EFFECTS OF ASPIRIN AND PROSTAGLANDIN E₂ ON SECONDARY PHASE AGGREGATION RESPONSES OF SCHIZOPHRENIC PATIENTS TREATED WITH CHLORPROMAZINE

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- 1 We investigated the effects of oral aspirin 900 mg upon the platelet aggregation responses to 5-hydroxytryptamine, dopamine or N-dimethyl dopamine in schizophrenic patients chronically treated with chlorpromazine.
- 2 Most subjects showed enhanced aggregation responses (that is appearance of secondary phase aggregation) to these agents prior to aspirin administration.
- 3 When aggregation was re-tested 23.5 h after aspirin the secondary phase of the enhanced aggregation responses was abolished.
- 4 Addition of 0.14-0.3 $\mu\text{mol/l}$ prostaglandin E₂ 15 s after inducing aggregation partially restored the secondary phase.
- 5 The observed secondary phase of aggregation may be due to increased prostaglandin synthesis or release.

Introduction

We have recently shown that platelet aggregation responses to 5-hydroxytryptamine (5-HT), dopamine (DA) and N,N-dimethyl dopamine (DMDA) are enhanced in schizophrenic patients treated with chlorpromazine (CPZ) (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975; Boullin, Grahame-Smith, Grimes & Woods, 1975; Boullin, Green & Grimes, 1975; Boullin & Grimes, 1976).

We are interested in investigating this phenomenon from two aspects: First, enhanced platelet aggregation may be used as a pharmacological index of the efficacy of phenothiazine therapy and provide a further guideline to therapy. Second, the mechanism whereby enhancement is produced is clearly of importance and forms the rationale for the work described here.

The effect of CPZ therapy on 5-HT, DA and DMDA aggregation responses is to cause the appearance of the so-called 'secondary phase' of aggregation typically observed with adenosine diphosphate (ADP). The 'secondary phase' of aggregation involves the release of intracellular ADP from within the platelet (for a recent review see Weiss, 1975). Recent work implicates prostaglandin E₂ (PGE₂) in the production of 'secondary phase' aggregation produced by ADP or adrenaline and Shio & Ramwell (1972) have shown that this phase was enhanced by the addition of PGE₂ to plasma.

Silver, Hernandovich, Ingberman, Kocsis & Smith (1974) showed that ADP or adrenaline induced aggregation is associated with increased concentrations of PGE₂ and PGF_{2 α} in plasma and that the production of PGE₂ was especially enhanced. Moreover, Silver *et al.* (1974) showed that oral ingestion of aspirin or indomethacin, which are well known inhibitors of platelet aggregation (O'Brien, 1970), also inhibited prostaglandin synthesis.

It therefore occurred to us that the secondary phase of aggregation commonly seen with 5-HT and other amines in CPZ treated schizophrenics might be due to alterations in prostaglandin synthesis. If this were the case then the secondary phase should be blocked by oral administration of acetyl salicylic acid but should be restored on addition of exogenous PGE₂. This paper describes a series of experiments designed to test this hypothesis.

Methods

Subjects

Four male and one female chronic schizophrenic in-patients were tested. Table 1 gives data on age, sex and medication. All five patients had been receiving chlorpromazine for more than 1 year and

Figure 1 Platelet aggregation responses to 50 $\mu\text{mol/l}$ 5-HT added to 1 ml platelet rich plasma (PRP) stirred at 1000 rev/min at 37°C. Aggregation is measured as light transmittance (mv, ordinate) in relation to time (min, abscissa).

Upper record (a) is response before giving acetyl salicylic acid (900 mg) orally. Lower record (b) is superimposed response in the same CPZ treated patient 23.5 h after oral acetyl salicylic acid.

had previously shown enhanced aggregation to 5-HT. Blood was taken from each patient at 09 h 30 min and aggregation responses investigated at 10.00 h. Aspirin (acetyl salicylic acid BP, 900 mg) was given orally, then the blood was collected 23.5 h later and the aggregation responses re-evaluated as described below.

Platelet aggregation

This was measured as described previously using 5-HT and ADP (Boullin *et al.*, 1975a and b) and dopamine (DA) and dimethyl dopamine (DMDA) (Boullin & Grimes, 1976). Responses were quantitated as aggregation rate and change in optical density ΔOD and expressed as a percentage of the maximal ADP response.

Table 1 Age, sex and daily CPZ dose of schizophrenic patients

Patient	Age (years)	Sex	CPZ dose (mg/day)
1	61	M	200
2	59	F	100
3	36	M	600
4	29	M	100
5	63	M	150

Results

Four out of the five patients showed enhanced aggregation responses to 5-HT prior to administration of acetyl salicylic acid. Table 2 shows the values for rate of aggregation and ΔOD obtained with 5-HT and DMDA, before and after the oral administration of acetyl salicylic acid (900 mg).

Figure 2 Platelet aggregation responses to dopamine, and N-dimethyl dopamine. Experimental procedures as described in Figure 1. (1) Responses to N-dimethyl dopamine. (2) Responses to dopamine.

Upper records, (a), are responses before giving acetyl salicylic acid (900 mg) orally. Lower records (b), are responses in the same CPZ treated patient 23.5 h after oral acetyl salicylic acid. The records are superimposed in Figure 2 (1).

When the responses to 5-HT and ADP were tested 24 h later (23.5 h after acetyl salicylic acid) this dose of acetyl salicylic acid was insufficient to change either the initial rate or ΔOD produced by ADP. On the other hand, the characteristic biphasic response seen in the four patients prior to acetyl salicylic acid was abolished and, as shown in Figure 1, the response to 5-HT after acetyl salicylic acid was monophasic.

The effect of acetyl salicylic acid was to either completely prevent the appearance of the secondary phase of aggregation to 5-HT or to produce a rapidly reversible response. Table 2 shows that the changes in aggregation responses produced by aspirin were confined to the secondary phase,

represented as decreased ΔOD , and there was no effect on the initial rate of aggregation.

As previously described by Boullin & Grimes (1976) both DA and DMDA produced irreversible aggregation responses in schizophrenic patients treated with CPZ.

Two patients showed enhanced aggregation with DA; dopamine responses are difficult to elicit (Boullin *et al.*, 1975) but the enhanced response seen in the two patients prior to acetyl salicylic acid was almost abolished after acetyl salicylic acid (Figure 2a).

Three patients showed irreversible aggregation with DMDA and similar effects of aspirin to those seen with 5-HT were observed (Figure 2b).

Table 2 Values of 5-HT rate and ΔOD and DMDA rate and ΔOD , expressed as a % of maximal ADP responses, before and after the oral administration of acetyl salicylic acid (900 mg)

Subject number	5-HT rate		5-HT ΔOD		DMDA rate		DMDA ΔOD	
	Before	After	Before	After	Before	After	Before	After
1	41.5	42.0	101.5	28.5	15.2	20.7	42.7	27.2
2	51.0	48.1	72.7	21.1	27.8	25.0	74.0	29.3
3	34.0	39.1	19.6	3.8	—	—	—	—
4	23.1	22.6	52.2	17.6	33.0	24.2	52.4	18.6
Mean	37.4	38.0	61.2	17.8	25.3	23.2	56.4	25.0
$\pm$ s.e. mean	5.9	5.45	17.2	5.2	5.3	1.3	9.3	3.3
P	NS		<0.05		NS		<0.05

Figure 3 Effect of PGE₂ on aggregation responses 23.5 h after acetyl salicylic acid (900 mg) orally.

Platelet aggregation responses to 5-HT (1) and N-dimethyl dopamine (2) are shown. Experimental procedure as described in Figure 1.

Lower records, (a) are responses to 5-HT and N-dimethyl dopamine after acetyl salicylic acid. Upper records, (b) are the superimposition of a second recording from the same CPZ treated patient made 5 min later when PGE₂ (0.3 μmol/l (1)) or (0.14 μmol/l (2)) was added during the maximum rate of aggregation (arrowed). Note appearance of a secondary phase of aggregation in (1) and the abolition of the gradual decrease in aggregation in (2).

Effects of PGE₂

We made many individual tests to attempt to reintroduce a secondary phase of aggregation after giving acetyl salicylic acid. These were only partially successful as shown in Figure 3. We found that the best effects were obtained when PGE₂ (0.14–0.3 μmol/l) was added approximately 15–20 s after the induction of aggregation with 5-HT or DMDA, during the period of the primary phase of aggregation when the initial rate of aggregation was maximal. Figures 3(1) and 2 show that addition of these concentrations of PGE₂ resulted in the appearance of the beginnings of a secondary phase of aggregation, which stabilized after 5 min and did not return to the base line. If PGE₂ was added prior to the aggregating agent there was either no effect or inhibition with higher doses (greater than 1.5 μmol/l).

Discussion

These experiments throw some light upon the mechanism whereby platelet aggregation responses are altered in schizophrenic patients treated with CPZ and other neuroleptics as described in the preceding paper (Orr & Boullin, 1976).

The experiments described here show that a single treatment with a normal therapeutic dose of acetyl salicylic acid produces an inhibition of platelet aggregation responses induced by 5-HT, DA and DMDA which is very similar to that described by others for ADP induced aggregation and believed to be due to inhibition of prostaglandin synthesis (O'Brien, 1970; Silver *et al.*, 1974).

We might therefore conclude that the inhibition of the secondary phase of platelet aggregation induced by 5-HT, DMDA, or DA in CPZ treated patients could be due to an inhibition of prostaglandin synthesis. This conclusion is supported by the fact that secondary phase aggregation can be partially restored by addition of PGE₂ after oral acetyl salicylic acid. However, we do not conclude that PGE₂ is the only prostaglandin involved, and further studies will be necessary with other prostaglandins and the prostaglandin precursor arachidonic acid which induces irreversible aggregation (Silver *et al.*, 1974).

We have now shown that irreversible aggregation responses with a pronounced secondary phase of aggregation can be produced by treatment with chlorpromazine, fluphenazine, flupenthixol (Boullin *et al.*, 1975; Orr & Boullin, 1976) and with thioridazine (Boullin & Woods, unpublished).

observations). These responses can be produced with 5-HT, DA and DMDA. As the preceding paper has shown that there appears to be a relationship between the clinical state of the patient and the presence or absence of irreversible aggregation to 5-HT (Orr & Boullin, 1976), two questions may be posed: Do the effects of acetyl salicylic acid explain the secondary phase of aggregation? Are the prostaglandins involved in the mechanism of action of the neuroleptics in regard to their clinical effects? The latter question presupposes that neuroleptic drugs affect prostaglandin synthesis, storage, release or catabolism.

Regarding the first question, we have no reason to assume that the experiments with acetyl salicylic acid have done anything else than throw light on the mechanism of development of the secondary phase of aggregation produced by 5-HT, DA and DMDA. We believe that the development

of the secondary phase in fact involves PGE₂ and possibly other prostaglandins and that acetyl salicylic acid prevents this. A single dose of acetyl salicylic acid did not affect the clinical state of our CPZ treated patients, and we do not believe that an investigation of the combined effects of acetyl salicylic acid and neuroleptic drugs is warranted. Secondly, we have no information upon the effects of CPZ or other neuroleptics relating to prostaglandin effects in the brain but it is possible that the effects of CPZ and prostaglandins may be linked via effects on brain adenylate cyclase, and these possibilities are currently under investigation in our laboratory.

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