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EFFECTS OF SINGLE DOSES OF TRANYLCYPROMINE ON PLATELET MAO AND AMINE UPTAKE IN NORMAL SUBJECTS

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Abstract—Twelve normal volunteers were administered single doses of *dl*-tranylcypromine, 10 and 20 mg, and placebo under double-blind conditions using a balanced cross-over design. Blood samples were taken pretreatment and at intervals thereafter and assayed for platelet MAO activity and platelet uptake of 5HT, dopamine and metaraminol. Platelet MAO activity was markedly and significantly decreased after both doses of tranylcypromine and remained low for at least 24 hr. By contrast, amine uptake into the subject's platelets was slightly but not significantly reduced. *In vitro* studies yielded parallel results, tranylcypromine being much more potent in inhibiting platelet MAO than platelet amine uptake. Therefore, it seems unlikely that the antidepressive activity of the MAOI's might correlate more closely with their ability to inhibit catecholamine uptake than with their power to inhibit MAO, as has been suggested.

Although several current hypotheses of the biological correlates of affective disorders are based in part on the fact that some anti-depressive drugs are irreversible inhibitors of monoamine oxidase (MAO), no conclusive evidence supports any special effectiveness of these compounds in patients with endogenous depressions [1, 2]. Indeed there is no compelling evidence that any psychotropic effects are directly related to MAO inhibition.

Surprisingly, despite MAOI drugs having been available for 20 yr, only a few studies have directly monitored the time-course of human MAO inhibition by these drugs, and even fewer have attempted to correlate estimates *in vivo* of enzyme activity with clinical response to MAOIs [3–5].

In vitro studies with rat brain slices suggested that the anti-depressive effectiveness of MAOI drugs might correlate more closely with their ability to inhibit catecholamine uptake than with their power to inhibit MAO. Tranylcypromine (Parnate) was considered a highly effective clinical antidepressive and metaraminol uptake inhibitor, and a moderately effective inhibitor of MAO whereas phenelzine was classified as a powerful MAOI and moderately potent clinical antidepressive and inhibitor of uptake [6]. Concomitant effects *in vivo* of these drugs on human MAO and uptake activities have not been reported to date.

We therefore undertook a study in which tranylcypromine effects on human platelet MAO and amine uptake activities were determined in concert with its effects on a psychophysiological battery. This paper presents the results of the biochemical measures; the psychophysiological effects and the correlation between these sets of measures will be reported elsewhere [7].

MATERIALS AND METHODS

Subjects. Twelve normal, drug-free volunteers (eleven males), aged between 22–32 yr, and of average body build, took part in this experiment. The female subject was not on contraceptive steroids. They were fully informed about the experimental procedure and dangerous interactions of MAOI drugs and sympathomimetic amines. They were in good health with normal blood pressure, pulse rate and E.C.G.

Drugs. Tranylcypromine sulphate (*trans-dl*-2-phenylcyclopropylamine $1/2\,H_2SO_4$, Parnate, 10 mg) and matching placebo tablets were available. Two doses, 10 and 20 mg, were chosen after preliminary experiments had shown them to produce less than total MAO inhibition. Their effects were compared with those of placebo, each subject receiving all three treatments. Tablets were ingested with 100 ml of tap water at time zero.

Design. The experiment was performed under double-blind conditions, with balanced cross-over design [8]. Subjects came to the laboratory at 9 a.m. after fasting overnight. Blood samples were taken three times on each of the three occasions (pretreatment and 1 and 3 hr after treatment). Extra samples of blood were taken from six subjects, 5 and 24 hr after treatment. Two weeks intervened between occasions to minimise carry-over of drug effects.

Biochemical methods

Preparation of platelets. Blood samples were taken by antecubital venepuncture with all-plastic syringes and siliconed needles. All glassware was silanized. Whole blood (30 ml samples) was anti-

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coagulated with freshly prepared sodium citrate (3.8% w/v, 1 ml/100 ml of blood) and immediately centrifuged at 4° (15 min, 180 *g*) to sediment red and white cells. The platelet-rich plasma (PRP) was carefully transferred to another glass centrifuge tube and further processed:

For MAO assay the PRP was centrifuged at 2000 *g* 4° for 20 min. The platelet plug was washed and suspended in ice-cold sucrose (0.3 M) and sonicated at low power for 30 sec to homogenize the enzyme distribution. The protein content was determined and adjusted to final concentration between 100–200 g/0.1 ml [9]. Between determinations the samples were kept frozen at −20°.

For uptake studies the PRP was centrifuged at 120 *g* for 5 min (4°) to decrease residual contamination by other cells and 1 ml of the resulting PRP was transferred to a silanized thin-wall, conical tube for uptake determinations.

MAO assay. Platelet MAO activity was determined by the method of Robinson, Lovenberg, Keiser and Sjoerdsma [10], as described by Collins and Sandler [11].

In vivo *studies.* The samples from the first two subjects were assayed with four substrates at concentrations equal to half their respective K_m: ^{14}C-phenylethylamine (NEN, 1.5 μM); 14C-benzylamine (ICN, 50 μM); ^{14}C-tryptamine (NEN, 6.5 μM); and ^{14}C-tyramine (Radiochemical Centre, 16 μM). The K_m value for PEA is lower than that generally accepted but it was established in preliminary experiments and is not dissimilar from some recent published values [12, 13]. Phenylethylamine (PEA) was also used at a $3 \times K_m$ concentration (9 μM). Preliminary experiments indicated that the reaction proceeds linearly in relation to time of incubation (at least up to 35 min) and to the protein content of the samples (up to 500 μg/0.1 ml) with the 4 substrates. The activities towards all substrates were highly significantly correlated and a single substrate (PEA, 9 μM) was used for the remaining samples. Pilot investigations revealed that platelet MAO is easily inhibited by higher concentrations of PEA. Results are expressed in nanomoles of product per mg of platelet protein per 30 min of incubation (37°, pH = 7.4).

In vitro *studies.* The effects *in vitro* of tranylcypromine sulphate on platelet MAO were determined using the same assay method with ^{14}C-benzylamine (final conc. = 0.5, 1.0, and $5.0 \times K_m$) and ^{14}C-PEA (final conc. = 0.5, 1.0 and $2.5 \times K_m$) as substrates. The enzyme was pre-incubated for 30 min at 37° with the drug in five different concentrations between 2×10^{-8} and 2×10^{-7} M (final conc.) before the addition of substrate and the reaction was allowed to proceed for a further 30 min (37°, pH = 7.4). The results were plotted to give the I_{50} and the curves were fitted by regression [13–15].

Uptake assay. The platelet uptake of 5-hydroxytryptamine (5HT), dopamine (DA), and metaraminol (MA) was determined by modification of the method of Sneddon [16].

In vivo *studies.* ^{14}C-5HT and ^{14}C-DA (Radiochemical Centre, sp. act. = 55 mCi/m-mole and 50 mCi/m-mole, respectively) were diluted in 0.001 N HCl to a final concentration of 10^{-6} M (5HT) and 10^{-5} M (DA). [^{3}H]MA (NEN, sp act. 300 mCi/m-mole) was diluted with non-radioactive MA in 0.001 N HCl to a final concentration of 10^{-5} M. Duplicate samples of PRP (1 ml) were pre-incubated for 10 min at 37° and the radioactive amine was rapidly added in a volume of 100 μl. Uptake was stopped by immersing the tubes in ice after 5 min (5HT) or 10 min (DA and MA) of incubation. Platelets were precipitated by centrifugation at 4000 *g* for 5 min (4°). Platelet-poor plasma was completely sucked off using a Pasteur pipette and the pellet washed once with cold saline. The inside of the tubes above the platelet sediment was wiped with filter paper to remove any remaining drops. Preliminary experiments showed that after resuspension of platelets and recentrifugation the supernatant was practically free of radioactivity. The platelet pellet was lysed in 1 ml of 0.1 N NaOH and the radioactivity was released by freezing and thawing. A 0.2 ml sample was mixed with 10 ml of insta-Gel (Packard) for liquid scintillation counting, and another 0.1 ml sample was used for protein determination [9]. Uptake was expressed as picomoles of amine per mg of platelet protein/min of incubation.

In vitro *studies.* The *in vitro* effect of tranylcypromine on the uptake of the three amines was studied by adding the drug dissolved in physiological saline (0.1 ml, final conc. = 10^{-6}, 10^{-5}, and 10^{-4} M) to the PRP 10 min before the addition of the amines. Pilot studies confirmed that 10 min preincubation with the inhibitor was sufficient for maximal results. The radioactive amines were used at four different concentrations (5-HT = 2×10^{-7}, 4×10^{-7}, 8×10^{-7}, 2.5×10^{-6} M; DA and MA = 2×10^{-6}, 4×10^{-6}, 8×10^{-6}, 2×10^{-6} M).

Analysis of the data. The results of the experiment *in vivo* were submitted to analysis of variance. Differences between treatment effects were obtained from the treatment × times interaction and their significance estimated against the within-subjects, within-occasions error variance. A Tukey "*t*"-test for differences between means with significant F-ratios was then computed [17].

RESULTS

MAO activity in vitro. Tranylcypromine sulphate was a non-competitive inhibitor of platelet MAO activity towards both substrates. with an I_{50} of 2.1×10^{-8} M for benzylamine and 3.9×10^{-8} M for PEA. Examples are shown in Figs 1 and 2.

MAO activity in vivo. Platelet MAO activity (PEA) was significantly decreased after the two doses of tranylcypromine ($F = 13.1$; 4,40 d.f.; $P < 0.01$). No significant difference was found between the two doses which produced a mean inhibition of 40 per cent at the first hr and 70 per cent at the third hr after treatment (Fig. 3). MAO activity remained inhibited to the same extent 24 hr after treatment.

Although preliminary data had suggested a return to pre-treatment levels after 1 week, order-effect analysis indicated a residual inhibition with the third week's mean activity being slightly but significantly

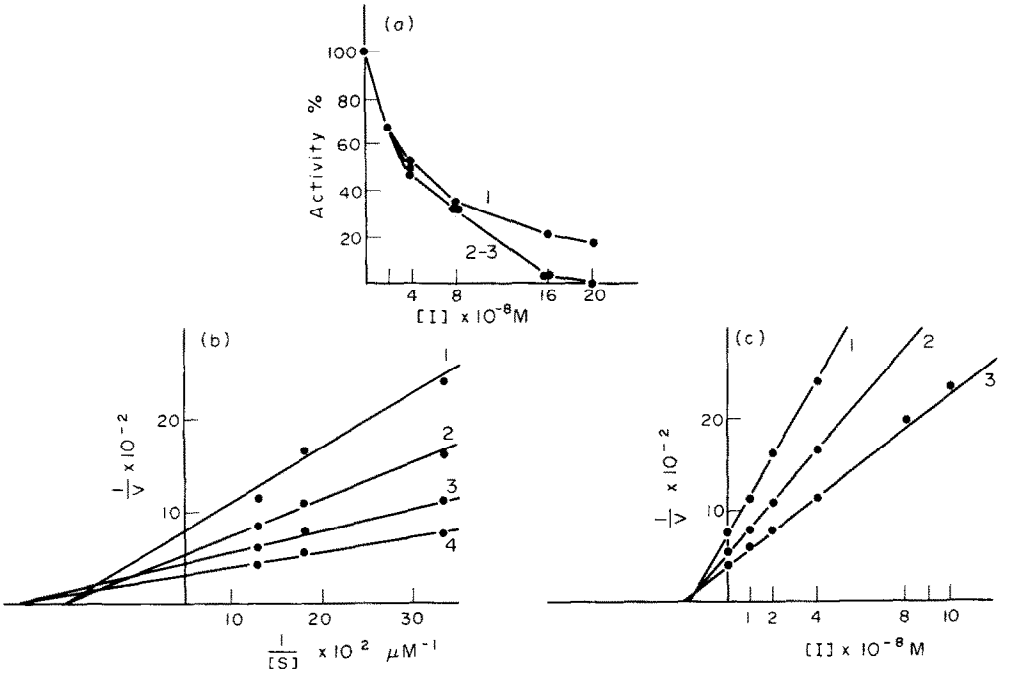

Fig. 1. Inhibition of MAO activity with benzylamine as substrate (platelets of subject nine). (a) Inhibitor concentration (abscissa); per cent activity (ordinate). 1: (S) = 491 μM, $I_{50} = 2.1 \times 10^{-8}$ M; 2: (S) = 94 μM, $I_{50} = 2.0 \times 10^{-8}$ M; 3: (S) = 48 μM, $I_{50} = 2.0 \times 10^{-8}$ M. (b) Lineweaver–Burk plot suggesting non-competitive inhibition. (c) Dixon plot suggesting non-competitive inhibition.

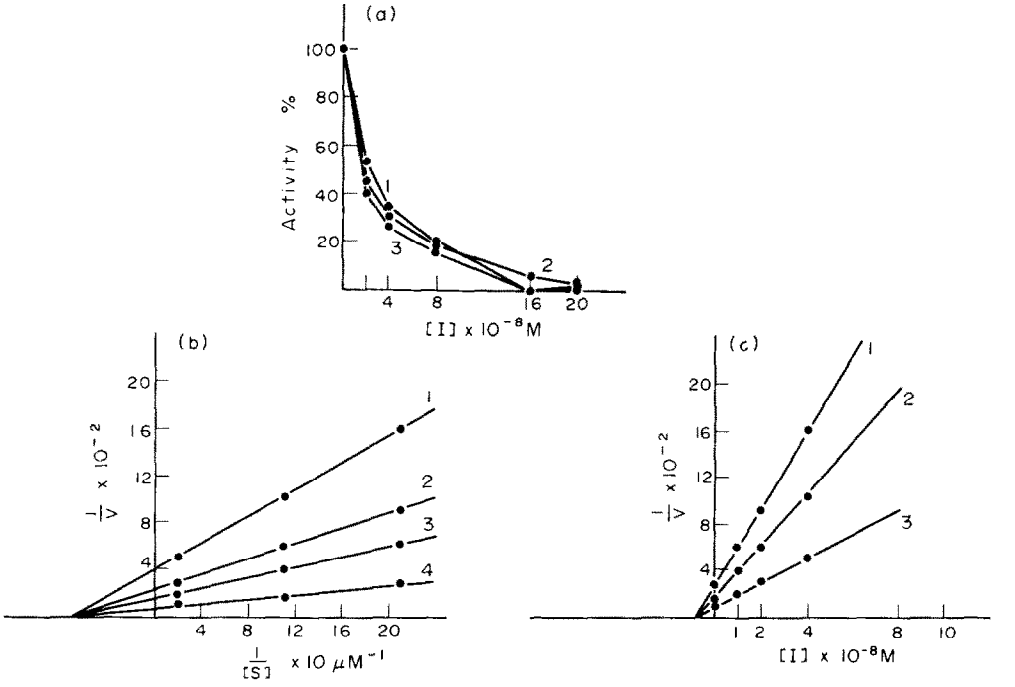

Fig. 2. Inhibition of MAO activity with B-PEA as substrate (subject nine). (a) Inhibitor concentration (abscissa); per cent activity (ordinate). 1: (S) = 7.7 μM, $I_{50} = 4.6 \times 10^{-8}$ M; 2: (S) = 3.6 μM, $I_{50} = 3.9 \times 10^{-8}$ M; 3: (S) = 1.8 μM, $I_{50} = 3.6 \times 10^{-8}$ M. (b) Lineweaver–Burk plot suggesting non-competitive inhibition. (c) Dixon plot suggesting non-competitive inhibition.

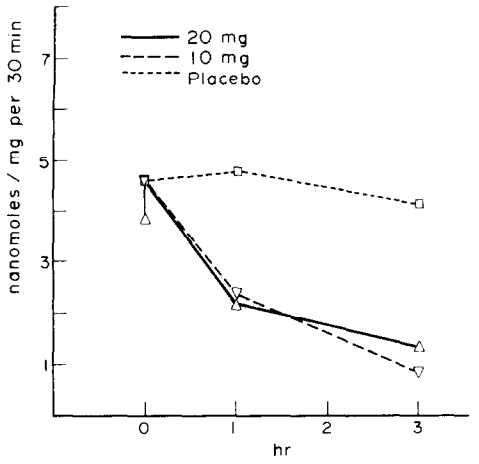

Fig. 3. Mean platelet MAO activity with β-PEA as substrate for twelve subjects before, 1 and 3 hr after placebo (□.....□), 10 mg (▽-----▽) and 20 mg (△——△) tranylcypromine. The vertical bar at time 0 is 0.05 critical difference: means lying further apart than this value are different at the 0.05 level of significance or beyond.

lower than that of the first ($F = 4.19$; 2,20 d.f.; $P < 0.05$).

Platelet uptake in vitro. Tranylcypromine slightly inhibited the uptake of 5HT, DA and metaraminol with a K_i of 0.9×10^{-4}, 1.07×10^{-4} and 1.75×10^{-4} M respectively.

Platelet uptake in vivo. 5HT uptake was slightly but not significantly reduced by 20 mg tranylcypromine at 1 hr. 5HT uptake was also slightly reduced by the 10 mg doses at 24 hr in the first six subjects from which extra samples were taken at 5 and 24 hr. DA uptake was decreased at 1 hr and to a lesser degree at 3 hr but the differences were not significant. Metaraminol uptake was also not significantly altered by tranylcypromine.

DISCUSSION

Racemic tranylcypromine was a much more potent inhibitor *in vitro* of monoamine oxidase than of amine uptake by human blood platelets. The K_i's for MAO inhibition were smaller than previously reported, but differences in substrates, substrate concentrations, and enzyme preparations make comparisons difficult [10]. Platelet uptake inhibition constants were higher than those reported for other

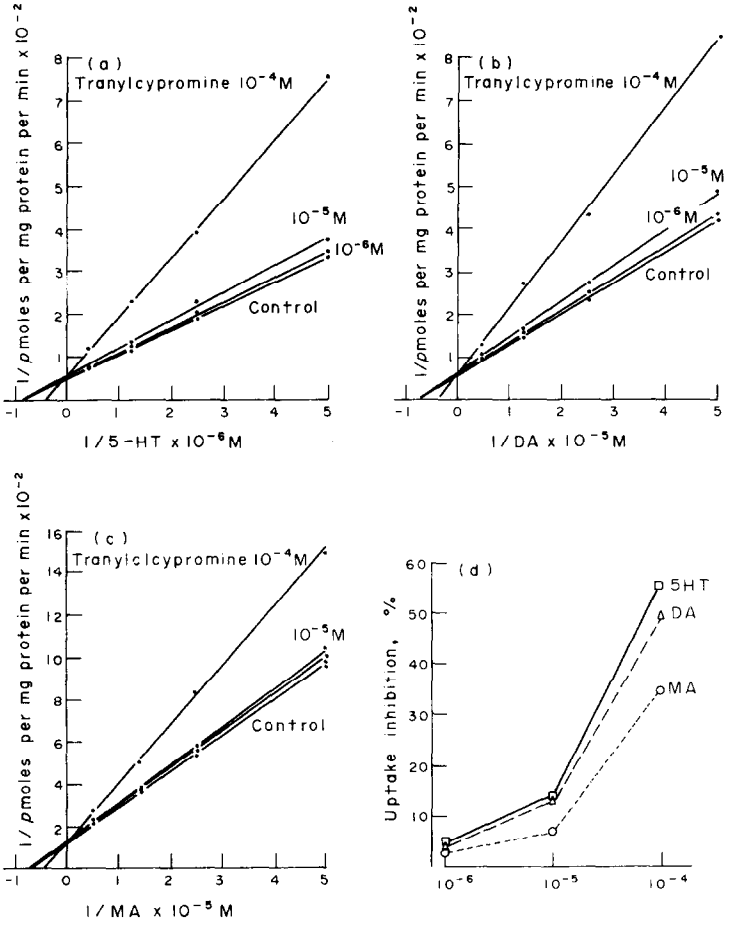

Fig. 4. Lineweaver–Burk plots for inhibition by tranylcypromine of platelet uptake of (a) 5HT; (b) dopamine; and (c) metaraminol, suggesting reversible inhibition at high concentrations. (d) Plot of concentration of amine (abscissa) against per cent uptake inhibition (ordinate) for the three amines.

tissues, but are in close agreement with the lack of effects *in vivo* in the present study and with the very slight inhibition of rat brain noradrenaline uptake after intracisternally administered tranylcypromine [6, 18–20].

The inhibition *in vivo* of platelet MAO was very marked after both doses of tranylcypromine, reaching a peak at the third and remaining at the same level for several hr. That single small doses of this drug can so quickly produce such an intense inhibition of platelet MAO limits the usefulness of this cell for attempts to relate MAO inhibition to clinical response as even a drug defaulter ingesting 10 mg of tranylcypromine 2 hr before blood sampling is likely to have 70 per cent inhibition of the enzyme. This may not be the case for less potent drugs, with which it may be possible to relate a range of enzyme activities to the known range of clinical responses to MAOIs.

Contrary to some suggestions tranylcypromine seems a potent and effective inhibitor of MAO but not of amine uptake activities. The available information on effects *in vivo* of tranylcypromine and phenelzine on human amine oxidase do not support a classification of the latter drug as the more potent or effective inhibitor [10, 21]. A better "correlation" between ability to inhibit re-uptake and clinical effectiveness of MAOIs as postulated by Hendley and Snyder therefore seems unlikely [6].

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