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STUDIES ON THE EFFECTS OF SOME BIOGENIC AMINES ON PLASMA FIBRINOGEN LEVEL OF RATS AND RABBITS

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Abstract—Intramuscular administration of iproniazid caused significant elevation of plasma fibrinogen in rats and rabbits. The elevation may be due to accumulation of monoamines, as the drug inhibited plasma monoamine oxidase quite strongly. This possibility was further supported by the fact that the direct administration of 5HT also elevated the plasma fibrinogen level by about 50 per cent. The effect of 5HT was more pronounced (about 75 per cent) when rabbits were pretreated with reserpine. The above observation has also been confirmed by reserpine-treated rats where different biogenic amines like 5HT, tryptamine, tyramine and epinephrine at low doses elevated plasma fibrinogen level. These biogenic monoamines possibly mediated their action through synthesis of protein since the increase of plasma fibrinogen level after 5HT administration was prevented by pretreatment of animals with actinomycin D or cyclohexamide.

It has recently been reported from our laboratory that plasma fibrogen level is significantly increased in eclampsia, a pathogenic condition of pregnancy, compared to normal pregnant women [1]. Earlier reports indicate that the monoamine oxidase (MAO)† is reduced in placenta in eclampsia [2]. The MAO activity in subcellular fractionation of the eclamptic sample reveals that the reduction is more pronounced in the soluble supernatant fraction [3]. In order to examine whether these two observations are related to each other, iproniazid, a potent inhibitor of MAO, was injected into pregnant and non-pregnant rabbits. The results indicate that iproniazid administration to the pregnant rabbit reduces MAO and elevates plasma fibrinogen level. However the plasma fibrinogen level is also found to increase in non-pregnant rabbit under the same experimental conditions. This finding suggests that a relationship might exist between the reduced MAO and increased plasma fibrinogen value without having any special significance to pregnancy. In the present communication we report the effect of some biogenic monoamines on the plasma fibrinogen level in rabbits and rats.

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† *Abbreviations*—5HT, serotonin; MAO, monoamine oxidase.

MATERIALS AND METHODS

Estimation of fibrinogen. Plasma fibrinogen was assayed 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 [4]. The incubation mixture consisted of 0.1 ml plasma, 2 NIH units of thrombin or 0.5 ml calcium chloride (0.25%)

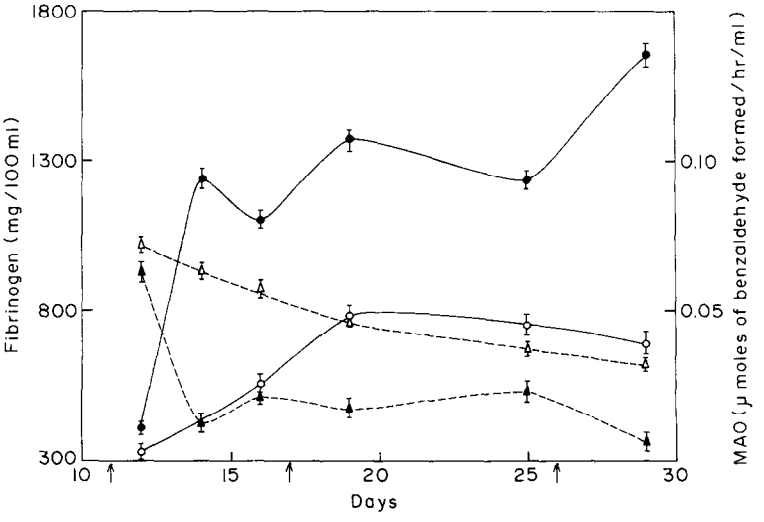

Fig. 1. Effect of iproniazid on the plasma fibrogen level and plasma monoamine oxidase of pregnant rabbits. Continuous lines and dashed lines represent fibrinogen level and monoamine oxidase activity respectively. Arrows at the *x*-axis indicate days of iproniazid injection. △ and ○ Control; ▲ and ● iproniazid.

and 0.5 ml sodium chloride (0.154 N) in a final volume of 1.1 ml. After incubation (30 min) at 37°, the clot was removed carefully and washed with sodium chloride (0.154 N) and dissolved in 0.5 ml sodium hydroxide (3%). Protein content of the dissolved clot was determined, after the addition of 0.5 ml biuret reagent, by measuring the optical density at 560 mu in a Carl Zeiss PMQ II spectrophotometer.

Assay of MAO. The plasma MAO activity was determined according to the modified method of McEwen [5]. The incubation mixture contained 0.6 ml sodium phosphate buffer (0.2 M; pH 7.4) and 0.4 ml of benzylamine (10 mM) and 0.5 ml plasma in a final volume of 3.0 ml. After 30 min of incubation at 37° the reaction was stopped by the addition of 0.15 ml of 60% perchloric acid and the benzaldehyde formed was extracted with 5 ml of cyclohexane. The absorbancy was measured at 242 mu in a Zeiss PMQ II spectrophotometer in 1 ml cuvette of 1 cm light path. Enzyme activity is expressed as μmoles of benzaldehyde formed/hr/ml.

Treatment with reserpine and amine. Rats or rabbits were treated with reserpine at a dose of 5 mg/kg body wt, intraperitoneally. After 20–22 hr various amines were injected intramuscularly with various doses as indicated in the tables and blood was collected after 24 hr from the injection of the amines.

RESULTS

There was a linear production of benzaldehyde from benzylamine in presence of plasma MAO for a period of 60 min beyond which estimations were not made. In all our subsequent experiments with plasma MAO, we restricted the incubation time to 30 min so that the initial rate of MAO could be correctly measured in our assays. Figure 1 shows that administration of iproniazid (100 mg/kg body wt) on days 11, 17 and 26 of gestation to pregnant rabbits caused significant decreases of plasma MAO activity as well as increases of plasma fibrinogen level as measured on days 12, 14, 16, 19, 25 and 29 of pregnancy. It can be seen further that, although there was a gradual decrease of MAO activity with the progress of gestation period, the decrease was many times more in iproniazid treated animals. As the MAO activity decreased the fibrinogen level increased with the progress of time.

The above experiment tempted us to investigate whether a single dose of iproniazid elevates the plasma fibrinogen level and whether the effect of iproniazid is specific to pregnant rabbits. Table 1 shows that the single dose of iproniazid elevated the plasma fibrinogen level and that the effect is not restricted to pregnant rabbits only. After 42 hr from iproniazid administration the MAO activity was inhibited by about 65 per cent, whereas at the same time the plasma fibrinogen level was elevated by 210 per cent. The results presented in Table 1 and Fig. 1 suggest that iproniazid might have elevated the plasma fibrinogen level through accumulation of biogenic amines. In order to test the above hypothesis, 5HT, one of the biogenic amines, was administered in rabbits, and plasma fibrinogen level was estimated. Table 2 shows that fibrinogen was elevated by 35 per cent after 24 hr of 5HT injection. The per cent elevation was more at 48 hr and 72 hr and the values were approximately 40 and 45 per cent respectively. It may be noted that a fairly high dose of 5HT was required to obtain this effect; hence in the next experiment, reserpine was injected to deplete the endogenous amines, and the action of 5HT on plasma fibrinogen was examined. Table 3 shows that a low dose of 5HT (5 μg/kg body wt) had a very slight effect, whereas with increasing doses of 5HT the fibrinogen level increased gradually. The increase was very pronounced compared to the previous experiment, as 45 μg of 5HT produced an elevation of 75 per cent in this experiment. Incidentally, reserpine itself did not affect the fibrinogen level. From the above results it seems clear that 5HT elevates plasma fibrinogen level. As rats are more

Table 2. Effect of repeated dose of 5HT on plasma fibrinogen level in rabbit

Hours after 5HT injection	Plasma fibrinogen (mg%)		
	Control	Control + 5HT *	Stimulation (%)
24 hr	437.1 ± 10.5	575.3 ± 13.2	35
48 hr	430.5 ± 15.1	600.8 ± 15.5	42
72 hr	425.6 ± 12.2	625.2 ± 17.4	47

* Total 100 μg of 5 HT/kg body wt injected intramuscularly in 2 equal doses at 2 hr intervals. (The control animals received same volume of normal saline at the same time.)

Table 1. Effect of iproniazid on plasma monoamine oxidase and fibrinogen level of non-pregnant rabbit

Time	Monoamine oxidase activity (μmoles/hr/ml)			Fibrinogen level (mg/100 ml)		
	Control	Control + iproniazid *	Inhibition (%)	Control	Control + iproniazid *	Stimulation (%)
0 hr†	0.056 ± 0.005	0.058 ± 0.004		382.2 ± 10.5	320.5 ± 13.2	
42 hr‡	0.054 ± 0.008	0.019 ± 0.006	64	325.6 ± 12.1	1000.0 ± 22.4	212

* 100 mg/kg of iproniazid was injected intramuscularly. Control group received same volume of saline.
† Blood was collected just before saline or iproniazid administration.
‡ Blood was collected after 42 hr of saline or iproniazid administration.

Table 3. Effect of varying dose of 5HT on plasma fibrinogen level in reserpine-treated rabbit

Group	Dose of 5HT (μg/kg of the body wt)	Plasma fibrinogen (mg%)	% Stimulation
Control	—	430.0 ± 12.20	—
Control + reserpine	—	425.6 ± 11.95	—
Control + reserpine + 5HT	5.0	475.0 ± 13.75	—
	12.0	525.0 ± 16.62	23
	22.5	576.2 ± 15.15	34
	45.0	750.0 ± 22.10	74

The results given above are average of three sets of experiments.

Table 4. Effect of different amines on plasma fibrogen level in reserpine-treated rat

	Dose of the amines (μg/100 gm body wt)	Plasma fibroginogen (mg%) Mean ± S.E. (*n*)*
Control	—	437 ± 10.6 (12)
Control + 5HT	2.5	556.0 ± 52.32 (5)
	5.0	816.5 ± 12.78 (10)
	10.0	713.0 ± 37.82 (6)
Control + tryptamine	2.5	800.0 ± 38.6 (6)
	10.0	725.0 ± 22.01 (6)
Control + tyramine	2.5	896.0 ± 50.60 (5)
	10.0	747.0 ± 50.70 (6)
Control + epinephrine	0.5	650.0 ± 14.20 (5)
	2.0	825.0 + 20.15 (5)
	5.0	900.0 ± 22.05 (5)

* n = number of rats.

economical and convenient as laboratory animals, the effect of biogenic amines on reserpinized rats was tested in the next experiment.

Table 4 shows that, besides 5HT, tryptamine, tyramine and epinephrine also elevated the plasma fibrinogen level.

In order to investigate whether the effect of 5HT is mediated through protein synthesis, 5HT was injected in actinomycin D- or cyclohexamide-treated rats. The preliminary results indicate that these inhibitors of protein synthesis prevented the increase of plasma fibrinogen level in actinomycin D- or cyclohexamide-treated rats (Table 5). Actinomycin D or cyclohexamide did not significantly affect the fibrinogen level when they were injected (results not shown).

DISCUSSION

In the present communication we report the effect of some monoamines on the plasma fibrinogen levels of rats and rabbits. Reduction of monoamine oxidase and elevation of plasma fibrinogen level as a result of administration of iproniazid in rabbits directly points to the involvement of monoamine in raising the fibrinogen level. Due to the unavailability of a spectrofluorometer, microquantity changes of any specific amine in circulation could not be measured, although Koren *et al.* [6, 7] demonstrated the gradual rise of placental 5HT during pregnancy. Hence the effects of administration of different biogenic amines on plasma fibrinogen level in both normal and reserpine-treated animals were tested.

Table 5. Effect of serotonin on plasma fibrinogen level in actinomycin D- and cyclohexamide-treated rats

Group	Plasma fibrinogen value (mg %)
Control	450.0 (4)
Control + 5HT*	825.0 (4)
Control + 5HT* + cyclohexamide	550.0 (4)
Control + 5HT* + actinomycin D	565.0 (4)

Numbers in parentheses indicate the number of rats.

* 5 μg/100 g body wt of 5HT injected intramuscularly. 50 μg/100 g body wt of antinomycin D injected. 150 μg/100 g body wt of cyclohexamide injected.

Following iproniazid administration, plasma MAO decreased as expected, but elevation of plasma fibrinogen level was found to be interesting. Recently, it has been reported from our laboratory that fibrinogen level is elevated in eclampsia [1] and reduction of MAO in eclampsia has already been reported earlier [2] and confirmed by us [3]. Hence, it was thought interesting to see whether iproniazid administration could be used to produce an experimental animal model for eclampsia. However, our attempts to produce this were frustrated, as iproniazid administration in non-pregnant animals also elevated the fibrinogen level. However, this experiment opened up another horizon of investigation, that is, the role of biogenic amines as regulators of plasma fibrinogen level. Slow and steady increase of plasma fibrinogen level [8] and low but significant reduction of MAO with progress of gestation in normal pregnancy has already been reported [6]. Our results also confirm these findings (see Fig. 1). The situation has actually been magnified on iproniazid administration.

Reserpine is known to deplete biogenic amines [9]. In our experiment also, much lower doses were required to significantly elevate the fibrinogen level in reserpine-treated animals than in their normal counterparts. In reserpinized rat the tyramine, epinephrine and tryptamine are more effective than 5HT in the elevation of plasma fibrinogen level. It has already been reported that plasma amine oxidase in some species has very low activity towards serotonin [10, 11]. In the present report it is not clear whether the effect of these biogenic amines is mediated through monoamine oxidase, as all the biogenic amines, including serotonin, tested in these experiments, elevated plasma fibrinogen level. From the preliminary results it seems that the effect of these biogenic amines is mediated through protein synthesis. However, it is difficult to say at the moment whether the effects of these amines are specific to fibrinogen biosynthesis or apply to generalized protein synthesis. The half-life of fibrinogen is 2–4 days [12] and the further possibility that these amines increase the fibrinogen level by decreasing the rate of degradation cannot be ruled out. Studies are in progress to investigate the effect of these amines on fibrinogen biosynthesis and degradation.

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