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THE EFFECT ON HUMAN MONOAMINE OXIDASE ACTIVITY OF SUBCUTANEOUS INJECTIONS OF ADRENALINE

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Abstract—Subcutaneous injections of adrenaline in human subjects resulted in small but significant increases in platelet MAO activity 20 min after administration. There were also significant increases in platelet counts 20, 40 and 60 min after injection with counts returning to baseline values after 80 min. It is suggested that the increase in platelet MAO activity produced by stress, at least in as far as it is mimicked by the effect of subcutaneous injections of adrenaline, is too small to have an important bearing on the controversy over the activity of the enzyme in schizophrenia.

INTRODUCTION

The activity of platelet monoamine oxidase (MAO, -monoamine -O_2 oxidoreductase (deaminating), E.C. 1.4.3.4) has been reported to be significantly reduced in schizophrenic patients by some groups of workers [1–5] and to be normal by others [6–10].

A number of factors are known to affect the activity of platelet MAO, including sex [11], phase of the menstrual cycle [12] and iron deficiency [13]. Reductions in platelet MAO activity have also been reported in migraine [14, 15] and in Down's Syndrome [16]. However there is no satisfactory explanation for the conflicting reports of the activity of platelet MAO in schizophrenia.

Recently, Gentil *et al.* [17, 18] reported that subcutaneous injections of adrenaline produced rapid elevations in platelet MAO activity, in human subjects, and suggested that 'stress' might be an important determinant of platelet MAO activity. In view of the relevance of these reports to the controversy over the activity of the enzyme in schizophrenia we attempted to verify the findings of Gentil and his colleagues.

MATERIALS AND METHODS

Chemicals. Adrenaline bi-tartrate in aqueous solution (1:1000) was obtained in sealed ampoules from MacCarthy's Ltd., Essex. Water, as placebo, was obtained in sealed ampoules from Antigen Ltd., Ireland. Benzylamine (free base, Sigma, London) was converted to the hydrochloride and re-crystallized from ethanol. [*methylene*-^{14}C] Benzylamine HCl 12.5 mCi/m-mole was obtained from ICN Pharmaceuticals, CA, and diluted with non-radioactive benzylamine HCl to the required specific activity. All other chemicals were of the highest grade commercially obtainable.

Experimental design. Twelve, male volunteers (mean age 28.5 years $\pm$ 4.5) were randomly allocated to two groups of six subjects receiving apparently identical injections of adrenaline bi-tartrate (14.3 μg/kg) or placebo (water). The injections were administered subcutaneously over the left deltoid. Blood was drawn from an in-dwelling catheter in the ante-cubital vein immediately prior to the injection and at 20, 40, 60 and 80 min after the injection. 20 ml of blood was mixed with 5 ml ACD anticoagulant for subsequent determination of platelet MAO activity and 5 ml of blood was collected into EDTA for determination of platelet counts. Until all estimations were completed only the physician administering the injections knew which subjects were receiving adrenaline or placebo.

Determination of platelet MAO activity. Platelet MAO activity was determined by a radiometric technique based on the method of Robinson *et al.* [19]. Briefly, platelets were separated by differential centrifugation as described previously [9]. Platelet samples were frozen and thawed twice and 100 μl (100–250 μg protein) of the preparation was incubated in a reaction volume of 0.5 ml containing [^{14}C]benzylamine as substrate at 10^{-3} M (specific activity 0.3 μCi/μmole) and at the much lower concentration of 2.1×10^{-5} M (specific activity 4 μCi μmole) as used by Gentil *et al.* [17] and phosphate buffer pH 7.2, 0.04 M final concentration. Incubations were carried out at 37° for 30 min. Reactions were stopped by the addition of 50 μl of 6 N HCl and the products extracted into 2 ml of toluene. The amount of product formed was quantified by scintillation counting and platelet protein content determined by a phenol reagent technique [20]. Results were expressed as nmoles of product formed/mg platelet protein/30 min. All assays were carried out in duplicate.

Determination of platelet counts. Platelet counts were determined by an automated technique using a Coulter Counter (model S).

RESULTS

Figure 1 illustrates the effect of adrenaline or placebo injections on platelet MAO activity at both concentrations of benzylamine in the enzyme assay. At the lower substrate concentration (2.1×10^{-5} M) there was a small but not significant increase in

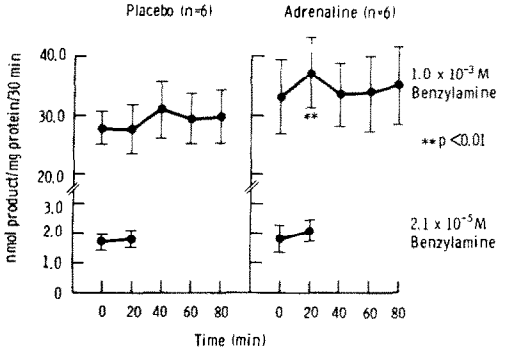

Fig. 1. The effect of placebo and adrenaline on platelet MAO activity.

platelet MAO activity in both groups of subjects. At the 10^{-3} M benzylamine concentration there was a significant increase in platelet MAO activity ($P < 0.01$, paired t test) only in the group receiving adrenaline and only in the 20 min post-injection samples.

The individual changes in platelet MAO activity 20 min after the injection of adrenaline are presented in Table 1.

Figure 2 shows the effect of adrenaline and placebo on platelet counts. In the group receiving adrenaline there was a marked and significant increase in platelet counts 20 min ($P < 0.001$), 40 min ($P < 0.05$) and 60 min ($P < 0.05$) after the injection with counts returning to baseline after 80 min. Placebo injections had no significant effect on platelet counts.

DISCUSSION

The increase in platelet MAO activity observed after adrenaline injections when benzylamine at 10^{-3} M was used as substrate in enzyme assays was small (i.e. from 33.2 ± 6.3 to 37.3 ± 5.9 nmoles of product formed/mg protein/30 min) but this increase occurred systematically in every sample resulting in a paired t test being statistically significant ($P < 0.01$). However, at the lower substrate concentration of 2.1×10^{-5} M used by Gentil *et al.* [17] there was no significant increase in platelet MAO activity after administration of adrenaline.

The marked elevation in platelet counts in all subjects 20 min and to a lesser extent 40 and 60 min after the injection of adrenaline but not placebo differs from the findings of Gentil *et al.* [18]. Aster [21] reported that there was generally, but not systematically, an increase in platelet counts after subcutaneous or intramuscular injections (0.5–1.0 mg) of adrenaline in man. Lower doses, given by slow infusion led to systematic increases (averaging 45 per cent) in platelet counts after 15 min with a return to baseline values 10–15 min after cessation of the infusion. Bearing in mind the expected slower effect of a subcutaneous injection Aster's findings are not dissimilar to those of the present study. Moreover, the dose of adrenaline administered by Aster (0.5–1.0 mg) was smaller than in this study (0.8–1.4 mg) and may account for his failure to observe a systematic increase in platelet counts after subcutaneous injection. Using ^{51}Cr labelled platelets Aster demonstrated the release of stored platelets from the spleen after adrenaline administration and such an efflux would explain the increase in platelet counts observed in this investigation.

Gentil *et al.* [17] suggested that platelets stored in the spleen and released in response to adrenaline administration might contain a more active form of MAO and account for the observed increase in enzyme activity. We pooled platelets from those subjects who had received adrenaline and also from those who had received placebo for determination of Michaelis Constants. The K_m values of MAO for benzylamine obtained from double reciprocal plots were not significantly different for the two groups—2×10^{-4} M for subjects receiving adrenaline and 1.6×10^{-4} M for those on placebo. So although there does appear to be an increase in platelet MAO activity after subcutaneous administration of adrenaline it seems unlikely that there are significant

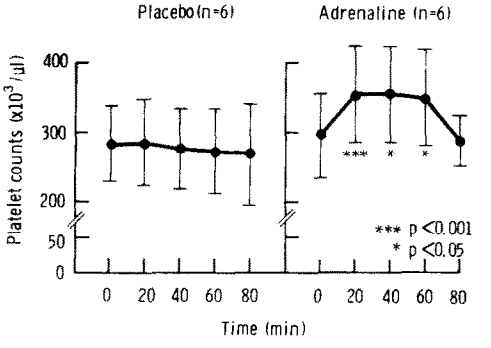

Fig. 2. The effect of placebo and adrenaline on platelet counts.

Table 1. Human platelet MAO activity (nmole product/mg protein/30 min) before and after subcutaneous injection of adrenaline (14.3 µg/kg body weight)

Subject	Pre-injection	+20 min	% Change
1	43.1	47.1	+9.3
2	30.5	31.8	+4.3
3	29.0	32.6	+12.4
4	33.4	39.0	+16.8
5	37.5	40.0	+6.7
6	25.8	33.1	+28.3
Mean ± S.D.	33.2 ± 6.3	37.3 ± 5.9	13.0 ± 8.7
	(Paired $t = 4.61$, $P < 0.01$)		

changes in the characteristics of MAO after adrenaline administration, and a release by adrenaline of newly formed platelets containing MAO of a higher specific activity would fit our data.

The investigations of platelet MAO activity in schizophrenia have been carried out at or near saturating substrate concentrations [1–10] and not at the very low concentrations used by Gentil and his coworkers. Tryptamine and tyramine have been the most commonly used substrates and Gentil *et al.* [18] did not observe a significant increase in platelet MAO activity after subcutaneous administration of adrenaline where tyramine or tryptamine were used as substrates in the assay procedure. We suggest, therefore, that the elevation in platelet MAO activity observed with benzylamine as substrate, which may be attributable to 'stress' at least in as far as it is mimicked by subcutaneous injections of adrenaline, is unlikely to play an important part in resolving the current controversy over the activity of platelet MAO in schizophrenia.

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