THE RELIABILITY OF 5-HYDROXYTRYPTAMINE INDUCED PLATELET AGGREGATION RESPONSES IN SCHIZOPHRENIC PATIENTS TREATED WITH NEUROLEPTIC DRUGS

J.M. KNOX, M.W. ORR, R. ALLEN & M.G. GELDER

University of Oxford, Department of Psychiatry,
Littlemore Hospital Research Unit, Oxford OX4 4XN

D.G. GRAHAME-SMITH

MRC Unit and University Department of Clinical Pharmacology,
Radcliffe Infirmary, Oxford OX2 6HE

- 1 The reliability of 5-hydroxytryptamine induced platelet aggregation responses in psychiatric patients treated with neuroleptic drugs was investigated by examining the effects of a number of technical factors which could account for the variability of responses observed in some patients.
- 2 Variation in incubation time, temperature, aggregometer stirrer speed, and in the time interval between withdrawal of the blood sample and testing, had no significant effects on the consistency of responses.
- 3 Dilution of platelet rich plasma (PRP) led to enhanced platelet aggregation responses becoming non-enhanced.
- 4 Overcentrifugation of blood samples during the preparation of PRP caused a decrease in the incidence of enhanced platelet aggregation responses observed in chronic schizophrenic patients receiving chlorpromazine.
- 5 The reliability of platelet aggregation responses as indices of the action of neuroleptic drugs in psychiatric patients remains doubtful.

Introduction

We have recently reported (Orr, Knox, Allen, Gelder & Grahame-Smith, 1981) a series of clinical studies in which we have shown that enhanced platelet aggregation responses in psychiatric patients receiving a variety of neuroleptic drugs do not occur as consistently or as often as expected from previous work (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975; Boullin, Knox, Peters, Orr, Gelder & Grahame-Smith, 1978). We have shown that our previous findings with chlorpromazine do not generalise readily to other neuroleptic drugs, and that some patients show considerable variability in platelet aggregation responses. We therefore suggested that the relevance of the human blood platelet as a pharmacodynamic index of neuroleptic drug action which could be used as a clinical guide to treatment in schizophrenia was open to question. We also suggested that further work on the relationship between the effects of neuroleptic drugs on platelet aggregation responses and therapeutic effect was unlikely to be fruitful until the sources of variability in platelet aggregation responses could be explained and the mechanism

whereby enhancement of aggregation occurred is better understood.

This paper reports a series of experiments designed to examine a number of possible technical factors which could account for the variability in platelet aggregation responses. We also report an attempt to validate any possible findings from these experiments by applying these to our study of patients. The following possible technical factors were examined: (a) dilution of platelet rich plasma (PRP), thus reducing the number of platelets in the sample, (b) variation of incubation time, (c) variation in the temperature at which platelet aggregation studies were carried out, (d) variation in the speed at which platelets were stirred during testing, as this could have had effects on collision rates, and (e) the time interval between blood sampling and subsequent testing.

Methods

Plasma (20 ml) was withdrawn from patients receiving neuroleptic drugs, or from normal controls. In

patients, samples were withdrawn before the morning dose of medication was given. Platelet rich plasma was prepared and platelet aggregation responses obtained as described elsewhere (Orr & Boullin, 1976).

Dilution of platelet rich plasma

Six patients who were known to show enhanced platelet aggregation were studied. Platelet rich plasma was then diluted by increasing quantities of platelet free plasma and the effect of increasing dilution was observed.

Variation in incubation time

PRP samples from patients who were receiving neuroleptic drugs and who had previously shown either normal responses or enhanced responses were incubated at 37°C for periods of time ranging from 3–60 min before testing.

Variation in temperature

Blood samples were withdrawn from three schizophrenic patients receiving a large dose of flupenthixol, and from a normal volunteer. None of these had shown enhanced aggregation previously. Platelet rich plasma was prepared in the usual way and aggregation responses were tested at temperatures ranging from 24°C to 48°C.

Variation in the stirrer speed

Blood samples were withdrawn from patients who were known to show enhanced aggregation and the samples of PRP were stirred at different rates ranging from 40–900 rev/min and the effects of platelet aggregation response were observed.

Variation in the time interval between blood withdrawal and testing

Blood samples were withdrawn from patients receiving neuroleptic drugs and known to show enhanced or normal aggregation and PRP was then prepared at varying intervals after collection of the sample.

Results

Effects of dilution of PRP

Dilution of platelet rich plasma with platelet free plasma produced striking effects on previously enhanced aggregation responses. Figure 1 shows that the area under the aggregation curve (ΔOD), which represents the magnitude of the aggregation response,

Figure 1 The effects of dilution of platelet rich plasma (PRP) with platelet free plasma on 5-hydroxytryptamine (5-HT) induced platelet aggregation responses, expressed as a percentage of the response induced by adenosine diphosphate (ADP), in schizophrenic patients treated with neuroleptics.

fell with increasing dilution of PRP and enhanced responses became non-enhanced when PRP samples were diluted by 30%. The effects of dilution are presumably due to a decrease in the number of platelets in the sample of PRP, and this can also be achieved by over-centrifugation of blood samples during preparation of PRP.

Boullin *et al.* (1978) reported platelet aggregation enhancement in only two of thirteen chronic schizophrenic patients receiving chlorpromazine; on examination of the techniques followed we found that blood samples were centrifuged at 180–200 g during preparation of PRP, and this could have led to a decrease in the number of platelets in PRP. We therefore re-tested twelve of the thirteen patients on a separate occasion and showed that, if blood samples were centrifuged at 100 g, the frequency with which enhanced responses could be observed increased so that half the patients now showed enhanced aggregation.

Variation in incubation time

This did not produce any significant effects on platelet aggregation responses. Plasma samples showing enhanced platelet aggregation continued to do so, irrespective of the length of time for which the sample was incubated at 37°C.

Variations in temperature

The only effect that could be observed was at 48°C, when a previously enhanced platelet aggregation response became reversible.

Variation in the stirrer speed

This showed no significant effects on platelet aggregation at speeds above 400 rev/min. Stirrer speeds below this led to smaller responses, presumably because of effects on collision rate.

Effects of the age of the sample

PRP samples preserved at room temperature for up to 6 h produced the same responses as freshly prepared PRP, and no significant effects could therefore be observed.

Discussion

We have examined a number of possible technical factors which could have accounted for the variability in platelet aggregation responses seen in patients receiving neuroleptic drugs. Of the various possibilities tested, only dilution of platelet rich plasma proved to

be a significant factor in that a relatively minor dilution led to enhanced responses becoming non-enhanced. We validated this finding in our re-examination of a group of chronic schizophrenic patients receiving chlorpromazine, in whom enhanced responses could be elicited more readily if blood samples were centrifuged at a slower speed during the preparation of PRP, and we believe that over-centrifugation may account, at least in part, for the low incidence of enhanced aggregation responses in this group of patients reported by Boullin *et al.* (1978). There is no obvious reason why dilution of PRP should have these effects, as it has been shown by Boullin, Grahame-Smith & Grimes (1975b) that enhanced aggregation is due to a change in the properties of platelets themselves rather than to any factor in the plasma, and one would therefore have expected abnormal or enhanced responses irrespective of the number of platelets in the sample.

The experiments reported here suggest that false negatives, i.e. normal reversible responses rather than enhanced responses, may not be uncommon and provide a further source of within patient variability.

Our reservations with regard to the relevance of platelet aggregation responses as a pharmacological index of neuroleptic drug action would therefore remain unchanged.

This research was supported by MRC grant G977/879.

References

- BOULLIN, D.J., WOODS, H.F., GRIMES, R.P.J., GRAHAME-SMITH, D.G., WILES, D., GELDER, M.G. & KOLAKOWSKA, T. (1975). Increased platelet aggregation responses to 5-hydroxytryptamine in patients taking chlorpromazine. *Br. J. clin. Pharmac.*, **2**, 29–35.
- BOULLIN, D.J., GRAHAME-SMITH, D.G. & GRIMES, R.P.J. (1975). Resuspension of platelets: Enhanced 5-hydroxytryptamine induced aggregation in chlorpromazine treated patients due to changes in platelet properties. *Br. J. clin. Pharmac.*, **2**, 37–39.
- BOULLIN, D.J., KNOX, J.M., PETERS, J., ORR, M.W., GELDER, M.G. & GRAHAME-SMITH, D.G. (1978). Platelet aggregation and chlorpromazine therapy. *Br. J. clin. Pharmac.*, **6**, 538–540.
- ORR, M.W. & BOULLIN, D.J. (1976). The relationship between changes in 5-HT induced platelet aggregation and clinical state in patients treated with fluphenazine. *Br. J. clin. Pharmac.*, **3**, 925–928.
- ORR, M.W., KNOX, J.M., ALLEN, R., GELDER, M.G. & GRAHAME-SMITH, D.G. (1981). The effects of neuroleptic drugs on 5-hydroxytryptamine induced platelet aggregation in schizophrenic patients. *Br. J. clin. Pharmac.*, **11**, 255–259.

(Received May 30, 1980)