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INHIBITION OF 5-HYDROXYTRYPTAMINE-INDUCED HUMAN BLOOD PLATELET AGGREGATION BY CHLORPROMAZINE AND ITS METABOLITES

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1 Blood platelets from normal human subjects were isolated and aggregated *in vitro* with adenosine diphosphate (ADP) or 5-hydroxytryptamine (5-HT).

2 The effects of chlorpromazine and 7 major metabolites upon 5-HT-induced aggregation were investigated.

3 All the phenothiazines inhibited 5-HT-induced aggregation when added to platelet rich plasma 3 min prior to 5-HT.

4 There were no qualitative differences in the inhibitory effects, but inhibitory potency varied over a wide range. The decreasing order of potency was monodesmethylchlorpromazine, chlorpromazine, 7-hydroxychlorpromazine, didesmethylchlorpromazine, 3,7-dimethoxychlorpromazine, didesmethylchlorpromazine sulphoxide, chlorpromazine sulphoxide, chlorpromazine nitroxide.

Introduction

The purpose of this investigation was to define a pharmacological action of chlorpromazine (CPZ) which might be used as a technique to measure the pharmacological activity of CPZ and its metabolites in patients during therapy. Chlorpromazine and some of its metabolites are accumulated by human erythrocytes and platelets (Ahtee & Paasonen, 1965; Ahtee, 1966a; Solatunturi & Ahtee, 1968). Mills & Roberts (1967) showed that low concentrations of chlorpromazine (1 nmol/ml) inhibited platelet aggregation *in vitro*, induced by adrenaline and 5-hydroxytryptamine (5-HT). Most important, CPZ inhibited 5-HT-induced platelet aggregation more than the aggregation caused by adenosine diphosphate (ADP). The effective inhibitory concentrations of CPZ were comparable to those found in the plasma of patients treated with CPZ (Curry, 1971).

The clinical effects of prolonged CPZ treatment on human blood platelet aggregation responses have not been investigated. However, it is known that CPZ is extensively metabolized by man, and there are, theoretically at least, 168 possible metabolites (see Usdin, 1971). The poor correlation between CPZ plasma concentrations and the therapeutic responses of schizophrenic patients has led to the proposal that one or more of the chlorpromazine metabolites may be pharmacologically active within the human brain (Curry, 1971; Curry, Lader, Mould & Sakalis, 1972). Recently Sakalis, Chan, Gershon & Park (1973) have found a relationship between plasma concentrations of 7-hydroxylated CPZ metabolites and a good therapeutic response to CPZ treatment in schizophrenics, while a poor response appeared to be associated with the presence of CPZ metabolites with the sulphoxide moiety (CPZSO, NOR_1CPZSO, NOR_2CPZSO, 7OHNOR_1CPZSO, 7OHNOR_2CPZSO). We considered that these metabolites might show pharmacological activity by inhibiting platelet aggregation. This paper reports the effects of seven CPZ metabolites upon platelet aggregation responses induced by 5-HT.

Methods

Blood was obtained by venepuncture from normal male and female volunteers aged 18-46 years, who had not taken acetylsalicylic acid for at least 5 days and no other drug for at least 3 days. No females were receiving oral contraceptives. The blood was collected into 0.129 M sodium citrate and centrifuged at 120-150 *g* to prepare citrated platelet rich plasma (PRP) as described by Boullin, Green & Price (1972). Samples (1 ml) of PRP were incubated at 37°C and then platelet aggregation responses were recorded with the platelet aggregometer (Boullin *et al.*, 1972) coupled to an X-Y pen recorder. This type of instrument was found

to be particularly satisfactory for measurement of the platelet aggregation rate. We used either a Bryans Model 24000A4, (Bryans Southern Ltd, Mitcham, Surrey), with a chart speed of 6 cm/min giving deflections on the Y axis of 400 μV/cm, or a Tekman TE 200 (Tekman Ltd, Oxford) using a speed of 20 or 40 mm/min giving deflections on the Y axis of 200 μV/cm.

The PRP was stirred at 1000 rev/min at 37°C and the phenothiazines were added 3 min before aggregation was induced by the addition of 5-HT. Responses were quantitated as changes in optical density (light transmittance) expressed in arbitrary units, or as a rate of change (μV/minute).

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate and adenosine diphosphate (Sigma Chemical Co, St Louis, Mo, USA); chlorpromazine (May & Baker Ltd). CPZ metabolites were obtained from Mr D. Wiles, Littlemore Hospital, Oxford.

We used 7-hydroxychlorpromazine base (7OHCPZ); monodesmethylchlorpromazine maleate (NOR_1CPZ); didesmethylchlorpromazine maleate (NOR_2CPZ); chlorpromazine nitroxide dihydrate (CPZNO); 3,7-dimethoxychlorpromazine hydrochloride (CH_3OCPZ); didesmethylchlorpromazine sulphoxide hydrochloride (NOR_2CPZSO) and chlorpromazine sulphoxide, base (CPZSO). 7OHCPZ and CH_3OCPZ are photolabile (Usdin, 1971) and experiments with these compounds were carried out in a room lit with tungsten filament lamps obscured with yellow filters (Kodak Safelight OB).

All drug concentrations are expressed in terms of molarity of the base.

Results

5-HT induced platelet aggregation, although the effect was small in comparison with aggregation produced by ADP. The effects of 5-HT were approximately 20-30% of the maximal aggregation response produced by ADP, as determined by the change in optical density of PRP. Table 1 shows normal 5-HT aggregation rates, expressed as change in optical density measured on the pen recorder as μV/min, compared with those obtained with equimolar concentrations of ADP in the same subject. This Table shows that with 5-HT concentrations in the range 4-50 nmol/ml, the initial rate of aggregation was maximal and not concentration-dependent for either ADP (Boullin, *et al.*, 1972) or 5-HT. Moreover in no case did the 5-HT aggregation rate exceed 30% of the maximum rate obtained with ADP. The responses to 5-HT were small, transient and consisted of one phase only; irreversible aggregation typically seen with ADP, was seen with 5-HT in only 10% of 40 normal subjects. Also there was considerable individual variation in the aggregation response to 5-HT; maximal change in light transmittance was not obtained with any predictable 5-HT concentration and in some cases inhibition was observed with concentrations of 50 nmol/ml. These data confirm earlier work (O'Brien, 1964; Mills & Roberts, 1967; Baumgartner & Born, 1968). Accordingly we tested the responses of each subject with a range of 5-HT concentrations from 4.0 to 50 nmol/ml. Having established the 5-HT concentration producing the maximal aggregation effect, we then investigated the inhibitory actions of the phenothiazine compounds on the aggregation produced by this concentration.

Inhibition of 5-hydroxytryptamine aggregation by chlorpromazine and metabolites

Figure 1 compares the effects of 0.1, 1.0, 10.0 and 100 nmol/ml concentrations of CPZ, 7OHCPZ and CPZSO and illustrates the differing inhibitory potencies of CPZ and metabolites upon 5-HT-induced aggregation. The figure also shows that these phenothiazines did not produce qualitative differences in the 5-HT aggregation responses. That is, aggregation was invariably transient and reversible. Similar results were obtained with the 5 other chlorpromazine metabolites, although there were differences in their inhibitory potency. When these inhibitory effects were expressed in terms of percentage inhibition of the maximum rate of

Table 1 Maximum rates of platelet aggregation produced by 5-hydroxytryptamine (5-HT)

Dose of drug added (nmol/ml)	*5-HT (Rate of change of optical density)*	*ADP*	*5-HT response as % of ADP response*
4	1220	4114	29.7
$n = 7$	±271	±242	±4.7
20	1057	4490	23.5
$n = 11$	±242	±865	±4.3
50	1400	4207	33.3
$n = 5$	±278	±403	±5.5

Samples (1 ml) of PRP were aggregated with 5-HT or ADP at 37°C as described in the methods section. Maximal rates of aggregation (μV/min) were calculated from the initial rate of aggregation measured during 15 s after addition of drug. Values are the mean with s.e. mean.

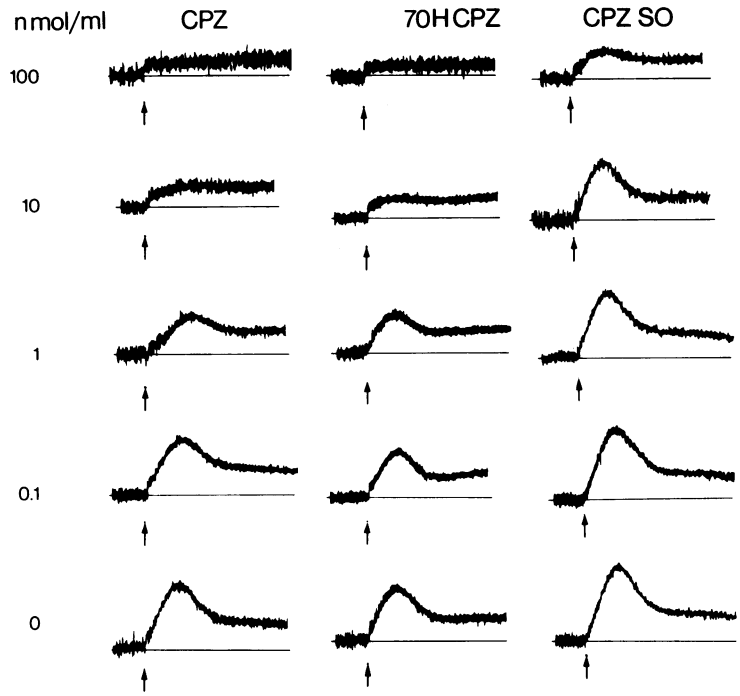

Figure 1 Inhibitory effects of various concentrations of chlorpromazine (CPZ), 7 hydroxy-CPZ (7OH CPZ) and CPZ sulphoxide (CPZSO) compared to control responses. CPZ compounds were added to PRP 3 min before induction of aggregation with 5-HT 20 μM (at the arrows).

aggregation produced by a standard dose of 5-HT, we obtained the data shown in Figure 2. This compares the inhibitory effects of the 7 metabolites with CPZ. Table 2, column I, shows the differences in potency calculated on the basis of the ID_{50}, the phenothiazine concentration required to produce 50% inhibition of the maximum 5-HT aggregation rate. These results indicate that there is a wide spectrum of inhibitory activity of the chlorpromazine metabolites, but all show some degree of pharmacological activity on platelet aggregation *in vitro*.

Table 2 Comparative activities of chlorpromazine (CPZ) metabolites in blocking amine sensitive cellular systems

Metabolites	*I 5-HT induced platelet aggregation* (ID_{50} *nmol/ml*)	*II Dopamine-sensitive adenylate cyclase* (ID_{50} *nmol/ml*)
NOR_1CPZ	0.9	6.0
CPZ	2.0	1.3
7OHCPZ	3.1	14.0
NOR_2CPZ	4.0	11.0
$3,7,CH_3OCPZ$	19.0	Not tested
NOR_2CPZSO	50.0	Not tested
CPZSO	68.0	> 100
CPZNO	120.0	50.0

Data in column I are from the present work; data in column II are from Miller & Iversen (1974).

Discussion

It has been established that chlorpromazine is metabolized to a large number of derivatives in man, and that some of these substances possess potent pharmacological activity (for review see Usdin, 1971).

In general, the pharmacological activity of CPZ metabolites in blocking 5-HT-induced platelet aggregation parallels their activity in other systems, particularly in the central nervous system. Posner, Hearst, Taylor & Cosmides (1962), Manian, Efron & Goldberg (1965) found that NOR_1CPZ and 7OHCPZ were the most active metabolites in a series of tests which measured animal behaviour. We found that the demethylated derivative, NOR_1CPZ, is more potent in inhibiting platelet aggregation than CPZ itself; that NOR_2CPZ and 7OHCPZ are somewhat less potent,

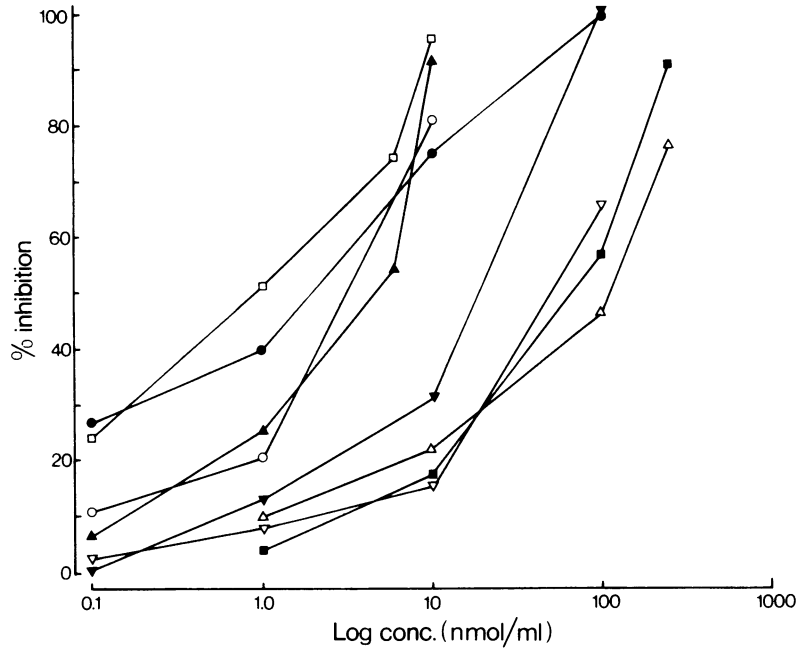

Figure 2 Comparative potency of chlorpromazine (CPZ) and 7 major metabolites in blocking 5-hydroxytryptamine-induced aggregation. Blockade of aggregation was assessed as described in methods section. Each point represents the mean value of 7 to 19 experiments with the standard error not exceeding 10% of the mean value. (●) CPZ; (○) 7OH; (■) CPZSO; (□) NOR 1; (▲) NOR 2; (▽) CPZ NO; (△) 3,7,MeO; (▼) NOR 2 SO.

while the N-oxide and sulphoxide metabolites have less than 1/10 the potency of CPZ. Some of these metabolites exhibit a remarkably similar order of potency to that seen with the dopamine-sensitive adenylate cyclase of rat striatum described by Miller & Iversen (1974) (Table 2, column II). Thus there is a precedent for the fact that CPZ metabolites have some pharmacological activity in the broadest sense in the brain and blood platelets. Differences in binding to platelet cells and plasma proteins may also influence pharmacological action of CPZ and metabolites. The Finnish group in Helsinki made an extensive investigation of the physicochemical properties of CPZ metabolites; their haemolytic effects on red cells, binding to platelets, and release of catecholamines from adrenal glands. CPZSO was bound less to serum proteins than CPZ or NOR_1CPZ (Ahtee, Mattila & Vapaatalo, 1967) and smaller amounts were taken up by platelets (Ahtee & Paasonen, 1966). Subcellular fractionation showed that most CPZSO was in the supernatant platelet fraction, whereas CPZ and NOR_1 CPZ were particulate bound (Solatunturi & Ahtee, 1968). The pharmacological activity of these 3 phenothiazines paralleled their platelet binding characteristics (Ahtee & Paasonen, 1965; Ahtee, 1966a), and their ability to release catecholamines from adrenal glands (Vapaatalo, Ahtee & Paasonen, 1966).

Comparison of the surface active properties of CPZ with NOR_1CPZ and CPZSO (Ahtee, 1966b) showed that CPZSO had the lowest potency. On the basis of this work, differences in pharmacological potency might be attributed to the differences in surface activity and protein binding. However the concentrations required to produce these effects were at least 10^{-4} M. Such concentrations of CPZ release 5-HT from platelets (Bartholini, Pletscher & Gey, 1964) and cause gross ultrastructural changes, including disruption of the plasma membrane with consequent leakage of the intracellular contents (Telkkä, Nyholm & Paasonen, 1964).

On the other hand, the pharmacological effects reported here and the effects described by Miller & Iversen (1974) upon brain adenylate cyclase were usually obtained with concentrations in the range 10^{-5} M to 10^{-8} M. In patients treated with CPZ we found that plasma concentrations of CPZ, NOR_1CPZ and CPZSO range between 30-400 pmol/ml. According to Fig. 2, CPZ and

NOR_1CPZ are both pharmacologically active in this concentration range.

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