INCREASED PLATELET AGGREGATION RESPONSES TO 5-HYDROXYTRYPTAMINE IN PATIENTS TAKING CHLORPROMAZINE

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1 The aggregation response of platelets induced by 5-HT was greatly increased in psychiatric patients receiving chlorpromazine therapy when compared with normal volunteers and psychiatric patients not receiving chlorpromazine.

2 Platelet aggregation responses to ADP were normal during chlorpromazine therapy, but 5-HT induced aggregation was increased in rate and the typical transient reversible response was converted to an irreversible response in all subjects. This was usually indistinguishable from the ADP response.

3 When chlorpromazine therapy was stopped, plasma concentrations of chlorpromazine, monodesmethylchlorpromazine and chlorpromazine sulphoxide fell rapidly within one week, whereas 5-HT induced platelet aggregation responses became normal after three weeks. The enhanced responses returned when chlorpromazine therapy was re-instituted.

4 It is possible that platelet aggregation responses to 5-HT *in vitro* could prove to be a useful index of the pharmacological effect of chlorpromazine *in vivo*.

Introduction

It has been reported (Mills & Roberts, 1967) that low concentrations of chlorpromazine (10^{-6} M) added to platelet rich plasma inhibited 5-HT induced platelet aggregation. We have recently confirmed and extended these findings showing that chlorpromazine (CPZ) and seven of its major metabolites (monodesmethylchlorpromazine, NOR₁CPZ; 7-hydroxychlorpromazine, 7-OHCPZ; didesmethylchlorpromazine, NOR₂CPZ; 3,7-dimethoxychlorpromazine, CH₃OCPZ; didesmethylchlorpromazine sulphoxide, NOR₂CPZSO; chlorpromazine nitroxide, CPZNO) are all inhibitors of 5-HT induced aggregation *in vitro* although they show different potencies (Boullin, Grahame-Smith, Grimes & Woods, 1975). The concentrations of CPZ, NOR₁CPZ and CPZSO used in these experiments were within the range of concentrations found in the plasma of patients being treated with chlorpromazine (Curry, 1971).

We have examined platelet aggregation responses in patients receiving chlorpromazine therapy and found that the responses were quite different from those observed *in vitro* (Mills & Roberts, 1967; Boullin *et al.*, 1975). 5-HT induced platelet aggregation was not inhibited but actually enhanced, with an increased initial rate of

aggregation and prolonged irreversible responses frequently indistinguishable from the typical ADP induced responses which were themselves unchanged.

Methods

The subjects studied were nine psychiatric patients (six males, three females) who had been receiving chlorpromazine therapy (150-900 mg/day) for longer than three years. Seven of these patients were receiving benzhexol (4-15 mg/day) in addition. In order to exclude the effects of environmental factors on platelet aggregation responses as far as possible, control subjects with one exception, were from the same hospital. Three were male psychiatric patients not receiving drugs (two with schizophrenia, one with chronic alcoholism). Three (one female, two male) were patients not on phenothiazines but receiving a variety of other drugs: benzhexol, biperiden, carbamazepine, cyclandelate, diazepam, ephedrine, potassium chloride, phenobarbitone, pimozide and theophylline.

Blood samples were taken 2 h after the morning

dose of chlorpromazine and collected into 0.129 M sodium citrate. Platelet rich plasma (PRP) was prepared and platelet aggregation measured as described by Boullin, Green & Price (1972). Aggregation was induced with 0.4 to 50 μ M 5-HT or adenosine diphosphate (ADP) added to PRP stirred at 1000 rev/min and maintained at 37°C.

In normal subjects the concentration of 5-HT required to produce maximal changes in light transmittance on aggregation could not be predicted. Usually maximal responses were obtained with concentrations not greater than 20 μ M and higher concentrations (up to 50 μ M) produced smaller responses. These data confirm earlier work (O'Brien, 1964; Mills & Roberts, 1967; Baumgartner & Born, 1968).

In schizophrenic patients, maximal aggregation responses were usually produced by 20 μ M 5-HT and higher concentrations did not produce inhibition. ADP responses in schizophrenic patients were qualitatively and quantitatively normal. We tested the response of platelets from schizophrenic patients with a range of 5-HT concentrations from 4 to 50 μ M and compared these with the maximal responses obtained with ADP.

Rates of change in optical density were expressed as μ V/min from recordings made on an x-y recorder (Boullin *et al.*, 1975).

Overall aggregation responses were measured by the total change in optical density during the first 210 s of aggregation after addition of drug to PRP. This was done by cutting out and weighing the area of recording paper under the aggregation curve record. The overall aggregation response to 5-HT was expressed as a percentage of the maximum ADP response in order to compensate for individual variations in the optical density of PRP (Boullin *et al.*, 1975).

The concentrations of CPZ, CPZSO and NOR₁CPZ were determined in plasma obtained at the same time as the blood used for the preparation of platelets, using a modification of the method described by Curry (1968).

Results

In normal volunteers 5-HT induced transient monophasic reversible aggregation (Figure 1, lower record). These normal responses were also seen in psychiatric patients not receiving therapy, and the three psychiatric patients receiving combinations of drugs but not chlorpromazine (see **Methods**).

The following effects were observed with 5-HT in patients receiving CPZ: (1) increased initial rate of aggregation (Table 1); (2) prolonged irreversible aggregation (Figure 1, trace b). The irreversible responses were often indistinguishable from those produced by ADP (Figure 1) and in some cases were biphasic.

Seven of the patients receiving chlorpromazine were also taking benzhexol. The latter drug did not affect platelet aggregation responses to 5-HT or ADP in one parkinsonian patient and two schizophrenic patients receiving benzhexol together with other non-phenothiazine drugs. *In vitro* 10⁻³ M benzhexol inhibited rather than enhanced 5-HT induced aggregation. This concentration is much higher than that achieved during therapy. ADP responses in all chlorpromazine treated subjects were normal and these were used as a comparative standard for 5-HT effects (see **Methods**).

Table 1 shows that in normal subjects the initial rate of 5-HT induced aggregation, expressed as changes in light transmittance (μ V/min), was 22.6% of the maximal rate induced by ADP. We confirmed our previous observations (Boullin *et al.*, 1972, 1975) that initial rates of aggregation were independent of the dose of ADP used in the concentration range studied (4-50 μ M). In subjects receiving CPZ the initial rate of 5-HT induced aggregation was almost doubled to 52.8% of the maximal ADP induced rate. An irreversible 5-HT aggregation response was seen in all subjects taking CPZ, although the dose of 5-HT required to produce the response differed from one patient to another within the range of 4-50 μ M.

The effect of interruption of chlorpromazine

Table 1 The mean $\pm$ s.e. mean platelet aggregation rates (μ V/min) produced by 5-HT or ADP

Aggregation induced by:	Control subjects (n = 13)		Chlorpromazine-treated patients (n = 9)
5-HT	771 $\pm$ 192	<i>P</i> < 0.01	1819 $\pm$ 246
ADP	3415 $\pm$ 323		3451 $\pm$ 347
		N.S.	
5-HT rate as % ADP rate	22.6		52.8

Figure 1 Normal and enhanced 5-HT induced platelet aggregation responses. The enhanced response to 5-HT (20 μM) (trace b) was obtained with PRP prepared from the blood of a schizophrenic patient who had received chlorpromazine (150 mg/day) for three years. This is compared with the responses to ADP (20 μM) (trace a) and 5-HT (20 μM) (trace c) in PRP prepared from the blood of a normal subject. Change in light transmittance (mV) is plotted against time (seconds).

therapy upon 5-HT aggregation responses was studied in three schizophrenic patients. The initial rates of aggregation returned to normal after cessation of therapy, but took three weeks to do so. They were measured three weeks after restarting therapy (Table 2) and were found to be enhanced again.

The increases in platelet aggregation rate do not completely describe the changes observed because they do not take into account the irreversible nature of the response. This is illustrated in Figure 2 which shows that the irreversible platelet aggregation response to 20 μM 5-HT had reverted to normal four weeks after stopping chlorpromazine therapy. When therapy was restarted, the irreversible response was detected when the subjects were retested four weeks later. These responses were compared to those obtained with 20 μM ADP in the same subject. The quantitative results for three patients are shown in Figure 3 where each point represents the area under the 5-HT induced aggregation curve expressed as a percentage of the area under the ADP induced curve. It is clear that the responses approached the normal range when CPZ therapy was discontinued and that enhanced responses re-appeared in two patients when therapy was re-introduced.

In addition to measuring the platelet responses

in these three patients, plasma concentrations of CPZ, NOR₁CPZ and CPZSO were also measured. CPZ concentrations ranged between 75 and 425 pmols/ml, but did not appear to be dose-dependent. In two of the three subjects CPZ

Table 2 Effect of interruption of therapy on the mean ± s.e. mean 5-HT induced platelet aggregation rate in CPZ treated patients

Time (weeks)	Aggregation rate (μV/min)	n
0 (on therapy)	1636 ± 292	3
After stopping therapy		
1	1525 ± 769	3
2	1400	2
3	861 ± 191	3
4	800 ± 202	3
After restarting therapy		
3	1770	1
4	2172	2
5	1685	2

The aggregation rate in 39 normal subjects was 771 ± 192 μV/min.

Figure 2 Changes in 5-HT induced aggregation responses during interruption of chlorpromazine therapy. Change in light transmittance (mV) is plotted against time (seconds).

concentrations showed wide variations, but in the other subject there was little variation (Figure 4). When therapy was interrupted, CPZ concentrations decreased to very low values within one week in all three patients and increased again in the two subjects when therapy was restarted. Similar changes were seen with the metabolites NOR₁CPZ and CPZSO, although plasma concentrations were lower than for CPZ itself. Comparison of the platelet response in the patient K.S. with the plasma CPZ and metabolite concentrations shows that following cessation of CPZ therapy the platelet response was enhanced at a time when the plasma concentrations of CPZ and its metabolites approached zero limits of detection (Figures 3 and 4 and Table 2).

We have attempted to reproduce the *in vivo* responses *in vitro* by addition of CPZ to normal PRP, but CPZ added *in vitro* always produces

inhibition of 5-HT induced aggregation, even after prolonged incubation (1 hour).

It is important to note that addition of CPZ, in concentrations normally present in the plasma of CPZ treated patients (0.1–10 µM), to samples of their PRP 3 min prior to 5-HT, blocked aggregation in the normal way (Figure 5). It is possible therefore that the *in vivo* responses are not the result of an immediate and direct action of CPZ itself on the platelet.

Discussion

The earlier work indicated that human platelet aggregation responses to 5-HT were ephemeral (O'Brien, 1964) and always reversible (Baumgartner & Born, 1968). However, recently it has been reported (Besterman & Gillett, 1973) that 8%

Figure 3 The effect of interruption of chlorpromazine treatment on 5-HT induced platelet aggregation in three patients. Each point represents the area under the 5-HT induced aggregation curve expressed as a percentage of the area under the ADP induced aggregation curve. The solid bars show the duration of treatment and the hatched bars represent the range of values seen in normal control subjects. CPZ treatment was restarted at the arrows. The dose of chlorpromazine received by the patients was: (a) K.S. 300 mg/day; (b) M.W. 150 mg/day; (c) L.M. 400 mg/day.

of normal subjects show an irreversible biphasic response to 5-HT. We have confirmed this result in 10% of 40 normal subjects. This effect differs from the enhanced response seen in most CPZ treated patients where 5-HT usually produces an aggregation response indistinguishable from the normal ADP response.

However, the change in 5-HT induced platelet aggregation does not seem to be a direct effect of CPZ itself for two reasons: firstly, CPZ added *in vitro* invariably inhibited the enhanced 5-HT induced aggregation response seen in CPZ treated patients. Also in two patients who had ingested overdoses of CPZ, 5-HT induced platelet aggregation was normal for two days thereafter. Secondly, after cessation of therapy the enhanced 5-HT responses persist 2-3 weeks after the plasma concentrations of CPZ, CPZSO and NOR₁CPZ approached zero.

Perhaps CPZ affects platelet membrane permeability, possibly by an action during megakaryocytosis, leading to increased permeability to 5-HT with release of ADP and the initiation of the secondary phase of platelet aggregation (Haslam, 1967). The lack of correlation between the rapid decline in plasma concentrations of CPZ and the slow disappearance of abnormal aggregation responses supports the view that the action is prolonged.

Baumgartner & Born (1969) and Baumgartner (1969) proposed that in rabbit platelets, which show a good aggregation response to 5-HT, aggregation induced by the indolylalkylamine involves the net transport of 5-HT across the platelet membrane. During this energy-dependent process ADP is formed intracellularly from ATP.

Obviously the role of 5-HT transport has to be considered in relation to aggregation of normal human platelets and the enhanced aggregation responses reported here. We were unable to take the quantities of blood required for the necessary extensive investigations. However, the experiments described in the following paper do suggest that the factor responsible for the enhancement of 5-HT induced aggregation in CPZ treated patients is localized within the platelet.

The multiplicity of CPZ metabolites (theoretically 168, Usdin, 1971), with a range of potency and pharmacological activity, makes correlation of plasma levels with therapeutic effects complex and difficult (Curry, 1971; Sakalis, Chan, Gershon & Park, 1973). This work was begun originally with the aim of finding a pharmacological effect of CPZ therapy which could easily be measured in patients. From the basic work it had been expected that in patients receiving CPZ therapy, 5-HT induced platelet aggregation would be inhibited, but the converse has proved to be the

Figure 4 Plasma concentrations of chlorpromazine (CPZ) (●), chlorpromazine sulphoxide (○) and nor-CPZ (■) during therapy with chlorpromazine (300 mg/day) in patient K.S. The graph shows the changes in concentration which occurred after cessation and re-introduction of therapy. The solid bars represent the times during which therapy was given.

Figure 5 Inhibition of enhanced 5-HT induced aggregation response by chlorpromazine *in vitro*. The upper record (a) shows a typical enhanced aggregation response induced by 5-HT (20 μ M). The lower record (b) shows that the enhanced response is reverted towards normal 3 min after the addition of chlorpromazine (10 μ M) to another sample of PRP taken from the same chronic schizophrenic patient receiving chlorpromazine (150 mg/day). Change in light transmittance (mV) is plotted against time (seconds).

case. Only further investigation will show whether the effect of CPZ *in vivo*, apparent as enhancement of 5-HT induced platelet aggregation, will correlate with its therapeutic effect in psychoses.

If this proves to be the case then the change in 5-HT induced platelet aggregation might 'by-pass'

all the problems involved in interpatient and inpatient variation in CPZ metabolism.

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