Uptake of dopamine and 5-hydroxytryptamine by platelets of patients with Parkinsonism

M. J. AMINOFF, R. S. B. EHSANULLAH, AND P. TURNER

From the National Hospital for Nervous Diseases and the Department of Clinical Pharmacology, St Bartholomew's Hospital, London

SUMMARY The uptake of tritiated dopamine and 5-hydroxytryptamine by platelets from 11 patients with Parkinsonism who were not receiving any medication, and from 11 control subjects matched for sex and for age within a decade was compared. No significant differences were found in the uptake of either amine. Our findings, therefore, provide no support for the belief that there is a generalised defect of dopamine systems in Parkinson's disease.

It has been known for some years that platelets accumulate 5-hydroxytryptamine (Humphrey and Toh, 1954; Hardisty and Stacey, 1955), noradrenaline (Born *et al.*, 1958), and dopamine (Boullin and O'Brien, 1970). Attention has recently been directed at the platelets as an easily accessible model of aminergic neurones.

Reduced levels of dopamine and 5-hydroxytryptamine have been reported in the corpus striatum of patients with Parkinson's disease, and the concentrations of their metabolites (homovanillic acid and 5-hydroxyindole acetic acid) are reduced in the cerebrospinal fluid. However, a number of the symptoms and signs of this disorder are difficult to explain on the basis of pathology localised to the corpus striatum, and Barbeau (1969) accordingly postulated the existence of a more generalised defect of dopamine metabolism. Little direct evidence for this belief was available until Barbeau and his colleagues (1975) reported an abnormality of dopamine uptake by platelets from patients with Parkinsonism, and suggested that the basic defect in this disorder involves membrane structure or membrane transport systems of dopaminergic systems in general. In an attempt to gain further insight into the pathophysiology of Parkinson's disease, we too have studied the uptake of biogenic amines by platelets from patients with this disorder.

Methods

Eleven patients (eight male and three female) with Parkinson's disease, ranging in age between 36 and 73 years, formed the test group. They were not on anti-Parkinsonism medication or on any drugs known to affect biogenic amine systems at the time of the study, and had not taken such drugs for at least the previous four weeks. The control subjects were 11 healthy volunteers without any disorders known to affect biogenic amine systems, and were not on any medication.

A 30 ml sample of venous blood was drawn from a patient with Parkinsonism, and from a control subject matched for sex, and for age within a decade. The order in which blood samples were obtained was randomised, but the second was always taken within 30 minutes of the first. Plastic syringes and tubes were used to prevent platelet aggregation. Each blood sample was put into a centrifuge tube containing 4 ml of anticoagulant. The anticoagulant used consisted of 25 g trisodium citrate, 13.7 g citric acid, and 20 g glucose in one litre of distilled water (Aster and Jandl, 1964). The blood was left at room temperature for three hours to separate, and the platelet rich plasma (PRP) removed. The remaining blood was centrifuged at 150 rpm for 5–10 min, and the plasma layer removed and added to the PRP already obtained. The tube was then swirled gently so that the platelets were dispersed evenly in the plasma.

About 0.2 ml of the PRP was withdrawn in each case so that the platelets could be counted, using a Coulter thrombocounter, model C. In order

Address for reprint requests: Dr M. J. Aminoff, Department of Neurology, School of Medicine, University of California, San Francisco, California 94143, USA.

Accepted 14 February 1978

to determine the uptake of 5-hydroxytryptamine or dopamine by PRP, 1 ml aliquots of PRP were incubated with the tritiated amine at 37°C in a water bath with a shaker. Uptake of 5-hydroxytryptamine was stopped after 5 min, and of dopamine after 10 min, by immersing the tubes in ice-cold water for a few minutes. The platelets were centrifuged out at 9000 rpm for 3.5 min, and the supernatant was decanted. One millilitre of 0.01 M KOH was added to the remaining platelet pellet, and left overnight. Platelets were resuspended with a rota mixer, and 100 μ l samples put into 10 ml Instagel and counted twice in a Packard Tri-Carb liquid scintillation spectrometer. The readings were converted to disintegration per minute (dpm) per 10^8 platelets. The tritium-labelled 5-hydroxytryptamine and dopamine were obtained from the Radiochemical Centre, Amersham. The uptake of each amine was studied at four different concentrations, 0.1, 0.2, 1.0, and 2.0 nmol/ml PRP.

The study was performed with the approval of the Committee for Human Experimentation of the National Hospital, London, and blood samples were obtained only from subjects who had given their informed consent.

Results

There was no significant difference in the platelet counts between the group of patients with Parkinsonism, and the control group of subjects. Thus, the mean platelet count was $441 (\pm \text{SEM } 50) \times 10^9/\text{l}$ in the former group, and $520 (\text{SEM } 80) \times 10^9/\text{l}$ in the latter.

Fig. 1 Mean ($\pm$ SEM) uptake of dopamine into platelets of untreated patients with Parkinsonism (●) and control subjects (Δ).

With regard to the uptake of dopamine or 5-hydroxytryptamine the grouped data are presented in Figs. 1 and 2. In analysing the results, we performed both paired and unpaired *t* tests on the data. We compared the results from each patient with a paired control subject. We also compared the mean and standard errors of the results obtained in the group of patients with Parkinsonism to those of the control group. We found no significant differences between patients and control subjects in the uptake of either amine. One patient appeared to have a very low platelet count, and an exceedingly high uptake level of both amines, but these findings were probably artefacts caused by platelet aggregation. This result alone is responsible for the mean value for amine uptake being higher in the test group than in the control group.

Discussion

There is increasing interest in the platelets as a model for the study of aminergic neurones and, more particularly, of the amine-storing presynaptic nerve terminals. As regards 5-hydroxytryptamine, such data as have accumulated suggest that the properties of platelets and of CNS serotonergic

Fig. 2 Mean ($\pm$ SEM) uptake of 5-hydroxytryptamine into platelets of untreated patients with Parkinsonism (●) and control subjects (Δ).

synaptosomes are sufficiently similar for the former to be accepted as a valid and appropriate model of the latter (Stahl, 1977).

We have found no difference between platelets from patients with Parkinsonism and those from normal subjects in the uptake of 5-hydroxytryptamine, supporting the observations of Boullin and O'Brien (1970). These authors, and Yamaguchi *et al.* (1972), also found that platelet 5-hydroxytryptamine levels were normal in patients with untreated Parkinsonism, but we have no further information on this point.

It is harder to justify acceptance of the platelets as a model of dopaminergic neurones at the present time, however, since a number of important differences may exist between them. Thus, Solomon *et al.* (1970) demonstrated that human platelets accumulate dopamine by an uptake mechanism which differs from the transport process for this amine in the corpus striatum. Indeed, the results of their more recent study led Stahl and Meltzer (1978) to suggest that, in contrast to the findings of Boullin and O'Brien (1970) and Solomon *et al.* (1970), dopamine accumulates in human platelets by passive diffusion, the dopamine then becoming bound to the amine-storage granules within the platelets. In particular, these authors found that the accumulation of dopamine by the platelets was not kinetically saturable and was not significantly inhibited by ouabain, metabolic inhibition, or by tricyclic antidepressant drugs. These findings, if confirmed by others, indicate that the characteristics of dopamine accumulation by human platelets are not comparable with those of dopamine transport into dopaminergic neurones of the central nervous system. Although human platelets could not be accepted as a valid model of dopaminergic neurones in such circumstances, they could still be regarded as a model for the binding of dopamine by amine storage granules (Stahl and Meltzer, 1978).

Boullin and O'Brien (1970) found that platelets from patients with Parkinsonism who were on treatment with L-dopa showed an increased affinity for dopamine, a decreased equilibrium concentration of dopamine after incubation for 90 min, and a greater efflux of dopamine from loaded platelets during a 10 min period of incubation, compared with platelets from normal subjects. In untreated patients with Parkinsonism, however, dopamine accumulation by platelets was normal, and the only abnormality that they could find was an enhanced efflux of dopamine from loaded platelets. Their findings accord with our own observations of normal uptake of dopamine by platelets of patients with Parkinsonism, but conflict with those

of Barbeau *et al.* (1975), who found that patients with Parkinsonism, whether untreated or treated either with anticholinergic or antihistaminic drugs or both, or with L-dopa with or without a peripheral dopa-decarboxylase inhibitor, had a diminished uptake of dopamine. Moreover, in contrast to Boullin and O'Brien (1970), Barbeau *et al.* (1975) found no difference in dopamine efflux from loaded platelets of normal subjects and patients with Parkinsonism.

We are unable to reconcile these differences between the findings of Boullin and O'Brien (1970) and ourselves on the one hand, and those of Barbeau *et al.* (1975) on the other concerning dopamine uptake. Presumably they do not relate to methodological differences since the approach of Boullin and O'Brien was adopted by Barbeau and his colleagues to facilitate comparison of their findings. Both these groups estimated uptake of dopamine after periods of incubation that were at least as long as the period we used (10 min), but were usually very much longer. Accordingly, the values for uptake that they found will have reflected any loss of dopamine occurring over this time due to spontaneous release from platelets, and—as indicated above—an enhanced efflux of dopamine from loaded platelets of patients with Parkinsonism was indeed found in one of these studies (Boullin and O'Brien, 1970), though not in the other (Barbeau *et al.*, 1975).

Our approach was somewhat different, since we were concerned primarily with the dopamine uptake process, and in particular with the velocity of initial uptake rather than the equilibrium concentration. For this reason we studied the uptake of dopamine during its linear phase, using incubation periods of only 10 min duration so that we could detect any increase or decrease in uptake. Our findings, and those of Boullin and O'Brien (1970), indicate that dopamine uptake by platelets from patients with untreated Parkinson's disease is normal. They, therefore, provide no support for the belief advanced by Barbeau (1969) that there is a generalised defect of dopamine systems in that disorder.

We are grateful to the physicians of the National Hospital, and to Dr G. M. Stern of University College Hospital, London, for referring patients for this study.

References

- Aster, R. H., and Jandl, J. H. (1964). Platelet sequestration in man. I. Methods. *Journal of Clinical Investigation*, **43**, 843–855.

- Barbeau, A. (1969). Parkinson's disease as a systemic disorder. In *Third Symposium on Parkinson's Disease*, pp. 66–73. Edited by F. J. Gillingham and I. M. L. Donaldson. Churchill Livingstone: Edinburgh.
- Barbeau, A., Campanella, G., Butterworth, R. F., and Yamada, K. (1975). Uptake and efflux of ^{14}C -dopamine in platelets: evidence for a generalised defect in Parkinson's disease. *Neurology (Minneapolis)*, **25**, 1–9.
- Born, G. V. R., Hornykiewicz, O., and Stafford, A. (1958). The uptake of adrenaline and noradrenaline by blood platelets of the pig. *British Journal of Pharmacology and Chemotherapy*, **13**, 411–414.
- Boullin, D. J., and O'Brien, R. A. (1970). Accumulation of dopamine by blood platelets from normal subjects and parkinsonian patients under treatment with L-Dopa. *British Journal of Pharmacology*, **39**, 779–788.
- Hardisty, R. M., and Stacey, R. S. (1955). 5-Hydroxytryptamine in normal human platelets. *Journal of Physiology*, **130**, 711–720.
- Humphrey, J. H., and Toh, C. C. (1954). Absorption of serotonin (5-hydroxytryptamine) and histamine by dog platelets. *Journal of Physiology*, **124**, 300–304.
- Solomon, H. M., Spirt, N. M., and Abrams, W. B. (1970). The accumulation and metabolism of dopamine by the human platelet. *Clinical Pharmacology and Therapeutics*, **11**, 838–845.
- Stahl, S. M. (1977). The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Archives of General Psychiatry*, **34**, 509–516.
- Stahl, S. M., and Meltzer, H. Y. (1978). The human platelet as a model for the dopaminergic neuron: kinetic and pharmacologic properties and the role of amine storage granules. *Experimental Neurology*, **59**, 1–15.
- Yamaguchi, K., Tomasi, L., Cote, L. J., Barrett, R., and Yahr, M. D. (1972). Serotonin content of platelet enriched plasma in Parkinson patients prior and during treatment with L-3, 4-dihydroxyphenylalanine (L-Dopa). *Biochemical Medicine*, **6**, 210–215.