Inhibition of Platelet Uptake of Serotonin in Plasma from Patients Treated with Clomipramine and Amitriptyline

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Summary. The inhibition of serotonin uptake by platelets has been measured in blood from 20 patients on amitriptyline (50–225 mg daily), 14 patients on clomipramine (25–200 mg daily), and in an untreated group of 21 depressed patients. A complete kinetic analysis was carried out in each patient. Using the increase in the kinetic parameter $K_m$ as a measure of uptake inhibition, there was a high correlation between the daily dose and inhibition within each drug group, clomipramine being about 10 times more potent than amitriptyline. The inhibition did not vary with age, sex, duration of treatment (up to 3 years), or concomitant use of moderate doses of benzodiazepines, neuroleptics or lithium. In the amitriptyline group the inhibition was significantly smaller in smokers than in non-smokers. The kinetic parameter $V_{max}$ was essentially unchanged in the amitriptyline group, and was markedly reduced in the clomipramine group, but without any correlation with dose. The mixed competitive-noncompetitive effect of clomipramine confirms previous in vitro findings.

Key words: tricyclic antidepressants, platelets, serotonin uptake inhibition; clomipramine, amitriptyline

The majority of tricyclic antidepressants have a marked inhibitory effect on the uptake of serotonin (5-hydroxytryptamine, 5HT) by platelets (Marshall et al., 1960; Lingjærde, 1977a) and in brain tissue (Carlsson et al., 1968; Hamberger and Tuck, 1973). Thus, 50% inhibition of serotonin uptake in human platelets was produced by approximately $2 \times 10^{-9} \text{M}$ clomipramine, $10^{-8} \text{M}$ imipramine and $4 \times 10^{-8} \text{M}$ amitriptyline, in a protein-free medium, at a serotonin concentration of $5 \times 10^{-7} \text{M}$ (Lingjærde, 1977b).

According to the hypothesis that some depressed patients have reduced serotonergic activity in the brain (Lapin and Oxenkrug, 1969; Asberg et al., 1976), the inhibition of serotonin reuptake may be one of the mechanisms behind the antidepressant effect of these drugs, although probably only in a particular subgroup of depressed patients. In other depressed patients, the inhibition of noradrenaline reuptake, or possibly some other effect, may be more important.

The inhibitory effect of antidepressants on serotonin uptake by platelets has also been demonstrated in platelet-rich plasma from patients treated with such drugs (Rysanek et al., 1965; Murphy et al., 1970; Lemberger et al., 1978). Probably as a result of the uptake inhibition, patients on tricyclic antidepressants and related drugs may have a reduced content of serotonin in their platelets (Marshall et al., 1960). It has also been reported that plasma from patients treated with clomipramine had an inhibitory effect on serotonin uptake by rat brain slices, whereas plasma from patients on amitriptyline or imipramine had little or no effect (Tuck and Punell, 1973).

Most tricyclic antidepressants are highly protein bound in plasma (Borgå et al., 1969), and it is generally believed that only the unbound portion of a drug has pharmacological activity. It has been demonstrated, for instance, that the inhibitory effect of clomipramine on serotonin uptake by brain slices is considerably smaller in plasma than in a protein-free buffer (Hamberger and Tuck, 1973). Furthermore, only the free fraction of nortriptyline was found to inhibit noradrenaline uptake by the rat iris, the difference between the inhibition in plasma and in protein-free buffer being in good agreement with the
degree of protein binding found by the ultrafiltration method (Borgå et al., 1970). Similarly for serotonin uptake by human platelets it has also been shown that antidepressants, such as doxepin and nomifensin, produce much stronger inhibition in a protein-free medium than in plasma (Lingjærde, 1976, 1977b), and again, this is thought to be due to protein binding in plasma.

Inhibition of serotonin uptake in plasma appears to be closely related to the biochemical effects in the brain, since Åsberg et al. (1977) found a very high correlation between the inhibitory effect on serotonin uptake by rat brain slices of plasma from clomipramine-treated patients and the decrease in 5-HIAA in CSF from the same patients.

Thus, it may be assumed that inhibition of platelet serotonin uptake in blood from patients treated with tricyclic antidepressants is an indication of the free plasma concentration of the drug and its active metabolites, and that this effect mirrors the action of the drug on serotonergic processes in the brain.

Since there are few data on the relationship between the dose of a given antidepressant and quantitative aspects of serotonin uptake inhibition in blood, e.g., the effect on the kinetic parameters \( K_m \) and \( V_{\max} \), the present study was done to elucidate this relationship for clomipramine and amitriptyline, which are among the most widely used antidepressants, and which are amongst those that cause the greatest inhibition of serotonin uptake. For practical reasons, the plasma concentrations of the drugs and their metabolites, and their clinical effect, were not measured in this study.

Material and Methods

Patients

The study comprised physically healthy inpatients and outpatients, who had been treated for a minimum of one week with a constant dose of amitriptyline or clomipramine, regardless of the diagnosis and dose level. Patients were included even if they received moderate additional doses of benzodiazepines, neuroleptics or lithium.

The patients were a rather heterogeneous group with regard to diagnosis, although about half of them suffered from unipolar recurrent depression of endogenous type. Since the study did not attempt a correlation of uptake inhibition and therapeutic effect, the diagnoses have not been further specified.

The drug and its dose were chosen by the individual patient’s physician and without obedience to any common guideline. The drugs were given daily, either in three divided doses, or as a single dose in the evening.

The clomipramine group comprised 8 female and 6 male patients, aged 34 to 74 years. One was investigated three times whilst taking different doses, and two were examined twice, also whilst on different doses. Thus, a total of 18 measurements were performed in the group. The daily dose of clomipramine varied from 25 to 200 mg; at the time of blood sampling, 7 patients had received the stated dose for one to two weeks, 8 had taken it from two to four weeks, and 3 for more than four weeks (longest period: 3 months). Seven patients received benzodiazepines in addition, six neuroleptics, and three lithium.

The amitriptyline group consisted of 10 female and 10 male patients, aged 32 to 69 years, each of whom was investigated only once. The daily dose of amitriptyline varied from 50 to 225 mg; at the time of blood sampling, it had been given for one to two weeks in eleven, from two to four weeks in five, and for more than four weeks in four patients (longest period: 3 years). Seven patients received additional benzodiazepines, and five additional neuroleptics.

The control group comprised 16 female and 5 male patients, aged 30 to 80 years, who had not received any antidepressant for at least two weeks. They were all suffering from various types of depression. Serotonin uptake characteristics in them did not differ significantly from those of a much larger group of healthy untreated controls.

Laboratory Methods

The principles of the method for measuring platelet serotonin uptake have been discussed elsewhere (Lingjærde, 1977a). Blood was taken with the subject in the fasting state in the morning, about 12 h after the last dose of drug, and was mixed with one tenth of its volume of 3% EDTA in 0.9% saline. Platelet-rich plasma (PRP) was prepared by centrifugation at a low speed (250 × g) for 15 min at room temperature. Aliquots of 0.5 ml PRP were mixed with 0.5 ml sodium phosphate buffer 55 mM pH 7.35 containing 70 mM NaCl and 7.5 mM KCl; the dilution was done partly to stabilize pH, and partly to prevent the substrate from being “used-up” too rapidly by an excessively high concentration of platelets. The dilution also reduced the concentration of drug and protein to 50% of the plasma level, which means that measurements of the absolute