Uptake and release of ^{14}C -5-hydroxytryptamine by platelets in affective illness

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SUMMARY In the search for paradigms of postulated abnormalities in indolamine function in the brain in affective disorders, 5-HT uptake and release was estimated in platelets from patients suffering from severe depressive illness. Neither of these measures was altered by the illness and the most likely explanation for the negative findings was that in this setting platelets are not a suitable model.

On the evidence available at the present time, it seems probable that there are abnormalities in amine metabolism in affective illness. Definition of these abnormalities is hampered by the inaccessibility of the brain and, in particular, that part of it likely to be involved in the functional disorder. The problem would be simplified if the inquiry could be narrowed down on the one hand to changes in transport or binding of amines, and on the other to alterations in their synthesis or degradation. Pletscher (1968) has suggested that the platelet is a suitable model for some of the processes occurring in amine-utilizing neurones in the central nervous system. In the present study we have extended this concept to see if the behaviour of platelets *in vitro* would shed any light on the nature of the abnormalities in amine metabolism in affective disorders—that is, would reveal the presence and nature of pathological processes rather than just act as a model for normal biochemical/physiological functions. Previous studies have shown that there are peripheral abnormalities in indolamine metabolism in severe depressive illness (Coppen, Shaw, Maleson, Eccleston, and Gundy, 1965).

The main criticism levelled at peripheral studies in psychiatric illness is that there are no *a priori* reasons for equating central events with those occurring elsewhere in the body. The contrary view could be argued, however, that parallel mechanisms might well respond similarly to, for instance, an abnormal hormonal milieu, and it is on such grounds that peripheral studies are justified.

METHODS

1. PATIENTS

All were patients suffering from severe depressive illness and were diagnosed on the basis of experiencing a proportion of such symptoms as depression of mood, diurnal variation of mood, early morning waking, loss of energy, interest, concentration, appetite and libido, delusions of guilt and unworthiness, etc. None had taken lithium, none had received phenothiazines or other tranquillizing drugs for a minimum of four weeks and none had had antidepressant therapy for at least two weeks. They were studied in the first few days after admission and again one week after full recovery induced by course of electroconvulsive therapy.

Control subjects were normal volunteers from hospital and laboratory staffs.

2. UPTAKE AND RELEASE OF 5-HYDROXYTRYPTAMINE (5-HT) BY PLATELETS

The methods used were modified from those of Stacey (1961). Venous blood was taken between 09.15 and 09.45 hours after overnight fasting, was mixed with one-tenth its volume of a solution containing disodium edetate (10 g/l.), sodium chloride (7 g/l.), and glucose (1 g/l.), and was spun at 1,000 *g* for five minutes to obtain platelet-rich plasma. Two millilitre aliquots were equilibrated at 37.5°C with 95% O_2 , 5% CO_2 , after which 0.2 ml. of a solution of ^{14}C -5-hydroxytryptamine creatinine sulphate (Radiochemical Centre) diluted with 'cold' amine (Koch-Light) to 1.68 mc/m-mole, 76.7 μmole , was added to each flask. Uptake of amine was arrested by rapid cooling to 4°C after 10, 20, and 90 minutes incubation and the platelets were separated by centrifugation.

TABLE
MEANS OF DATA

		Mean 5-HT content of platelets (m-mole 5-HT/mole platelet nitrogen)							
		Duration of incubation (min):							
		10		20		90			
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a. Uptake of 5-HT									
Depressed patients									
ill		0.60 (10)		0.94 (10)		1.39 (11)			
well		0.56 (10)		0.91 (10)		1.48 (11)			
Control subjects		0.68 (6)		1.00 (6)		1.59 (11)			
		Ill (5)				Well (4)			
		<hr/>							
				Duration of incubation (min):					
		20	45	80	120	20	45	80	120
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b. Concentration of 5-HT in 'loaded platelets' after prolonged incubation									
Mean 5-HT content (m-mole 5-HT/mole N) as % of value after 10 min									
		102	102	99	100	97	94	100	92

Numbers in parentheses.

Intergroup comparisons by *t* test.

Intraindividual comparisons by *t* test for paired comparisons.

No significant differences between means.

fugation at 2,500 *g* for 30 minutes at 4°C. Aliquots of a NaOH digest of the platelet plug were assayed for radioactivity and nitrogen content (microkjeldahl) and the results were expressed as m-mole 5-HT/mole platelet nitrogen.

In the release experiments, 25 ml. blood was used to prepare platelet-rich plasma which was incubated with ^{14}C -5-HT for 90 minutes. A further 25 ml. blood was centrifuged at 3,000 *g* for 10 minutes to give 'platelet-free' plasma.

The platelet-rich plasma was centrifuged in two 20 ml. tubes at 2,000 *g* for 3.5 minutes and the two lots of platelets were resuspended each in 0.5 ml. EDTA-glucose-saline solution. The platelet suspension was added to the 'platelet-free' plasma, aliquots of which were incubated as before (but without addition of further 5-HT) for 10, 20, 45, 80, or 120 minutes. 5-HT content of the platelets (m-mole 5-HT/mole platelet nitrogen) was expressed as a percentage of the content at 10 minutes.

RESULTS

The means of the data obtained are given in the Table. Uptake during neither the early phase of incubation, 10 and 20 minutes, nor after prolonged exposure to 5-HT for 90 minutes differed as between ill and well phases, and in both instances the range of values obtained was within that seen in the control subjects. Similarly, platelets from depressed subjects showed no signs of being unable to retain 5-HT on exposure to a 'low 5-HT' environment.

DISCUSSION

Uptake of 5-HT into the platelets during the first

periods of incubation reflects the rate of transport of amine. At 90 minutes the platelets are approaching saturation, so that the content of 5-HT at this time gives some indication of their binding capacity. Exposure of 5-HT loaded platelets to fresh plasma assesses their ability to retain the amine. Under the conditions of our experiment, all these processes were normal in depressed subjects both when they were ill and after full clinical recovery.

One explanation for our negative findings is that the postulated abnormalities in affective illness do not lie in uptake or binding mechanisms but rather in enzymic or other processes. It is much more likely, however, that the platelet is an unsuitable model for the disorder of amine metabolism occurring centrally in this illness.

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