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# Specific 5-hydroxytryptamine binding to rat platelets as a system to evaluate tricyclic antidepressants in plasma

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Tricyclic antidepressants (TAD) are the major drugs now used in the treatment of mental depression. However only 65% of the patients given TAD show a clinical improvement [1]. Interindividual variation in plasma steady-state TAD levels as a consequence of the differences in the metabolism of these compounds has been claimed to be the reason for the lack of effects in some patients [2]. The routine determination of blood levels of TAD is therefore of great importance especially since both low and high plasma values has been reported to be correlated with a poor clinical response [3]. A number of different procedures are currently used to monitor blood levels of TAD; these are often time-consuming and complex [1, 4, 5] and an obvious place exists in clinical medicine for a simple and rapid biological assay to detect TAD and their active metabolites in plasma. The binding of 5-hydroxytryptamine (5-HT) to rat platelets at 4° demonstrated the presence of three saturable sites. Interestingly, the medium affinity site was found to be extremely sensitive to the inhibition by clomipramine and imipramine [6]. We report here the use of such a system as a bioassay to evaluate the concentration of TAD in plasma.

## *Materials and methods*

Blood was obtained from rats (200–300 g body wt) anesthetized with ether by puncture of the carotid artery. Samples were anticoagulated with trisodium citrate (final concentration in blood 2 mg/ml) and centrifuged at room temperature for 10 min at 180 *g*. The supernatant platelet-rich plasma (PRP) was removed and kept for a maximal time of 2 h at room temperature. Cell-free plasma was prepared by centrifugation of the PRP for 20 min at 10,000 *g*.

For the radioreceptor assay polypropylene incubation tubes received, in order, 0.04 ml of PRP containing an average of $40 \times 10^6$ platelets, either 0.05–0.20 ml of plasma (from drug treated or drug-free animals) or 0.1 ml of drug for the standard curve, 0.05 ml of 1 $\mu$M 5-hydroxy-[G-$^3$H]tryptamine creatinine sulphate (15.5 Ci/mmole; Radiochemical Centre, Amersham, U.K.) and a modified Tyrode solution (containing 136 mM NaCl, 3 mM KCl, 12 mM $NaHCO_3$, 0.4 mM $NaH_2PO_4$, 1 mM $MgCl_2$, 5 mM glucose and 3.5 g/l bovine albumin) to 1 ml total volume. After 2 min at 4° the bound [$^3$H]-5-HT was separated from the free fraction by centrifugation at 14,700 *g* for 2 min in the cold. The pellet was then rapidly washed (< 5 sec) with 1 ml of Tyrode solution and further digested with 0.25 ml 19 M formic acid in an oven heated at 80° for 10 min. The pellet was dissolved in PCS (Radiochemical Centre) and counted in a Packard Tri-Carb 2425 liquid scintillation counter. Blanks without PRP were run in parallel. Moreover, for each assay the amount of unspecific binding was evaluated by adding a 100-fold excess of unlabelled 5-HT. Under these conditions, approximately 5% of the radioactivity remained in the pellet and this value was subtracted from the total [$^3$H]-5-HT bound in order to correct for extracellular space and for that which was unspecifically bound to the platelets. Remarkably, as compared with experiments described in previous reports [6, 7] a higher sensitivity of this system to the TAD came about when the PRP was diluted with Tyrode solution.

For the *in vivo* experiments, rats were killed after being treated intraperitoneally with a dose of the drug. Plasma was obtained as indicated above.

*Source of drugs.* Nortriptyline hydrochloride, amitriptyline hydrochloride, and chlordiazepoxide hydrochloride from Hoffman-La Roche (Basel, Switzerland); clomipramine hydrochloride, imiprazine hydrochloride and desipramine hydrochloride from Ciba-Geigy (Basel, Switzerland); viloxazine hydrochloride from ICI-Farma (Madrid, Spain); and chlorpromazine hydrochloride from Barcia (Madrid, Spain). All drugs used were dissolved in 0.9% (w/v) NaCl.

## *Results*

A number of preliminary experiments confirmed that [$^3$H]-5-HT binding to platelets was reached after 2 min of incubation at 4°. Under the conditions selected for the assay no significant active transport occurs [6] and Scatchard analysis of the binding within the concentration range 0.0001–10 $\mu$M of [$^3$H]-5-HT gave a curvilinear relationship from which three sites could be resolved. The apparent dissociation constants for these sites were $10^{-9}$ M, $4 \times 10^{-7}$ M and $3.5 \times 10^{-6}$ M. On the basis that, as in previous reports [6, 7], the number of high affinity sites was found to be much lower than the medium affinity sites, a

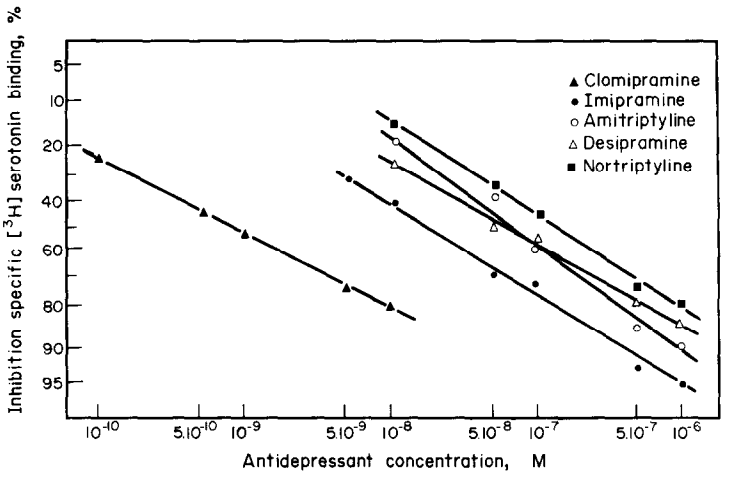


Fig. 1. Log-probit plots of tricyclic antidepressant inhibition of specific [$^3$H]-5-hydroxytryptamine ([$^3$H]-5-HT) binding to rat platelets. Different concentrations of the compound were incubated together with the labeled ligand, and the platelet-rich plasma as described in Materials and Methods. The bound [$^3$H]-5-HT was evaluated in the precipitate after high speed centrifugation and compared to the amount of ligand bound in control samples that were run in parallel.

concentration of $5 \times 10^{-8}$ M of [$^3$H]-5-HT gave binding that could be inhibited by about 90% by TAD (Fig. 1) while it was sensitive to cinanserin and D-LSD (results not shown).

As expressed in Fig. 1, a progressive increase in the concentration of TAD produced a linear displacement in the amount of radioactivity bound to the medium affinity 5-HT receptor with dibenzazepines showing a higher inhibitory potency than the dibenzocycloheptadiene derivatives (amitriptyline and nortriptyline). As a requirement for the validation of the assay no TAD was found to modify the grade of unspecific binding within the range of concentrations added to the incubation mixture.

By constructing a standard inhibition curve of specific $^3$H-ligand binding by known amounts of the drug, the quantity present in an unknown plasma sample could be easily determined. A log-probit plot was used to transform the displacement curve into a straight line so that percentage inhibition of [$^3$H]-5-HT binding could be converted into molar drug concentration. In this assay, when using plasma samples, no extraction or other purification procedure is required. Interestingly, up to 0.2 ml of TAD-free human or rat plasma could be used without observing any variation in the maximal amount of [$^3$H]-5-HT bound.

As shown in Table 1, this bioassay is sensitive enough to detect the drug levels generally found in plasma from patients treated with TAD. Moreover, even at brief periods of time (3 hr) after the intraperitoneal administration of either clomipramine (5 mg/kg) or imipramine (10 mg/kg), TAD doses that were found to be active in a rat model of depression [8], the amount of drug measured in rat plasma (clomipramine: 4.1–5.9 ng/ml; imipramine: 54.8–63.3 ng/ml) could be readily determined. In the plasma of a patient treated with a dose of 100 mg of clomipramine, levels of 9.7 mg/ml of the TAD were detected as early as 12 hr after the administration of the drug.

Occasionally, neuroleptic drugs are prescribed to be taken at the same time as TAD for the treatment of mental depression. More often, anxiolytics are used as coadjuvants of the antidepressive drugs. Concentrations of chlordiazepoxide (10 $\mu$M) higher than the levels expected to be found in plasma after its administration produced no significant change in the [$^3$H]-5-HT specific binding. However, chlorpromazine at 10 $\mu$M blocked the binding process by more than 80%.

Interestingly, viloxazine, one of the so-called atypical antidepressants that do not fulfill the traditional pharmacological criteria for antidepressive activity [9] was capable of displacing [$^3$H]-5-HT binding by 26%, at a concentration as low as $10^{-8}$ M.

*Discussion*

In the present study, the existence of three different binding sites for [$^3$H]-5-HT on rat platelets has been confirmed [6]. Moreover the medium-affinity site has been found to be sensitive to increasing concentrations of TAD

Table 1. Lower limit of sensitivity for the radioreceptor assay as compared to the concentration of tricyclic antidepressant in human plasma

| Drug | Lower limit of sensitivity (ng/ml) | Plasma levels (mean value in ng/ml) |
|---|---|---|
| Clomipramine | 0.1 | 137 [11] |
| Imipramine | 2.3 | 55 [1] |
| Desipramine | 6.0 | 303 [1] |
| Amitriptyline | 14.6 | 114 [12] |
| Nortriptyline | 20.0 | 96 [13] |

* The lower limit of sensitivity refers to the concentration of drug present in 0.15 ml of plasma that induces a 15% of inhibition of the specific [$^3$H]-5-HT binding. The mean values of the TAD levels in human plasma were compiled from different reports and correspond to concentrations of the compound in plasma after at least one week of treatment.

in such a way that unknown quantities of these compounds can be determined in the plasma of chronically treated patients or experimental animals.

Other methods commonly used to detect antidepressive drugs include gas–liquid chromatography (GLC) [10] and radioimmunoassay [1]. The radioreceptor assay described here is easier to perform and requires considerably less volume than GLC techniques. In addition, while the radioimmunoassay technique is also sensitive and relatively simple, the antibody used may be directed against a part of the TAD molecule that is not essential for activity. Consequently, pharmacologically-inactive metabolites may be detected and give false 'positives'. The radioreceptor assay detects all TAD and metabolites which interact with the 5-HT uptake receptor.

The assay is sensitive and can detect the commonly used antidepressants in plasma at one fifth to one thousandth the concentration found in the blood of patients receiving the drugs (Table 1). The assay is clearly not as specific as GLC or radioimmunoassay, although, as pointed out above, this does have its advantages. One problem is that certain other drugs which are not antidepressants may give false 'positives'. Thus, chlorpromazine, which is around 10% as potent as desipramine in reducing [$^3$H]-5-HT binding would contribute slightly (10%) to the apparent displacement of binding in patients receiving both chlorpromazine and an antideppressant. Other drugs such as the benzodiazepines which may be coadministered with antidepressants do not interfere however.

Recently, Innis *et al.* [14] have described a radioreceptor assay for antidepressants which utilizes the ability of these drugs to interact at central nervous system muscarinic receptors. This assay is comparable in specificity and sensitivity with that described here although it does involve extraction of plasma samples by organic solvents and is hence more time-consuming. Both assays indicate well the potential of radioreceptor assays in monitoring plasma drug levels as does that previously described for neuroleptic agents [15].

In summary, the radioreceptor assay described here is rapid, sensitive and simple to perform and offers a potential tool for routine application to determine plasma levels of TAD in large numbers of patients. It also provides a simple method of investigating pharmacokinetic aspects of TAD drug action in experimental animals.

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