b] | 39±3.28[b] | 153±0.27[b] | 45±3.10[a] | 14±2.09[a] | 12±0.98[a] |
| | Control | 39±0.48[a] | - | 57±0.98[a] | - | 6±0.26[a] | |
| | Cornea | 89±1.99[b] | 26±1.18[a] | 196±2.50[b] | 35±2.13[a] | 19± 0.17[a] | 27±0.28[b] |
| | Humor vitreus | 286±5.75[c] | 87±3.37[d] | 319±2.22[e] | 76±3.85[b] | 48±3.28[c] | 13±4.25[a] |
| **EYE** | Humor aqueous | 363±3.41[d] | 88±4.59[d] | 486±5.68[g] | 75±4.55[b] | 78±3.90[d] | 35±3.41[b] |
| | Lense | 54±2.36[a] | 27±2.03[a] | 98±1.10[b] | 35±2.76[a] | 12±1.07[a] | 12±0.23[a] |
| | Pupilla | 43±3.28[a] | 19±2.01[a] | 52±3.87[a] | 34±1.17[a] | 8±2.01[a] | 5±0.11[a] |
| | Retina | 367±5.78[d] | 78±3.65[d] | 497±5.96[g] | 86±3.44[b] | 97±2.25[e] | 65±4.52[c] |
| | Nervus opticus | 385±3.90[d] | 75±5.27[d] | 485±2.45[g] | 85±3.02[b] | 87±4.55[e] | 51±5.02[c] |
| **Liver** | Control | 59±3.12[a] | - | 55±3.26[a] | - | 66±4.21[d] | - |
| | Totally | 362±4.48[d] | 83±3.20[d] | 478±2.01[g] | 83±0.98[b] | 71±2.56[d] | 48±2.09[c] |
| | Control | 48±3.20[a] | - | 148±4.29[b] | - | 26±3.65[b] | - |
| **Heart** | Atriculus | 93±1.18[b] | 28±0.18[a] | 191±3.67[b] | 68±2.27[b] | 83±4.21[d] | 37±0.23[b] |
| | Ventriculus | 157±3.98[b] | 44±2.67 | 295±5.92[d] | 38±1.55[a] | 81±4.99[d] | 50±3.38[c] |
| | Mitral valvula | 55±1.09[a] | 14±1.05[a] | 479±3.65[g] | 44±2.22[a] | 82±0.98[d] | 35±2.88[b] |
| | Control | 41±2.65[a] | - | 61±0.23[a] | - | 10±0.93[a] | - |
| **Blood** | Platelets (nmol/$10^8$ platelets/h) | 56±1.01[a] | 48±203[b] | 64±0.14[a] | 35±0.18[a] | 16±2.01[a] | 78±3.59[d] |

* Control : Control experiments were conducted as total of organ or tissues.

** Values shown with different letters as vertical in the same column are significant ($p<0.01$) ; but values shown with same letter aren't significant ($p>0.05$).

51.

tissues in stres group have higher concentrations more than control ($P < 0.01$). MAO A and B were highest levels in thalamus and hypothalamus than other tissues of cerebrum ($P<0.01$).Total activities of both MAO and CA enzymes were found. in cerebrum, cerebellum, heart, liver and eye than control ($P<0.01$). However, CA concentartions were found less than that of MAO A and B acivations ($P<0.01$). Thalamus and hypothalamus are very important areas for anti-stress and entellectual functions of brain. Therefore, increasing of MAO A and B in thalamus and hypothalamus have vital importance. On the other hand, in *in vitro* tests, pargyline substance was significantly inhibited both MAO A and B fractions in cerebrum (thalamus, hypothalamus), cerebellum, medulla spinalis, heart (atriculus MAO B, ventriculus-MAO A, mitral valvula- MAO B), liver, eye (humor vitreus, humor aqueous, retina, nervus opticus) and blood ($P < 0.01$). That is, pargyline was significantly decreased MAO A and B activation in retina, humor vitreus, humor aqueous and nervus opticus ($P< 0.01$). This finding shown that MAO A and B activations in retina, humor vitreus, humor aqueous and nervus opticus have both chemical and psychophysiological effects. These data may effect the eye chemistry and eye tension.In our study, the starvation stress have incresaed significantly both MAO A and B and CA activations (Table 1). So that, atriculus, ventriculus and mitral valvula have been significantly affected by starvation and MAO A and MAO B activations were increased in these sections of heart. Pargyline is "chlorhydrate de *N*-méthyl *N*-propyne-2 yl benzylamine". Chlorhydrate and *N*-méthyl have toxic effects, therefore, these two agent may be effecting on inhibition of MAO [3,4,6,8,11,16].

On the other hand blood values of MAO fractions have been found less than other organ or tissues. In this case, MAO A and B fractions were increasing signifiacntly in organs as brain, eye, heart and liver. That is, according to the our investigation, MAO A and B augmentations may include all organs. Monoamine oxidase (MAO) A and B are isoenzymes located that play a central role in regulating tissue levels of biogenic amines. MAO-A preferentially metabolizes serotonin (5-HT) and norepinephrine (NE), whereas MAO-B has a higher affinity for phenylethylamine (PEA). Both forms of the enzyme metabolize dopamine (DA), though MAO-A in mice has a higher affinity for DA. When the meat produces of animals stressed consume, humanity may effect because of higher MAO A and B activations. These findings may be useful for studying the molecular mechanisms of monoamine functions [4,7,9,14,15].

On the other hand, carbonic anhyrase activation has been found higher in retina, thalamus, hypothalamus, nervus opticus, liver, atriculus, ventriculus and mitral valvula than other tissues or organs. CA enzyme has been the more inhibition in lobus temporalis, lobus occipitalis, retina, nervus opticus, ventriculus and blood *e.g.* acetazolamide has decreased significantly the activation of CA in these tissues ($P<0.01$). Carbonic anhydrase inhibitor, acetazolamide (Diamox)is N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide; *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-acetamide; 5-acetamido-1,3,4-thiadiazole-2-sulfonamide and molecular weight 222.2, molecular formula: $C_4H_6N_4O_3S_2$ [19,23,45, 47,49,50]. Patients with either acute or chronic overdosage with acetazolamide may show signs of dehydration with thirst, lethargy, confusion, poor skin turgor, and prolonged capillary refill time, but may have a paradoxical continued diuresis. Electrolyte abnormalities include hyponatremia, hypokalemia, and a non-anion gap hyperchloremic metabolic acidosis in the more than mild ingestion which may lead to further deterioration in mental status, production of seizures, electrocardiographic abnormalities, and arrhythmias. Prior renal insufficiency will lead to increased toxicity at a given dose. There are idiosyncratic reactions producing bone marrow suppression with hepatic and renal insufficiency. Acetazolamide may also precipitate in the renal tubules producing calculi with renal colic. Hypokalemia may lead to muscular weakness, hyporeflexia, and hypochloremic metabolic alkalosis. In chronic therapy, especially in geriatric patients, a chronic metabolic acidosis may lead to a chronic compensatory hyperventilation which increases pulmonary vascular resistance and decreases left ventricular function. This can be especially significant in patients on concurrent β-blocker or calcium channel blocker therapy. The ventricular fibrillation threshold may then be reduced [23,25,33,36,39,41,44,48].

By catalyzing a very simple physiological reaction, $CO_2$ hydration to bicarbonate and $H^+$ ions, carbonic anhydrases (CAs, EC 4.2.1.1), of which five genetically distinct families (α–ε) are presently known, are fundamental enzymes in all organisms over the phylogenetic tree. In vertebrates, including humans, the 14 different α-CA isozymes characterized so far are involved in a host of physiological and pathological processes, and modulation of their activity by means of specific inhibitors or activators leads to important pharmacological responses. CA inhibitors (CAIs) systemic or topically acting are clinically used ophthalmologic drugs for the management of glaucoma, cystoid macular edema, and retinopathies of diverse nature, being used alone or in combination therapies with other agents. In the last period, a large number of sulfonamides/sulfamates targeting the tumor-associated CA isozymes (CA IX and XII) have also been reported, with one such derivative, indisulam (E-7070) being in Phase II clinical trials for the treatment of solid tumors. On the other hand, Only one isozyme, CA V, is present in mitochondria, among the many α-CA isoforms isolated in animals (humans included). This isozyme was shown to be involved in several biosynthetic processes, such as ureagenesis, gluconeogenesis, and lipogenesis, both in vertebrates (rodents) as well as invertebrates (locust). Indeed, in several crucial biosynthetic processes involving pyruvate carboxylase, acetyl-CoA carboxylase, and carbamoyl phosphate synthetases I and II, bicarbonate not $CO_2$, is the real substrate of these carboxylating enzymes, and the provision of enough bicarbonate is assured by the mitochondrial isozyme CA V. (35,37,38,41,45).

They are widespread in nature, being found in animals, plants, and certain bacteria. In animals they play an important role in respiration by facilitating transport of $CO_2$ and are involved in the transfer and accumulation of $H^+$ and $HCO^{3-}$. In chloroplasts of plant cells their role may be related to photosynthetic fixation of $CO_2$. Mammalian carbonic anhydrases are of several forms differing in enzymatic properties, amino acid sequences and

inhibitor binding. In erythrocytes there is usually present both a high activity and a low activity form. The pH-rate profiles and pH-binding curves indicate that the same group with pKa of approximately seven is involved in both forms. All the isoenzymes have a molecular weight of approximately 30,000 and contain one zinc atom per molecule.. Bovine erythrocytes contain two electrophoretically separable forms designated A and B in order of mobility. Both have a high order of activity similar to the human variant "C" [17-51].

## Expermental

All the chemicals used for MAO A and B and carbonic anhyrase activities determination were obtained from Sigma Chemical Co. (St. Louis, MO) and Merck (Germany). Other reagents and solvents were of analytical grade.

*Animals, stress treatment and homogenisation of tissues*

Ten male goat (50-55 Kg, *Capra aegagrus hircus*, n = 5 control, n = 5 stress group) were purchased from the producers in Kütahya,Turkey. They were maintained in an air conditioned room with light from 07:00 to 19:00 h. The room temperature (22±1 °C) and humidity (55±10%) were controlled. They were fed water and food adlibitum. During experimental study, the starvation stress was carried out to the goats approximately between 7.00 and 18.00 hours during the day and 90 days.

Organ or tissues of normal goat used as control (no treatment) for cardiovascular studies were removed on the termination of the experiment immediately after sacrifice. The lenses were enucleated through posterior approach, washed with saline and their fresh weights were recorded. Ten lenses were pooled and a 10% homogenate was prepared in 0.1 M phosphate buffer saline (pH 7.4). After centrifugation at 5000×*g* for 10 min in a refrigerated centrifuge, the supernatant was collected and kept in ice for the determination of MAO and CA activities.

*Purification of MAO from the liver homogenates [7-16]*

MAO was purified from the goat liver (100-150 g) according to the method of Holt with some modifications. Homogenates of liver were prepared with 1:25 (w/v) in ice-cold potassium phosphate buffer, pH 7.4 with a polytron mechanical homogenizer. Homogenates were centrifuged at 1000 g, at 4 °C for 15 min. and the supernatant was used as the source of MAO and kept at -70 °C. Since, MAO-A activity decreased rapidly following homogenization, freshly prepared homogenates were used in kinetic studies of MAO-A activity. These crude homogenates were used as the MAO source for preliminary kinetic assays.

Mitochondrial MAO was purified by isolation of mitochondria from liver homogenates. Liver tissue (5-8 g) was homogenized 1:40 (w/v) in 0.3 M sucrose. Following centrifugation at 1000 g for 10 min., the supernatant was centrifuged at 10000 g for 30 min to obtain crude mitochondrial pellet. The pellet was incubated with either CHAPS of 1% or Triton X-100 of 1% at 37 °C for 60 min and centrifuged at 1000g for 15 min. The pellet was resuspended in 0.3 M sucrose and layered onto 1.2 M sucrose, centrifuged at 53000 g for 2 h and resuspended in potassium phosphate buffer. It was kept at 70 °C until use.

*Purification of MAO-B from goat platelets [7-16]*

Venous blood samples were pooled in stoppered laboratory vacutainer tubes containing sodium citrate as anticoagulant. Platelet-rich plasma (PRP) was prepared by sequential centrifugation: blood (300-500 mL) was centrifuged at 200 g for 15 min at room temperature; supernatant was aspirated, recentrifuged at 1200 g for 10 min. and kept. The ellet was resuspended in 0.5 mL of TRIS buffer (15 mM TRIS-HCl, 140 mM NaCl, 10 mM EDTA), pH 7.4 and centrifuged at 10000 g for 10 min. Supernatants were pooled. Platelet counts were determined on aliquots of pooled PRP diluted in Isoton II and counted twice on a thrombocounter (Coulter Electronics, STKS) The kinetic behavior of MAO-B activity of the pooled PRP was determined as described below by using benzylamine as substrate (in the concentration range of 0-10 mM) and the results were expressed as nmol/$10^8$ platelets/h.

*Measurement of MAO activity [7,8,9,11,12,14,15]*

Total MAO activity was measured spectrophotometrically according to the method of Holt. The chromogenic solution which is prepared for inclusion in the assay mixture consisted of 1 mM vanillic acid, 500 µM 4-aminoantipyrine, 4 U/mL$^{-1}$ peroxidase in 0.2 M potassium phosphate buffer, pH 7.6. Chromogenic solution was prepared daily and kept at

4°C until used. A standard assay mixture contained 167 µL chromogenic solution, 667 µL substrate (500 µM *p*-tyramine, 450 µM benzylamine or 500 µM serotonin) and 133 µL potassium phosphate buffer, pH 7.6. The mixture was preincubated at 37°C for 10 min before the addition of enzyme. Reaction was initiated by addition of the homogenate (100 µL) and absorbance increase was followed at 498 nm at 37°C for 60 min. Molar absorption coefficient of 4654 $M^{-1}cm^{-1}$ was used to calculate the initial velocity of the reaction.

*Selective measurement of MAO-A and MAO-B activities [1-16]:*

Goat liver homogenates were incubated with the mixed substrate *p*-tyramine at 500 µM to measure MAO A and at 2.5 mM to measure MAO B; 450 µM for benzylamine and 500 µM for serotonin following inhibition of MAO with inhibitors (50 µM) or at 500 µM to measure total MAO when no inhibitor had been included. Substrate concentrations were chosen with respect to our pervious studies on MAO activity of goat liver homogenates.

Aqueous solutions of clorgyline or pargyline (50 µM) were added to homogenates at the ratio of 1:100 (v/v) so the final inhibitor concentrations were 500 nM. Homogenates were incubated with inhibitors at 37 °C for 60 min prior to activity measurement.

In order to test the dependence of reaction upon enzyme concentration, a range of volumes of inhibitor-treated homogenates (25-100 µL) was made up to total volumes of 100 µL with potassium phosphate buffer and then incubated with *p*-tyramine, at 500 µM or 2.5 mM, to assay MAO-A and B activities, respectively.

*Purification of MAO from the other organ homogenates [2,4,5,6,7,8,9,11,14]:*

Purification of MAO from other tissues was carried out as that of liver producure above.

*Protein determination*

Protein contents were determined according to the method of Bradford with bovine serum albumin used as standard.

*Measurement of carbonic anhyrase activity in tissues [17-51]*

## *Assay*

*Method*

The electrometric method of Wilbur and Anderson [49] in which the time required (in seconds) for a saturated $CO_2$ solution to lower the pH of 0.012 M Tris$^{\oplus}$HCl buffer from 8.3 to 6.3 at 0°C is determined. The time without enzyme is recorded at $T_0$; with enzyme, T.

*$2 \times (T_0 - T)$A Unit of activity = T*

*Reagents*

- 0.02 M Tris$^{\oplus}$HCl buffer, pH 8.0. Store in an ice bath at 0-4 °C before and during use.
- Carbon dioxide saturated water. Bubble $CO_2$ gas through 200 ml ice cold water for 30 minutes prior to assay. During saturation process, store water at 0-4 °C in an ice bath.

*Enzyme*

Dissolve lyophilized powder at a concentration of 0.1 mg/ml in ice cold water. Stored in ice bath prior to use. Immediately prior to use dilute suspensions or lyophilized materials to a concentration of approximately 0.01 mg/ml in ice cold water.

*Procedure*

Blank Determination: Add 6.0 ml of chilled 0.02 M Tris⊕HCl buffer, pH 8.0 to a 15-20 ml beaker. Maintain temperature at 0-4 °C and record pH.

Withdraw in a 5 ml syringe, 4 ml of chilled $CO_2$ saturated water and add to Tris buffer. Immediately start a stop watch and record the time required for the pH to drop from 8.3 to 6.3. Record this time as $T_0$.

Enzyme Determination: Add 6.0 ml of chilled 0.02 M Tris⊕HCl buffer, pH 8.0 to a 20 ml beaker. Maintain temperature at 0-4°C and record pH. Add 0.1 ml of freshly diluted enzyme. Quickly add 4 ml of $CO_2$ saturated water and record the time required for the pH to drop from 8.3 to 6.3. Record this time as T.

*Calculation*

2x ($T_0$ – T) Units /mg = T X mg enzyme in reaction mixture

**References**


1. A.W.C. Bach, N.C. Ian, D.L. Johnson, C.W. Abell, M.E. Bembenek, S.W. Kwan, A.H. Seeburg and J.C. Shih,.*Proc. Natl. Acad. Sci.*, **85**, 4934 (1988).
2. C.J. Fowler and K.F. Tipton, *J. Pharm. Pharmacol.*, **30**, 111 (1984).
3. P.M. Yu, T.F. Tipton and A.A. Boultan, *Prog. Brain. Res.*, **106**, 85 (1995).
4. J. Woulters, *Current. Med. Chem.*, **5**, 137 (1998).
5. S.M. Benedetti, P. Dostert, Monoamine Oxidase: From Physiology and Patho-physiology to the Design and Clinical Application of Reversible Inhibitors, Academic Press, London, **23**, 67 (1992).
6. I.M. Anderson, *Br. Med. Bull.*, **57**, 161 (2001).
7. L. Oreland, Basic and Clinical Aspects. VSP Press, Utrecht, 219 (1993).
8. A Holt, D.F. Sharman, G.B. Baker and M.M. Pelcic, *Anal. Biochem.*, **244**, 384 (1997).
9. D.L. Murphy, C. Wright, M. Buchsbaum and A. Nichols, *Biochem. Med.*, **16**, 254, 1976.
10. M.M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
11. C.J. Fowler and K.F. Tipton, *Biochem. Pharmacol.*, **24**, 3329 (1981).
12. M.B.H. Youdim, *Experientia*, **44**,137 (1988).
13. K.F. Tipton, Inhibitors of monoamine oxidase and the pressor response to dietary amines in: Milestones in Monoamine Oxidase Research: Discovery of (-)-Deprenyl, K. Magyar and E.S.Vizi (Eds), Medicine Publishing House Co., Budapest, (2000).
14. M.H.B Youdim and P.M. Finberg, *Biochem. Pharmacol.*, **41**, 155 (1991).
15. D.L. Murphy, *Biochem. Pharmacol.*, **27**, 1889 (1978).
16. (a). C. Salles, R.H.S. Sauza, J.B. Salles and V.L.F. Bastos, *J Fish Biology* **58**, 1301 (2001). (b) Davis, R.:, *The Enzymes 5*, P. Boyer, H. Lardy, and K. Myrback, Academic Press, NY, 545 (1961).
17. H. Deutsch, S. Funakoshi, T. Fujita, N. Taniguch, and H. Hirai, *J Biol Chem* **247**, 4499 (1972).
18. J. Edwards, *Biochim. Biophys. Acta*, **2024**, 280 (1970).
19. Y. Edwards,, *Biochem. Soc. Trans.*, **18**, 171 (1990).
20. P. Engberg and S. Lindskog, *Eur. J. Biochem.*, **165**, 407 (1986)
21. T. Fawcett, J. Browse, M. Volokita and S. Bartlett, *J. Biol. Chem.*, **265**, 5414 (1990)
22. R. Fernley, R. Wright and J. Coghlan, *Biochem.*, **27**, 2815 (1988).
23. I. Fridovich, *J. Biol. Chem.*, **242**, 1445 (1967).
24. S. Funakoshi and H. Deutsch, *J. Biol. Chem.*, **245**, 4913 (1970).
25. T. Furtsch, D. Dierdorf and A. Cowley, *J. Am. Chem. Soc.*, **92**, 5758 (1970).
26. P. Göthe and P. Nyman, *FEBS. Lett.*, **21**, 159 (1972).
27. I. Greene, M. Arifullah and A. Kenny, *Pharmacology*, **45**, 278 (1992).
28. L. Henderson, D. Henriksson and P. Nyman, *Biochem. Biophys. Res. Commun.*, **52**, 1388 (1973).
29. , P. Henkarte, G. Guidotti and J. Edsall, *J. Biol. Chem.*, **243**, 2447 (1968).
30. R. Henkens, B. Kitchell, S. Lottich, P. Stein and T. Williams, *Biochem.*, **21**, 5918 (1982).
31. M. Kandel, A. Gornall, S. Wong and S. Kandel, *J. Biol. Chem.*, **245**, 2444 (1970).
32. K. Kannan, K. Fridborg, P. Bergsten, A. Liljas, S. Lovgren, M. Petef, B. Strandberg I. Waara, L. Adler, S. Falkbring, P. Göthe and P. Nyman, *J. Mol. Biol.*, **63**, 601 (1972).
33. R. Khalifah and J. Edsal, *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 172 (1972).
34. T. Kondo, K. Murakami, H. Isobe, N. Taniuchi and Y.A. Kawakammi, *Clin. Chim. Acta*, **191**, 169 (1990).
35. L. Lie-Injo, C. Lopez and P. Hart, *Clin. Chim. Acta*, **29**, 541 (1970).
36. A. Liljas, K. Hakansson, B. Jonsson, and Y. Xue, *Eur. J. Biochem.*, **219**, 1 (1994).
37. A. Liljas, K Kanna, P. Bergsten, I. Waara, K. Fridborg, B. Strandberg, U. Carlbom, L. Jarup, S. Lovgren and M. Petef, *Nature New Biol.*, **235**, 131 (1973).
38. S. Lindskog, L. Henderson, K. Kannan, A. Liljas, P. Nyman and B. Strandberg, *Enzymes*, **5**, 587 (1971).
39. S. Lindskog, *Biochim. Biophys. Acta*, **39**, 218 (1960).
40. B. Malmström, P. Nyman, B. Strandberg and B. Tilander, *Structure and Activity of Enzymes*, T. Goodwin, J. Harris and B. Hartley, Academic Press, NY, 121 (1964).
41. M. Mondrup and N. Anker, *Clin. Chim. Acta*,

**61**, 127 (1975).
42. Y. Pocker and C. Fong, *Biochem.*, **22**, 813 (1983).
43. Y. Pocker and N. Janjic, *J. Biol. Chem.*, **263**, 6169 (1988).
44. S. Podos and J. Serle, *Arch. Ophthalmol.*, **109**, 38 (1991).
45. J. Severinghaus, F. Hamilton and S. Cotev, *Biochem. J.*, **114**, 703 (1969).
46. W Siffert and G. Gros, *Biochem. J.*, **217**, 727 (1984).
47. P. Whitney, P. Nyman and B. Malmström, *J. Biol. Chem.*, **242**, 4212 (1967).
48. P. Whitney, *Anal. Biochem.*, **57**, 467 (1974).
49. K. Wilbur and N. Anderson, *J. Biol. Chem.*, **176**, 147 (1948).
50. T. Williams and B. Colman, *J. Exp. Bot.*, **45**, 153 (1994).
51. X. Zhu and W. Sly, *J. Biol. Chem.*, **264**, 8795 (1990).