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EFFECT OF SOME MONOAMINE OXIDASE INHIBITORS AND CYCLIC-AMP ON PLASMA FIBRINOGEN LEVEL OF RATS

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Abstract—*In vivo* administration of short acting or long acting inhibitor of monoamine oxidase elevates plasma fibrinogen level in rats. These inhibitors cannot elevate plasma fibrinogen level in rats pretreated with *p*-chlorophenyl alanine and α-methyl-*meta*-tyrosine, inhibitors of catecholamine and serotonin formation respectively. However, administration of epinephrine or serotonin under the above experimental condition elevates fibrinogen level indicating rise of biogenic monoamine, even for a short period, is a good stimulus for increase of plasma fibrinogen. Dibutyryl cyclic-AMP (c-AMP) also elevates fibrinogen level and shows additive effect with theophylline or serotonin.

Earlier reports from our laboratory show that administration of iproniazid decreased the plasma monoamine oxidase (MAO) and increased the fibrinogen level in both rabbits and rats [1]. We have observed that administration of some biogenic amines like epinephrine, serotonin, tyramine, etc. to rats and rabbits also elevated the plasma fibrinogen level [1].

It is well established that liver is the site of synthesis and secretion of fibrinogen [2, 3] and clorgyline (*N*-methyl *N*-propergyldichlorophenoxy-propylamine) and pargyline (*N*-methyl-*N*-2-propynyl-benzylamine) are known to be tissue MAO inhibitors [4]. Thus, it was thought interesting to study the effect of these inhibitors on liver MAO and plasma fibrinogen level and the results show that these inhibitors did elevate the plasma fibrinogen. However, it is still not clear whether the elevation of fibrinogen is due to a direct effect of MAO inhibitors or the effect is mediated through accumulated biogenic monoamines. In order to have some idea on this question two other compounds, which, unlike reserpine, deplete monoamine by inhibiting their formation, were tried in our experiments. DL-*p*-Chlorophenylalanine (PCP) reduces tissue serotonin by inhibiting tryptophan hydroxylase [5] and α-methyl-*m*-tyrosine (α-MMT) lowers tissue catecholamines by inhibiting aromatic amino acid decarboxylase [6], and also possibly by producing false transmitter [7]. The effect of harmaline and monoamines on rats pretreated with these two compounds are quite interesting and the results are incorporated in this communication.

In terms of duration of effect, MAO inhibitors have been classified in (i) long-acting type like pargyline and (ii) short-acting type like harmaline (1-methyl-7-methoxy-3,4-dihydrocarboline) [4].

Results obtained on the effects of pargyline and harmaline on the MAO activity and plasma fibrinogen level in normal rats are also included.

A number of amines seem to produce similar effect on plasma fibrinogen level [1] and it appears that there might be a common mode of action for all these monoamines and, if so, whether it is through c-AMP. Since epinephrine is known to elevate c-AMP level in liver [8], attempts have, therefore, been made to find out whether there is any involvement of c-AMP in this process.

MATERIALS AND METHODS

Serotonin, pargyline, theophylline, DL-*p*-chlorophenylalanine, α-methyl-*m*-tyrosine, epinephrine and dibutyryl c-AMP were purchased from Sigma Chemical Company and clorgyline was procured from May and Baker, U.K.

Treatment of rats

Female rats 100–120 g of the Wister Albino IICB inbred strain from the Institute colony were used. MAO inhibitors and theophylline were injected intraperitonially whereas serotonin, epinephrine and dibutyryl c-AMP were administered intramuscularly. The rats were killed at definite times and blood and liver were collected. In our previous report [1] rats were pretreated with reserpine to increase the sensitivity towards amine but in the present one, reserpine treatment was omitted to avoid complication.

Assay of MAO

Rat liver mitochondria was prepared according to the method of Grosso and Gawienowski [9]. Minced livers were homogenised in 3 vol. of 0.32 M sucrose medium containing 0.1 mM EDTA at pH 7.0 by a motor-driven Potter–Elvehjem homogenizer with Teflon pestle. The homogenate was diluted to 10 vol. and centrifuged at 3000 *g* for 3 min in a Sorval Model RC 2-B refrigerated centrifuge. The supernatant was again centrifuged at 35,000 *g* for 15 min. The resulting pellet was washed twice with 0.32 M sucrose, resuspended in 0.32 M sucrose and was used as the enzyme. The assay of liver MAO was carried out

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Table 1. *In vivo* effects of different doses of clorgyline and pargyline on liver MAO and plasma fibrinogen level of rats

System	MAO (o.d./30 min/mg protein) Mean ± SE	Percent inhibition of MAO	Plasma fibrinogen (mg %)	Percent stimulation
Control	0.23 ± 0.009		314 ± 33	
Pargyline (2 mg/kg)	0.086 ± 0.008‡	62	367 ± 17.98*	16.8
Clorgyline (2 mg/kg)	0.096 ± 0.0095‡	58	436 ± 31*	39
Pargyline (5 mg/kg)	0.053 ± 0.01§	76	445 ± 35†	41.7
Clorgyline (5 mg/kg)	0.058 ± 0.009‖	74	485 ± 39‡	54
Pargyline (10 mg/kg)	0	100	536 ± 20§	70
Clorgyline (10 mg/kg)	0	100	525 ± 10§	67

The control value is an average of 16 rats and all the other values are on average of 6 rats.
The rats were killed 24 hr after the injection of MAO inhibitors.
* Not significant against control; † $P < 0.05$; ‡ $P < 0.01$; § $P < 0.001$; ‖ $P < 0.002$ vs control.

according to the method of Green and Haughton [10] with slight modification. The incubation mixture contained in a final volume of 2 ml, approx. 3 mg of mitochondrial protein, 0.5 ml of 0.1 M sodium phosphate buffer of pH 7.4, 0.2 ml of 0.125 M semicarbazide hydrochloride of pH 7.0 and 0.2 ml of 0.1 M tyramine. The tubes containing incubation mixture were incubated in a shaker of 37° for 30 min. After incubation, 2 ml of 2:4 dinitrophenol solution (0.5 mg of 2:4 DNP per ml of 2 N HCl) was added to each tube and kept for 10 min. Four ml benzene was added to each tube, shaken well and centrifuged for 5 min. The benzene layer was separated carefully and added to 5 ml of 0.1 M NaOH solution. After vigorous shaking the benzene layer was discarded and the alkali layer was kept in a boiling water bath for 10 min, cooled to room temperature and the o.d. was measured at 450 mμ in a Carl Zeiss PMQII spectrophotometer. The enzyme activity was expressed as o.d./30 min/mg protein against a reagent blank. The protein was estimated according to the method of Lowry *et al.* [11] using bovine albumin as the standard.

Estimation of plasma fibrinogen

Fibrinogen was estimated from clear, platelet free plasma by allowing it to clot in the presence of thrombin or calcium and by measuring the protein content of the clot according to Raymond and Wilkinson [12] as reported earlier [1].

RESULTS AND DISCUSSIONS

The data presented in Table 1 show the effects of clorgyline and pargyline on rat plasma fibrinogen. Clorgyline seems to be more effective at lower doses. According to the recent concept there are two types of MAO, type A and type B [13]. Type A from rat liver is responsible for the oxidation of serotonin [14], adrenaline [15] and noradrenaline [16], whereas type B is responsible for the oxidation of benzylamine and 2-phenyl-ethylamine [17]. Tyramine, dopamine and kynuramine are substrates for both the forms [18]. At low doses clorgyline selectively inhibits A-form and pargyline selectively inhibits B-form, but at higher doses any of the inhibitors inhibit both A and B forms. Table 2 shows that when rats are pretreated with clorgyline which selectively inhibits MAO type A at a low dose, the effect of serotonin is magnified. This suggests that the amine itself and not its metabolites is responsible for the elevation of plasma fibrinogen level.

It has already been mentioned that MAO inhibitors may be classifed into long and short acting type in terms of their duration of effects. Effects of these two types of MAO inhibitors on inhibition of MAO and elevation of fibrinogen were next studied. Figure 1 shows in pargyline-treated rats, the liver MAO activity gets almost completely inhibited within 4–8 hr and continues to be inhibited even after 25 hr. The fibrinogen level of these rats starts increasing within 4 hr, reaches a maximum around 15 hr and

Table 2. Effects of serotonin on the plasma fibrinogen level of clorgyline-pretreated rats

System	Plasma fibrinogen (mg %) Mean ± SE	$(n)^a$	Percent stimulation
Control	339.5 ± 14.49	(6)	
Clorgyline (5 mg/kg)	485 ± 24*	(6)	42.6
Serotonin (10 μg/100 g)	456 ± 10†	(6)	34.11
Clorgyline (5 mg/kg) + serotonin (10 μg/kg)	574 ± 20‡	(6)	68.82

$(n)^a$ = No. of rats.
The rats were killed 24 hr after the injection of serotonin.
Clorgyline was injected 1 hr before serotonin injection.
* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ vs control.

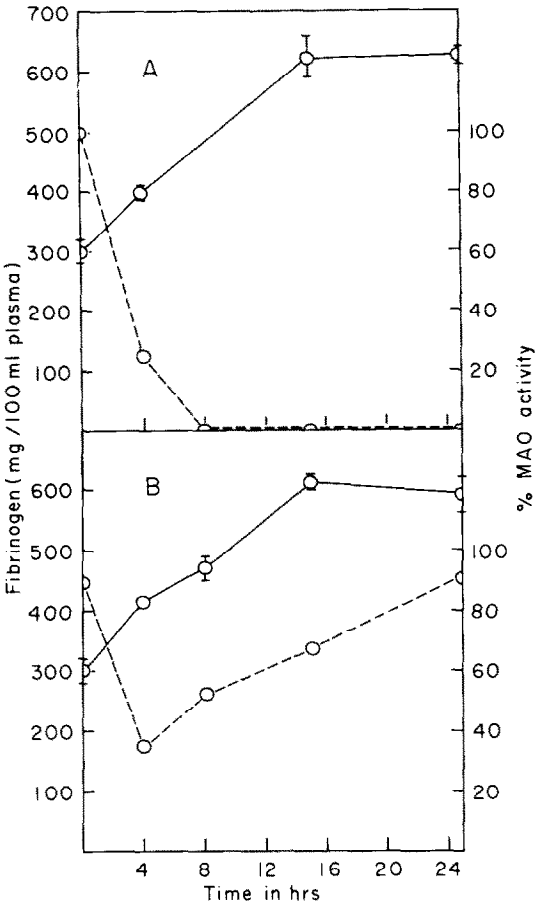

Fig. 1. Effect of pargyline (A) and harmaline (B) on plasma fibrinogen level and monoamine oxidase activity. Solid line indicates the plasma fibrinogen level and dotted line represents monoamine oxidase activity. The bars represent standard errors of mean and where no bars are shown these are less than the size of the symbols used. Five animals were used for each point.

continues to be at that level even after 25 hr. From this result and the results of our previous experiments, it seems likely that suppression of MAO level causes an increase in the level of endogenous monoamines which in turn elevates plasma fibrinogen. The fact that the injection of a similar type of MAO inhibitor clorgyline (as shown in Table 2) along with serotonin gives an additive result also supports the above hypothesis. On the other hand, harmaline, a short-acting inhibitor of MAO [4] produces maximum inhibition of liver MAO within 4 hr from the injection and then the inhibition is abolished gradually to reach almost to normal level in 25 hr. In this case also the fibrinogen level starts increasing within 4 hr as in the case of pargyline but continues to increase even when the inhibition of MAO activity is being reversed. The result indicates that no clear relationship exists between MAO inhibition and plasma fibrinogen level but suggests that a sudden rise in endogenous monoamines even for a short period due to harmaline action may be sufficient to trigger the reactions which ultimately lead to an elevation of plasma fibrinogen.

So far we have gathered evidence that epinephrine, serotonin, tyramine, etc. elevate plasma fibrinogen level. It appears that there might be a common mode of action and the effect of monoamines are mainly mediated through receptors. Since epinephrine activates both α- and β-receptors, but the elevation of c-AMP in liver is mediated only through β receptor [8], an attempt has been made to find out whether c-AMP elevates fibrinogen level like monoamines. The data presented in Table 3 shows that dibutyryl c-AMP elevates the fibrinogen level significantly. It is further seen that theophylline, which is known to elevate intracellular c-AMP level by inhibiting phosphodiesterase [19], not only showed the additive effect with dibutyryl c-AMP but elevates plasma fibrinogen level by itself and shows a concentration effect (Table 3). Theophylline also shows additive effect with serotonin and more than additive effect with dibutyryl c-AMP.

In order to verify whether a transient increase of monoamines can initiate fibrinogen formation, another set of experiments was designed. In this experiment, rats were injected with both DL-*p*-chlorophenyl-alanine (PCP) and α-methyl-*m*-tyrosine (α-MMT) to completely suppress the formation of serotonin and catecholamines and thereby deplete them. Delorme injected cats with a dose of 35–100 mg/kg body wt to produce maximum depletion of biogenic monoamines which occurred between 48–72 hr [20].

The drugs, PCP and α-MMT were injected both at a dose of 30 mg/kg/day to each rat (i.p.) at an interval of 1 hr for two consecutive days and on the 3rd day harmaline (with or without monoamine) was injected i.p. at a dose of 25 mg/kg. The rats were killed 25 hr after harmaline injection.

Table 3. Effect of dibutyryl c-AMP and theophylline on plasma fibrinogen level of rats

System	Plasma fibrinogen (mg %) Mean ± SE	$(n)^a$	Percent stimulation
Control	332 ± 31	(6)	
Dibutyryl c-AMP (200 μg/100 g)	448 ± 13.06†	(4)	34.93
Theophylline (2 mg/100 g)	381 ± 20*	(6)	14.75
(3 mg/100 g)	454 ± 32‡	(6)	36.74
(6 mg/100 g)	558 ± 24§	(6)	68.08
Theophylline (3 mg/100 g) + dibutyryl c-AMP (200 μg/100 g)	681 ± 16.32‖	(4)	105.0
Theophylline (3 mg/100 g) + serotonin (10 μg/100 g)	622 ± 32‖	(4)	80.87

$(n)^a$ = No. of rats.
* Not significant; † $P < 0.02$; ‡ $P < 0.05$; § $P < 0.01$; ‖ $P < 0.001$ vs control.
The rats were killed 24 hr after the injection of dibutyryl c-AMP, or serotonin. Theophylline was injected 1 hr before these two agents.

Table 4. Effect of harmaline, serotonin and epinephrine on the fibrinogen level of PCP and α-MMT treated rats

System	Fibrinogen (mg %) Mean ± SE	Stimulation (%)
Control	272 ± 13.85	
PCP + α-MMT	240 ± 12.68	
Harmaline	492 ± 49.2*	81
Harmaline + PCP + α-MMT	266 ± 14.14	
Harmaline + PCP + α-MMT + serotonin (10 μg/100 g B.W.)	362 ± 5.36*	33
Harmaline + PCP + α-MMT + epinephrine (5 μg/100 g B.W.)	597 ± 23.27†	119

* $P < 0.01$; † $P < 0.001$ vs control.

The data presented in Table 4 show that injection of harmaline has no effect on plasma fibrinogen level of PCP plus-MMT treated rats. However, administration of epinephrine or serotonin to PCP, α-MMT and harmaline injected rats produced marked elevation of fibrinogen level. The results presented in this communication clearly indicate that the MAO inhibitors elevate plasma fibrinogen through accumulation of biogenic monoamine as harmaline itself could not elevate the fibrinogen level in PCP plus α-MMT pretreated rats where formation of epinephrine and serotonin was blocked. Pretreatment of PCP, α-MMT and harmaline, however, does not affect the capability of epinephrine or serotonin to demonstrate their respective effect on plasma fibrinogen.

All these results clearly show that monoamines elevate the plasma fibrinogen level and the effect is due to monoamines and not due to their metabolite(s). Evidence presented in this communication further suggests that the effect of monoamines may be mediated through the elevation of c-AMP. Experiments will be conducted to determine the level of c-AMP under the above experimental conditions which we could not determine due to our limitations.

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