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Further studies on the inhibition of monoamine oxidation by monoamine oxidase inhibitors

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Since the discovery of monoamine oxidase (monoamine: O_2 oxidoreductase (deaminating E.C. 1.4.3.4.) many types of chemicals, both hydrazine and non-hydrazine, have been found as the inhibitor of this enzyme [1–3]. One of the results of these monoamine oxidase inhibitors (MAOI) is to elevate the level of biogenic amines in brain [4–6]. On the other hand, observations are also available where it is reported that these inhibitors may fail to elevate brain level of tryptamine [7, 8]. Perusal of literature on monoamine oxidase (MAO) shows that the earlier workers [9] used tetrazolium salts or other redox indicators for the determination of MAO activity, but they did not, at any time, mention the existence of an amine dehydrogenating system except Davison's suggestion [10] which was extended by others [11, 12]. Furthermore, histochemical studies with tetrazolium salt in the presence of monoamines have always been described as MAO activity in the literature [13, 14], although it was not known whether the amine dehydrogenating system (MADH) was identical with MAO or not. However, recent work on MADH [15–18] strongly suggest that such an enzyme probably exists, although further investigation on this direction seems necessary. Hence, comparative studies of MAO and MADH activities with potent monoamine oxidase inhibitors appear justified since it helps in understanding whether the two enzymes (MAO and MADH) are same or different. Relevant results of the effect of these inhibitors are summarised in this communication.

Whole brain homogenates of adult albino rats were prepared as a 10 per cent suspension in 0.25 M sucrose and incubations for enzyme activity were carried out at 38° for 30 min with a pre-incubation period of 5 min which was extended up to 20 min when inhibitors were added.

In vivo experiments were performed by administering intraperitoneal injections of MAO inhibitors (50 mg/kg of iproniazid or catron, 20 mg/kg of nialamide and 2 mg/kg of *trans*-PCP or Pargyline) daily to a group of six rats for 3 consecutive days in each case. Similarly, normal saline was administered to the six control rats for 3 consecutive days. The animals, both control and experimental, were sacrificed on the fourth day and the enzyme activities were determined.

The standard incubation mixture for MAO assay consisted of 0.02 M phosphate buffer pH 6.5, 0.0125 M semicarbazide pH 6.5, 0.01 M tyramine and 50 mg of tissue homogenate in a final volume of 2 ml. The enzyme activity was determined by measuring the aldehyde formation in a Bausch and Lomb colorimeter at 420 mμ according to the method of Green and Haughton [19]. Enzyme units were expressed in terms of μ moles of 2:4-dinitrophenylhydrazones of *p*-hydroxyphenylacetaldehyde formed per min per g of tissue.

The reaction mixture for MADH assay contained 0.025 M phosphate buffer pH 7.0, 0.5 mg neotetrazolium chloride (NTC), 0.01 M tyramine and 100 mg of tissue homogenate in a final volume of 2 ml. NTC reduction was measured at 520 mμ in a Bausch and Lomb colorimeter according to the method of Lagnado and Sourkes [11]. Air was used as the gas phase in aerobic experiments and anaerobic experiments were carried out *in vacuo* in Thunberg tubes [20]. Enzyme unit was expressed as μmoles of diformazan formed per min per gram tissue.

The effects of intraperitoneal administration of MAO inhibitors (both hydrazine and non-hydrazine derivatives) on MAO and MADH activities of rat brain are shown in Table 1. All these inhibitors showed a marked inhibition in case of MAO and the per cent of inhibition as caused by these inhibitors were 85–95 per cent. With non-hydrazine inhibitors like *trans*-PCP and pargyline, MADH activity appeared normal as shown by the control rats. On the contrary, hydrazine derivatives like iproniazid, catron and nialamide caused a slight inhibition of MADH activity which was 12–15 per cent in aerobic condition and 5–12 per cent in anaerobic condition.

Table 2 shows the *in vitro* effect of MAO inhibitors on MAO and MADH activities. In MADH systems (both aerobic and anaerobic conditions), the inhibition produced by hydrazine derivatives was less than its corresponding MAO inhibition. At the same concentration, where MAO was inhibited, MADH showed no trace of inhibition, for example, 1×10^{-5} M iproniazid, 5×10^{-5} M catron or 1×10^{-3} M nialamide. It is also found from Table 2 that the hydrazine derivatives caused less inhibition anaerobically than the aerobic MADH systems. Simple electrolytes except azide which failed to inhibit MAO inhibited both

* The following abbreviations are used: MAO, monoamine oxidase; MADH, monoamine dehydrogenase; *trans*-PCP (Tranylcypromine), *trans*-2-phenylcyclopropylamine; NTC, neotetrazolium chloride; MAOI, monoamine oxidase inhibitor; S.E.M., standard error of mean.

Table 1. Effect of *in vivo* administration of certain monoamine oxidase inhibitors on rat brain MAO and MADH activities

Sl. No.	System	MAO activity μmoles of 2:4-DPN hydrazone of *p*-hydroxy phenylacetaldehyde formed/g/min (Mean ± S.E.M.)	MADH activity μmoles of diformazan formed/g/min (Mean ± S.E.M.) Aerobic	Anaerobic
1.	Normal rats	24.18 ± 1.32	4.56 ± 0.71	8.85 ± 1.48
2.	Iproniazid treated rats	2.49 ± 0.23	0.56 ± 0.05	0.47 ± 0.03
3.	*Trans*-PCP treated rats	3.61 ± 0.36	4.56 ± 0.71	8.85 ± 1.48
4.	Pargyline treated rats	3.02 ± 0.08	4.56 ± 0.71	8.85 ± 1.48
5.	Catron treated rats	1.23 ± 0.13	0.68 ± 0.03	1.33 ± 0.11
6.	Nialamide treated rats	2.49 ± 0.31	0.68 ± 0.03	1.06 ± 0.09

Table 2. *In vitro* effect of monoamine oxidase inhibitors on monoamine oxidation of rat brain

Sl. No.	System	Final Concentration of inhibitors (M)	MAO activity μmoles of 2:4-DNP hydrazone of *p*-hydroxyphenylacetaldehyde formed/g/min (Mean ± S.E.M.)	MADH activity μmoles of diformazan formed/g/min (Mean ± S.E.M.) Aerobic	Anaerobic
1.	Normal	—	24.18 ± 1.32	4.56 ± 0.71	8.85 ± 1.48
2.	Iproniazid	1×10^{-3}	2.94 ± 0.18	1.38 ± 0.11	4.43 ± 0.35
		5×10^{-4}	3.34 ± 0.23	3.04 ± 0.20	6.81 ± 0.40
		1×10^{-4}	8.48 ± 0.77	4.20 ± 0.29	8.40 ± 0.57
		1×10^{-5}	14.59 ± 1.12	4.56 ± 0.71	8.85 ± 1.48
3.	Catron	1×10^{-2}	Nil	0.31 ± 0.02	0.91 ± 0.10
		1×10^{-3}	2.50 ± 0.24	2.28 ± 0.21	4.42 ± 0.32
		5×10^{-4}	3.21 ± 0.19	2.73 ± 0.15	5.77 ± 0.53
		1×10^{-4}	4.88 ± 0.04	3.66 ± 0.26	6.66 ± 0.92
		5×10^{-5}	6.12 ± 0.41	4.56 ± 0.71	8.85 ± 1.48
4.	Nialamide	5×10^{-3}	Nil	2.26 ± 0.20	4.86 ± 0.37
		1×10^{-3}	Nil	4.56 ± 0.71	8.85 ± 1.48
		5×10^{-4}	6.01 ± 0.54	4.56 ± 0.71	8.85 ± 1.48
5.	Sodium azide	1×10^{-3}	24.18 ± 1.32	3.78 ± 0.42	7.75 ± 1.04
6.	Copper sulphate	1×10^{-4}	14.50 ± 1.02	2.07 ± 0.26	5.75 ± 0.74
7.	Ferric chloride	1×10^{-3}	12.09 ± 1.33	2.54 ± 1.33	5.53 ± 0.76
8.	Silver nitrate	1×10^{-3}	14.51 ± 0.95	1.52 ± 0.20	4.45 ± 0.40
9.	Mercuric chloride	1×10^{-4}	12.17 ± 0.99	2.85 ± 0.38	5.23 ± 0.64
10.	α,α'-dipyridyl	1×10^{-3}	24.18 ± 1.32	2.98 ± 0.28	6.22 ± 0.43
11.	*O*-phenanthroline	1×10^{-3}	15.74 ± 1.39	2.50 ± 0.19	4.90 ± 0.32
12.	~ *p*-chloromercuri benzoate (~ *p*-CMB)	5×10^{-4}	12.16 ± 0.95	2.51 ± 0.17	5.76 ± 0.63
13.	Chlorpromazine	1×10^{-3}	14.48 ± 0.82	4.56 ± 0.71	8.85 ± 1.48

the enzymes. Again chelating agents like *O*-phenanthroline and *p*-CMB inhibited both, MAO and MADH, while 8 (OH)-quinoline failed to inhibit either MAO or MADH. α, α'-dipyridyl is the only inhibitor which inhibited MADH, but not MAO. It is also observed that cupferron and rotenone had no effect on either MAO or MADH systems, while chlorpromazine inhibited MAO only.

Other potent MAO inhibitors like Pargyline and trans-PCP produced inhibition of MAO activity at varied concentrations. But these compounds failed to inhibit MADH system, both aerobically and anaerobically. Hydroxylamine was unique in this series as it inhibited 50 per cent of MAO and MADH (aerobic and anaerobic) activities at 1×10^{-2} M concentration.

D-amphetamine, L-amphetamine, ephedrine and mescaline (at 10^{-2} M concentration) did not cause any inhibition when added to the MADH system. It was also observed that D-amphetamine produced more inhibition (67 per cent) than its isomer (50 per cent) in the case of MAO. Saturated fatty acids like palmitic acid and stearic acid failed to inhibit either MAO or MADH. On the other hand, unsaturated fatty acids (oleic acid, arachidonic acid, linoleic acid and palmitoleic acid) only inhibited MAO activity at a final concentration of 5×10^{-3} M and degree of inhibition was observed in between 25 and 36 per cent depending upon the nature of inhibitor.

Carbonyl reagents like KCN at 4×10^{-3} M inhibited both MAO and MADH (aerobic and anaerobic) systems and the rate of inhibition was 60 per cent for all the three systems. Semicarbazide produced 100 per cent inhibition for MADH, while thiosemicarbazide did not show any inhibition for MADH. Semicarbazide and thiosemicarbazide were not used for MAO since semicarbazide was added for the estimation of MAO in the aldehyde assay procedure where the resultant aldehyde from the oxidation of amine was trapped by semicarbazide.

Most of the inhibitors employed for *in vitro* studies are potent MAO inhibitors, but these inhibitors either failed to inhibit MADH or inhibited MADH at higher dosages as it has been observed with hydrazine derivatives. Again, hydrazine compounds inhibit MADH more pronouncedly *in vitro*, although *in vivo*, they inhibit slightly. In the presence of hydrazine derivatives, anaerobiosis reduces per cent inhibition of MADH. It is reported [3] that the interaction between MAO and hydrazine derivative is influenced by the presence of oxygen and the actual inhibitor is only a product of reaction of the parent compound. However, formazan production is sensitive to hydrazine derivatives, cyanide, semicarbazide and hydroxylamine. KCN is added in the conventional manometric technique of MAO assay to arrest the activity of other oxidative enzymes, but here, in aldehyde procedure, it inhibits MAO and this inhibition is probably due to a formation of a complex compounds with semicarbazide.

The above results suggest that MADH is probably a different enzyme. So, it behaves differently with the potent MAO inhibitors. Recently, Guha and Ghosh [15] have suggested that MADH is probably different from MAO. Meanwhile, the electrophoretic separations that have been reported [22, 23] made use of assay with tetrazolium salt (nitro-blue tetrazolium) as electron acceptor. But it has been proved to be liable to artifacts [24] and its value for monoamine detection appears doubtful [25, 26]. This fact is further supported from the present observation that MADH behaves differently from MAO. At the same time, the above results may explain the previous observations [10, 11] that brain tryptamine level is not elevated by MAO inhibitors, since tryptamine is actively metabolised by brain MADH [21].

However, it has been suspected for some time now that the oxidative deamination of monoamines is due to more than one enzymes [27, 28]. So, it could be assumed from

the present investigation that this MADH is another enzyme which takes part in the physiological inactivation of biogenic amines. Of course, further work in this direction is essential, especially to elucidate its actual role in connection with monoamine metabolism.

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Effects of chlorpromazine and DL-amphetamine on calcium uptake by adrenal chromaffin cell membrane

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Biochemical investigations have shown that plasma membrane of adrenal medulla is concentrated in the microsomal fraction [1, 2]. Electron microscopy has shown that vesicles form from this plasma membrane-rich fraction [1, 2], and biochemical evidence suggests that these plasma membrane vesicles take up calcium in the presence of ATP [2]. This calcium transfer mechanism may be of importance in regulating calcium-mediated secretion. Agents that modify this transfer mechanism may also modify secretion in the intact organ. Recent evidence suggests that caffeine and certain inorganic cations may act, at least in part, by interfering with this plasma membrane calcium transfer mechanism [3]. Caffeine, Zn^{2+}, Cd^{2+} and Hg^{2+} produce a relatively prolonged catecholamine release from perfused adrenal medulla [4, 5]. These agents probably cause secretion by mobilizing intracellular calcium [4] and by inhibiting calcium extrusion from the adrenal medullary cell [3].

It has also been proposed that chlorpromazine and amphetamine evoke catecholamine release from the adrenal medullary cell by mobilizing intracellular calcium pools [4]. The purpose of the present investigation was to determine if chlorpromazine and amphetamine also inhibit adrenal medullary plasma membrane calcium transport.

Bovine adrenal glands were obtained from a local slaughterhouse and carried on ice to the laboratory and used within 2 hr post-mortem. Approximately 5 g of medullary tissue was dissected free from the cortex and homogenized in 10 vol. of 0.32 M sucrose using a conical all-glass homogenizer (Duall tissue grinder, Kontes Glass Co.). The homogenate was centrifuged at 800 g_{max} for 10 min to separate nuclei and cell debris. The supernatant obtained was centrifuged at 27,000 g_{max} for 10 min to separate mitochondria and chromaffin granules. This supernatant was centrifuged at 105,000 g_{max} for 60 min. The final pellet which represented purified microsomes was resuspended in 5 ml of 0.32 M sucrose.

For Ca^{2+}-stimulated adenosinetriphosphatase (Ca^{2+}-ATPase) activity, a 0.1-ml aliquot of the sample was made 0.1 per cent with respect to sodium desoxycholate and incubated for 20 min at 37° in a Dubnoff metabolic shaker