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Relationship between internal Na^+/K^+ and the accumulation of ^{14}C-5-hydroxytryptamine by rat platelets

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Summary

1. 5-Hydroxytryptamine (5-HT) transport has been investigated in rat blood platelets poisoned with dinitrophenol-sodium fluoride or ouabain.

2. The inhibition of transport produced by different concentrations of the metabolic inhibitors has been correlated with changes in the internal Na^+ and K^+ concentrations of the platelets.

3. Platelets poisoned in a high K^+ medium maintained a high internal K^+ concentration in the absence of cellular metabolism. When transferred to Krebs solutions containing different concentrations of Na^+ they accumulated 5-HT by a process that was related to the magnitudes of the internal and external Na^+ concentrations.

4. The results are consistent with the hypothesis that the spontaneous movement of ions through the platelet membrane is capable of providing, at least in part, the energy requirements for 5-HT transport.

Introduction

The accumulation of 5-hydroxytryptamine (5-HT) by blood platelets is a saturable, Na^+-dependent process obeying Michaelis–Menten kinetics (Humphrey & Toh, 1954 ; Born & Gillson, 1959 ; Lingjaerde, 1969 ; Sneddon, 1969). A detailed kinetic study of the role of Na^+ in this uptake process (Sneddon, 1969) suggests that the mechanism of uptake takes the form of a carrier mediated transport system in which the affinity of the carrier for 5-HT is influenced by the relative amounts of Na^+ and K^+ at the transporting surface, and in this respect 5-HT transport exhibits characteristics similar to the transmembrane movement of sugars, amino-acids and biogenic amines in a wide variety of tissues and cells (Christensen & Riggs, 1952 ; Crane, 1965 ; Kipnis & Parrish, 1965 ; Eddy, Mulcahy & Thomson, 1967 ; Eddy & Hogg, 1969 ; Bogdanski, Tissari & Brodie, 1970 ; Schultz & Curran, 1970 ; Smith & Ellory, 1971).

Inherent in many discussions of carrier mediated transport is the hypothesis that the transported species can be accumulated intracellularly without directly utilizing ATP, but by making use of the energy available from the tendency of Na^+ and possibly K^+ to move spontaneously across the cell membrane down their respective concentration gradients (Riggs, Walker & Christensen, 1958 ; Crane, 1964 ; Vidaver, 1964 ; Eddy, 1968a, b ; Schultz & Curran, 1970). Thus the transport of non-electrolytes can be visualized as essentially a physical process in which the cellular energy is not directly involved, but is expended to maintain the asymmetric distribu-

tion of cations across the cell membrane. An essential requirement for such a system is that the intracellular Na^+ concentration is maintained at a low level relative to the extracellular concentration and that the Na^+ is continually removed from the cell against a concentration gradient by means of the sodium pump linked to Na^+/K^+ stimulated ATPase.

The present experiments are an attempt to test the hypothesis that 5-HT may be accumulated by blood platelets without the direct intervention of cellular energy, but by utilizing the energy inherent in the tendency of Na^+ and/or K^+ to move spontaneously down their respective concentration gradients.

Methods

Blood platelets were obtained from adult male rats as previously described (Sneddon, 1969).

Measurement of ^{14}C-5-hydroxytryptamine uptake in poisoned platelets

The platelet pellet was resuspended in 16 ml of incubation medium (NaCl, 121 mM ; KCl, 4·17 mM ; KH_2PO_4, 1·18 mM ; $MgSO_4$, 1·18 mM ; Tris (hydroxymethyl) methylamine (Tris)-HCl pH 7·2, 24·87 mM) with or without ouabain, or a mixture of dinitrophenol-sodium fluoride (see **Results** for the concentrations used) to a final protein concentration of approximately 1 mg/ml. The suspension was preincubated for 15 min at 36° C, then 1 ml samples were distributed in twelve 4 ml plastic centrifuge tubes, and the platelets precipitated by centrifugation. The supernatant was decanted and the platelet pellets were gently resuspended in 1 ml samples of the original medium containing ^{14}C-5-HT (0·1 μCi/ml) and the incubation continued for a further 5 minutes. ^{14}C-5-HT uptake was stopped by the addition of 2 ml choline–Tris-HCl (Tris-HCl, 24·87 mM, choline chloride, 129·13 mM, pH 7·2) and the immediate separation of the platelets by rapid centrifugation. The supernatant was decanted, the inside of the tubes was wiped dry with tissue paper, and the platelet pellet was washed once by resuspending in 4 ml Tris-choline. This washing procedure took approximately 90 s from resuspending the pellet in Tris-choline until the final pellet was obtained by centrifugation. After carefully drying the inside of the centrifuge tubes the pellets were resuspended in 1 ml of deionized water. Samples (0·1 ml) were taken for liquid scintillation counting and for the determination of protein by the method of Lowry, Rosenbrough, Farr & Randall (1951). All tubes were run in triplicate.

^{14}C-5-HT uptake and sodium gradients

The internal Na^+ and K^+ concentrations in blood platelets were manipulated by incubation for 15 min at 36° C in a medium containing dinitrophenol, 0·75 mM, sodium fluoride, 20 mM, and different external concentrations of Na^+ and K^+ (see **Results**). After preincubation, ^{14}C-5-HT uptake was determined as before.

Determination of internal Na^+ and K^+

Identical tubes were treated as above except that the ^{14}C-5-HT was replaced by an equivalent amount of non-radioactive 5-HT. After the final wash, the platelet pellets were dissolved overnight in 0·1 ml of concentrated nitric acid and the volume

was made up to 4 ml with deionized water. After centrifuging to remove non-soluble material, the Na^+ and K^+ concentrations were determined on the supernatant with an EEL flame photometer by reading against standard solution containing both Na^+ and K^+ in the molar ratio 2:1 through the range 0·1 to 0·01 mM Na^+ (0·05–0·005 mM K^+). Extracellular contamination of the final pellet by electrolytes in the incubation medium was estimated using ^{14}C-inulin. After subtracting the appropriate value for the inulin space, the Na^+ and K^+ content of the platelet pellet was expressed as μmol/mg platelet protein.

Drugs

All solutions were made up in deionized water. Dinitrophenol and ouabain were obtained from the Sigma Chemical Co. 5-Hydroxytryptamine-3′-14C-creatine sulphate, sp. ac. 58 mCi/mM, and inulin (carboxylic acid-C14) sp. ac. 1·62 Ci/mg were obtained from the Radiochemical Centre, Amersham.

Results

If the sodium gradient hypothesis provides an explanation for the mechanism of accumulation of ^{14}C-5-HT, it can be predicted that the degree of inhibition of uptake at different concentrations of metabolic inhibitors will be related to changes in the intracellular Na^+ concentration. The first experiments were designed to test this prediction by comparing the inhibitory effect of different concentrations of the metabolic inhibitors ouabain (Na^+:K^+ activated ATPase), dinitrophenol (oxidative phosphorylation) and sodium fluoride (glycolysis) on ^{14}C-5-HT accumulation and the changes in intracellular Na^+ and K^+.

Ouabain

The experiments were performed as described in **Methods**. After preincubation for 15 min with either 10^{-3}M, or 5×10^{-4}M or 10^{-4}M ouabain, the platelets were collected by centrifugation and resuspended in fresh incubation medium containing the same concentration of ouabain and 0·1 μCi/ml ^{14}C-5-HT or non-radioactive 5-HT, and the incubation continued for a further 5 minutes. Samples of the washed pellets were processed either for the determination of radioactivity or internal Na^+ and K^+.

TABLE 1. *Effect of different concentrations of ouabain on the internal Na^+ and K^+ concentrations of blood platelets and on the accumulation of ^{14}C-5-HT*

Concentration of ouabain	Na^+ content (μmol/mg protein)	K^+ content (μmol/mg protein)	Total (Na^+ K^+) (μmol/mg protein)	Na^+:K^+ ratio	^{14}C-5-HT uptake (c.p.m./mg protein)	*n*
10^{-3}M	0·2280±0·018	0·2092±0·016	0·4371±0·029	1·161±0·121	3059±301	27
5×10^{-4}M	0·2410±0·028	0·2180±0·007	0·4589±0·032	1·015±0·078	3653±546	12
10^{-4}M	0·2047±0·019	0·2326±0·011	0·4373±0·022	0·92 ±0·10	4905±547	15
None (controls)	0·1916±0·023	0·3165±0·029	0·5081±0·047	0·63 ±0·052	6310±657	15

^{14}C-5-HT accumulation measured after incubation for 5 min at 36° C. *n*, Number of observations used to calculate the mean ±S.E.M. of the individual values.

Ouabain significantly inhibited the uptake of ^{14}C-5-HT at 10^{-3}M, although the inhibitory effect was much less apparent when the concentration was reduced tenfold (Table 1). The corresponding values for the internal Na^+ and K^+ are given in the same table. Preincubation with 10^{-3}M ouabain resulted in a fall in internal K^+ concentration and a rise in internal Na^+ so that the ratio $Na^+:K^+$ was doubled. However, lower concentrations of ouabain had a similar but less marked effect; the smaller the increase in internal Na^+ concentration, the greater the accumulation of ^{14}C-5-HT. The inhibitory action of a given concentration of ouabain varied from experiment to experiment, so that relatively large standard errors are obtained when the results are grouped together for analysis. However, from the internal $Na^+:K^+$ ratio it is possible to calculate the extent of the Na^+ gradient during the period when the platelets are exposed to ^{14}C-5-HT, and if a graph of log ^{14}C-5-HT uptake against the internal Na^+ concentration is plotted (Fig. 1), it will be seen that the ^{14}C-5-HT uptake correlates with the internal Na^+ concentration rather than the concentration of ouabain used for the preincubation. The larger the increase in internal Na^+ with a given concentration of ouabain, and hence the smaller the sodium gradient, the less ^{14}C-5-HT is accumulated during the 5 min incubation. The scatter in the individual results has been reduced by taking $\log_{10}$ on the values for ^{14}C-5-HT uptake.

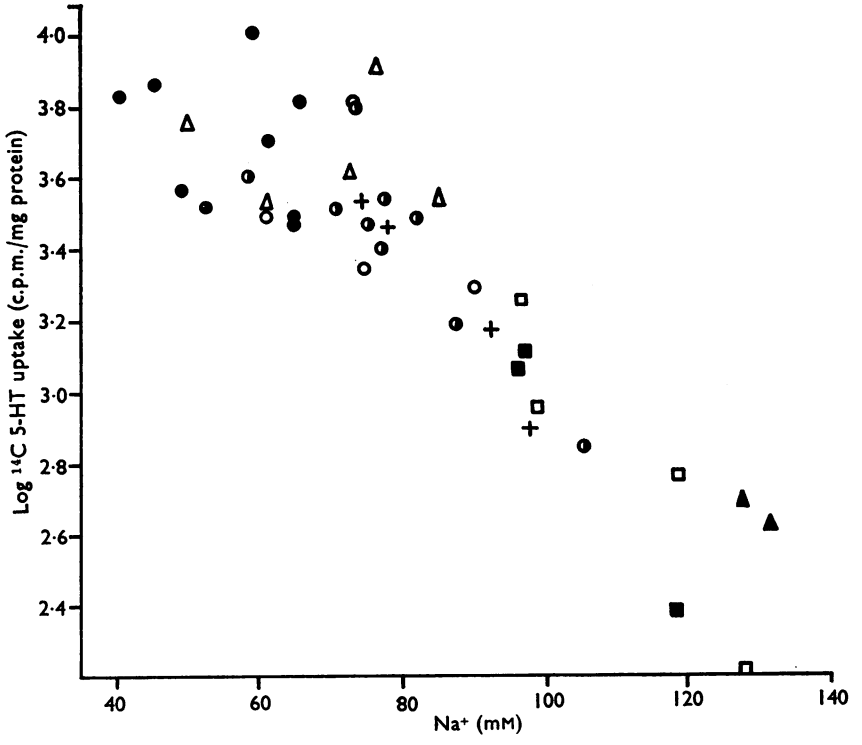

FIG. 1. Plot of log ^{14}C-5-HT uptake (c.p.m./mg protein) as a function of the internal Na^+ concentration in the transient steady state after preincubation with different concentrations of metabolic inhibitors. During the 15 min preincubation and during the subsequent ^{14}C-5-HT uptake the platelets were resuspended in one of the following concentrations of inhibitors: ouabain (10^{-4}M) △, (5×10^{-4}) ○, (10^{-3}) ◑; NaF/DNP ($4\times10^{-2}/1{\cdot}5\times10^{-3}$) ▲, ($2\times10^{-2}/7{\cdot}5\times10^{-4}$) □, ($10^{-2}/3{\cdot}75\times10^{-4}$) ■, ($2\times10^{-3}/0{\cdot}75\times10^{-4}$) +. Controls, ●. Correlation coefficient of log 5-HT uptake versus internal Na^+ concentration was calculated to be $-0{\cdot}894$ ($P<0{\cdot}001$).

TABLE 2. *Effect of different concentrations of sodium fluoride (NaF) and dinitrophenol (DNP) on the internal Na^+ and K^+ concentrations of blood platelets and the accumulation of ^{14}C-5-HT*

Concentration of inhibitors	Na^+ content (μmol/mg protein)	K^+ content (μmol/mg protein)	Total ($Na^+ + K^+$) (μmol/mg protein)	$Na^+ : K^+$ ratio	^{14}C-5-HT uptake (c.p.m./mg protein)	*n*
NaF (4×10^{-2} M) DNP ($1{\cdot}5\times10^{-3}$ M)	0·3924±0·014	0·0880±0·012	0·4806±0·027	4·83±0·03	430± 34	6
NaF (2×10^{-2} M) DNP ($7{\cdot}5\times10^{-4}$ M)	0·3635±0·022	0·1623±0·031	0·5201±0·052	3·07±0·43	866±188	12
NaF (10^{-2} M) DNP ($3{\cdot}75\times10^{-4}$ M)	0·3198±0·005	0·1919±0·023	0·5200±0·023	1·98±0·27	1255±214	12
NaF (2×10^{-3} M) DNP ($0{\cdot}75\times10^{-4}$ M)	0·2553±0·022	0·2133±0·026	0·4689±0·045	1·32±0·27	2181±332	12
None (controls)	0·1719±0·012	0·3365±0·031	0·5076±0·040	0·54±0·05	4401±532	12

^{14}C-5-HT accumulation measured after incubation for 5 min at 36° C. *n* = Number of observations used to calculate the mean ± S.E.M. of the individual values.

Inhibition of ATP production

The relationship between the internal Na^+ concentration and ^{14}C-5-HT accumulation was investigated further by inhibiting the formation of ATP. Washed rat blood platelets exhibited both oxidative phosphorylation and glycolysis (Detwiler & Zivkovic, 1970) and inhibition of ATP production was achieved by using a mixture of dinitrophenol (DNP) and sodium fluoride (NaF). Platelets were preincubated with different concentrations of a fixed ratio of DNP–NaF for 15 min, then resuspended in medium containing the same concentration of inhibitors plus ^{14}C-5-HT. The effects of the different concentrations of DNP–NaF on the internal Na^+ and K^+ concentrations and on the accumulation of ^{14}C-5-HT are shown in Table 2. All concentrations of DNP–NaF tested markedly decreased ^{14}C-5-HT accumulation, and this was associated with an increase in the intracellular Na^+ concentration and a fall in the K^+ concentration. As in the case of ouabain, the degree of inhibition of ^{14}C-5-HT accumulation correlated with the increase in internal Na^+ concentration, rather than the concentration of the metabolic inhibitor used (Fig. 1). Calculation of the correlation coefficient for log ^{14}C-5-HT uptake versus the internal Na^+ concentration for all the data in Fig. 1 ($r = -0.894$, p 0·001) indicates the high degree of correlation between internal Na^+ and ^{14}C-5-HT uptake.

Effect of varying amounts of Na^+ and K^+ in poisoned platelets

If the energy for the accumulation of ^{14}C-5-HT is provided, in part, by the inward-directed Na^+ gradient, it would be expected that an induced Na^+ gradient would stimulate ^{14}C-5-HT accumulation. Attempts to establish an artificial Na^+ gradient in normal respiring blood platelets by suddenly increasing the external Na^+ concentration to 200 or 300 mM always resulted in a decreased accumulation of ^{14}C-5-HT. This effect is apparently due to platelet damage in the hypertonic medium, as similar results could be obtained when the medium was made hypertonic by the addition of small volumes of 5 M LiCl or choline chloride. To overcome this difficulty a suitable test system was devised where it was possible to vary the internal Na^+ and K^+ concentrations in platelets that had been poisoned with metabolic inhibitors. This was achieved by preincubation of washed platelets with a mixture of sodium fluoride (20 mM) and dinitrophenol (0·75 mM) in an incubation medium where the

TABLE 3. *Na^+ concentration and subsequent ^{14}C-5-HT accumulation in platelets prepared in various incubation media in the presence of NaF* (20 mM) *and DNP* (0·75 mM)

Cells poisoned for 15 min in medium containing		^{14}C-5-HT accumulated for 5 min in medium containing		Intracellular Na^+ μmol/mg protein		^{14}C-5-HT uptake (c.p.m./mg
Na^+ (mM)	K^+ (mM)	Na^+ (mM)	K^+ (mM)	Before 5-HT	After 5-HT	protein)
18	108	121	5	0·1633±0·009	0·2483±0·023	1247±198
36	90	121	5	0·2418±0·017	0·2687±0·032	750±18
72	54	121	5	0·2931±0·018	0·3659±0·012	526±18
121	5	121	5	0·5157±0·032	0·5018±0·025	211±7

Intracellular Na^+ and K^+ were determined after preincubation and 5 min after resuspending the platelets in 121 mM Na^+ incubation medium containing 5-HT. All incubation media contained 20 mM NaF 0·75 mM DNP throughout the experiment. ^{14}C-5-HT uptake has been corrected for extracellular trapping in the final pellet, and the mean±S.E.M. are given for four determinations from two separate experiments.

external concentrations of Na^+ and K^+ was maintained at 126 mM. After pre-incubation the platelets were collected by centrifugation and resuspended in standard incubation medium (121 mM $Na^+ + 5$ mM K^+), containing the metabolic inhibitors and either ^{14}C-5-HT or an equal concentration of non-radioactive 5-HT. Platelets prepared in a medium of high K^+ and low Na^+ maintained a low internal Na^+ concentration relative to K^+ during the period of preincubation, and following exposure to a high Na^+ environment these platelets accumulated approximately five times as much ^{14}C-5-HT as platelets poisoned in a high Na^+ medium which had a raised internal Na^+ concentration (Table 3).

If platelets prepared in a low Na^+ medium were exposed to standard incubation (121 mM Na^+) for 5 min before the addition of ^{14}C-5-HT, they did not accumulate ^{14}C-5-HT during a further period of incubation. This suggests that the factor or factors involved in the accumulation of ^{14}C-5-HT are dissipated in the high sodium medium whether or not 5-HT is present.

In the previous experiment an artificial sodium gradient was established in poisoned blood platelets by keeping the external Na^+ concentration constant and varying the internal Na^+ concentration. An alternative method of establishing a sodium gradient is to maintain a very low internal Na^+ concentration and vary the external Na^+ concentrations. The results of such an experiment are shown in Fig. 2. Blood platelets were poisoned with NaF/DNP in a medium containing 108 mM K^+ and 18 mM Na^+. After preincubation, the platelets were centrifuged and samples resuspended in medium of progressively increasing Na^+ concentrations (18 mM ; 36 mM ; 72 mM and 121 mM). Platelets prepared in this manner maintain a low internal Na^+ content relative to K^+ and transferring samples to solutions of high Na^+ effectively establishes a sodium gradient from the external medium to the inside of the cell.

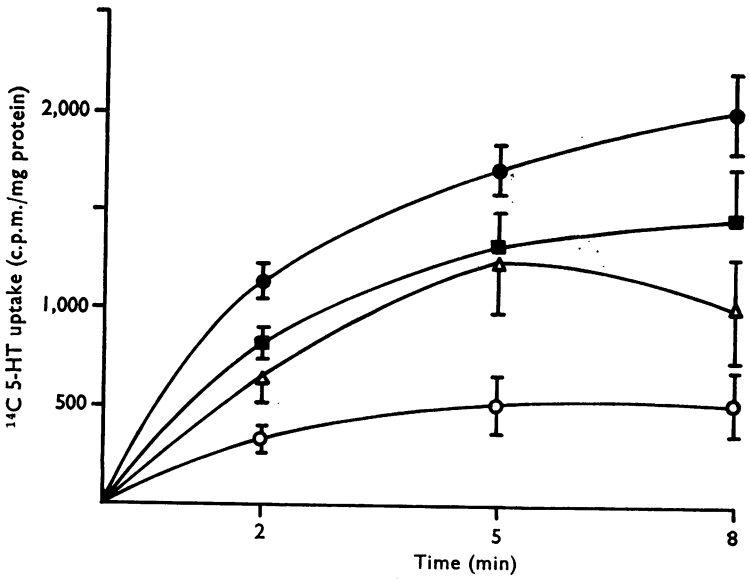

FIG. 2. Uptake of ^{14}C-5-HT in blood platelets poisoned with 20 mM NaF/0·75 mM DNP in an incubation medium containing 108 mM K^+ and 18 mM Na^+. After preincubation for 15 min the platelets were resuspended in increasing concentrations of Na^+. ○, 18 mM ; △, 36 mM ; ■, 72 mM ; ●, 121 mM.

From Fig. 2 it can be seen that samples exposed to different Na^+ concentrations accumulated ^{14}C-5-HT according to the amount of Na^+ in the resuspending medium; the greater the concentration of Na^+ the larger the amount of ^{14}C-5-HT accumulated. At all concentrations of Na^+ the influx of ^{14}C-5-HT was greatest during the first 2 min of exposure to Na^+, at a time when the gradient between external and internal medium will be greatest, and the maximum influx of Na^+ will be occurring. Within 5 min ^{14}C-5-HT accumulation appears to have reached a maximum value, which with the largest concentration of Na^+ was approximately 20% of the uptake occurring in normal respiring cells incubated under the same conditions.

Discussion

From a study of the kinetics of Na^+ dependent 5-HT transport in blood platelets (Sneddon, 1969) it was postulated that the transport process could be resolved into a number of events. 1. Carrier molecules on the outer phase of the blood platelet membrane form a complex with 5-HT in a reversible reaction which comes to equilibrium. The formation of this complex is controlled by the external Na^+ concentration. 2. A small fraction of these complexes traverses the membrane to the inner phase. 3. Some of the complexes then dissociate, liberating 5-HT in the platelet. This dissociation is assisted by the high internal K^+ concentration competitively displacing Na^+ from the carrier, so reducing the affinity of the carrier for 5-HT. 4. The carrier molecules traverse the membrane to the outer phase and enter the cycle again.

These characteristics are essentially similar to those postulated for the transmembrane movement of a wide variety of non-electrolytes (Christensen & Riggs, 1952; Vidaver, 1964; Crane, 1965; Kipnis & Parrish, 1965; Eddy *et al.*, 1967; Eddy & Hogg, 1969; Bogdanski *et al.*, 1970; Schultz & Curran, 1970).

Inhibitors of cellular metabolism abolish the active transport of 5-HT in blood platelets (Sano, Kakimoto & Taniquchi, 1958; Born & Gillson, 1959; Weissbach & Redfield, 1960), and it has often been assumed that these effects reflect a direct link between the transporting process and the energy producing metabolic reactions. This paper offers an alternative explanation of the relationship between cellular metabolism and the transport of 5-HT.

The observation that 5-HT uptake is Na^+ dependent and that there is an asymmetric distribution of Na^+ and K^+ between the platelet and the external medium suggested that the unequal distribution of monovalent cations could provide part of the energy required for 5-HT transport as was originally suggested for amino-acid transport by Riggs *et al.* (1958). According to this hypothesis (see **Introduction**), inhibition of active Na^+ extrusion should, in time, result in the disappearance of the Na^+ gradient across the cell membrane and the active transport of 5-HT should no longer be possible, and as the active extrusion of Na^+ is ultimately dependent upon the metabolism of ATP, the effect of inhibitors of ATP formation should resemble that of ouabain on the inhibition of 5-HT transport.

The method adopted to test this postulate was to preincubate blood platelets with different concentrations of ouabain, or with inhibitors of ATP productions, in an attempt to impair the efficiency of the Na^+ extrusion mechanism by varying degrees depending upon the concentration of the inhibitor used. As the platelets were preincubated in a medium containing 121 mM Na^+, a new extracellular Na^+, intra-

cellular Na^+ ratio would be established indicating a new transient steady state between Na^+ influx and efflux. The lower the efficiency of the Na^+ extrusion mechanism, the higher the internal Na^+ concentration, and the smaller the amount of 5-HT accumulated by the platelets. These predictions appear to be satisfied experimentally (Tables 1 & 2, Fig. 1); there is a significant correlation between the intracellular Na^+ level and the subsequent 5-HT uptake, indicating that as the intracellular Na^+ concentration is raised, effectively reducing the Na^+ gradient, 5-HT uptake is progressively reduced.

These experiments also offer an explanation of why relatively high doses of ouabain are required to inhibit 5-HT transport, as it is only at concentrations in the order of 10^{-3}M that significant intracellular accumulation of Na^+ occurs. As the concentration of ouabain used in the experiments is sufficient to inhibit the ouabain sensitive Na^+/K^+ activated ATPase (Mason & Saba, 1969; Moake, Ahmed, Bachur & Gutfreund, 1970), the results also indicate that factors other than the ouabain sensitive sodium pump are involved in Na^+ fluxes in blood platelets. After preincubation with ouabain the intracellular Na^+ concentration increases less than when the total ATP production is inhibited with a mixture of DNP/NaF (Tables 1 & 2), so that, although Na^+ fluxes are restricted by ouabain, the platelets are capable of maintaining an asymmetric distribution of Na^+. Similarly, the reduction in 5-HT uptake following ouabain is less than that following treatment with DNP/NaF, but is of the same order (approximately 60%) as that found following omission of K^+ from the incubation medium (Sneddon, 1969).

The experiments described above only provide circumstantial evidence as to the possible role of Na^+ gradients in 5-HT transport. If 5-HT is accumulated by such a mechanism during respiration, one would expect 5-HT to be accumulated in the absence of respiration, provided the appropriate ion gradients could be maintained between the platelet and the suspending medium. This has been achieved by poisoning platelets in a medium in which the K^+ concentration is large relative to Na^+ so that the poisoned platelets maintain a high internal K^+ concentration.

The results of such experiments (Table 3 and Fig. 2) show that blood platelets poisoned with a mixture of dinitrophenol and sodium fluoride accumulate 5-HT in amounts that are related to the relative magnitudes of the internal and external Na^+ concentrations, accumulating 5-HT when the external Na^+ is greater than the internal Na^+. It is unlikely that energy derived from ATP is involved in this accumulation of 5-HT, as the metabolic inhibitors were used in sufficient concentrations to inhibit all ATP production. Also, platelets poisoned in an incubation medium containing 121 mM Na^+, so that at the end of preincubation Na^+ inside equalled Na^+ outside, did not accumulate significant amounts of 5-HT when transferred to fresh incubation medium, indicating that a Na^+ concentration difference between the inside and the outside is a requirement for 5-HT uptake.

Although the possibility that the uptake observed represents 5-HT exchange between the platelet and the external medium has not been ruled out by the experimental results, it is considered unlikely that an exchange process could explain all the 5-HT accumulation that occurs in poisoned platelets, because: 1. It is difficult to see why this did not occur when internal Na^+=external Na^+ (Fig. 2). 2. If exchange is the explanation of 'uptake', it is difficult to see why this is so dependent upon the relative magnitudes of the external and internal Na^+ concentrations. In general the experimental results are in agreement with Christensen's hypothesis

(Riggs *et al.*, 1958) that the spontaneous movement of Na^+ or K^+, or both ions, serves to provide the energy to accumulate a non-electrolyte. The present experiments do not permit any conclusions as to the adequacy of the Na^+ gradient in the 5-HT transport process in respiring cells, although the maximum accumulation that can occur in poisoned cells under the influence of a Na^+ gradient is approximately 20% of that occurring in respiring platelets incubated under the same conditions. It is also difficult to dissociate the effects of an inwardly directed Na^+ gradient from an outwardly directed K^+, as both groups of inhibitors used in the present experiments had marked effects on the internal K^+ concentration, so that K^+ gradients would tend to be in the opposite direction to the Na^+ gradients and so contribute a driving force to the 5-HT transport mechanism. Quantitative analysis of Christensen's hypothesis for amino-acid transport (Vidaver, 1964; Crane, 1964; Eddy, 1968a, b), however, indicates that in other tissues the Na^+ gradient is a more important factor in driving accumulation than the K^+ gradient.

Many facets of monoamine transport mechanisms have yet to be analysed in detail, but it is perhaps pertinent to note that 5-HT transport in blood platelets exhibits similar characteristics to 5-HT transport in synaptosomes, in that it is a Na^+ dependent process and part of the energy for uptake may be derived from an inwardly directed Na^+ gradient (Tissari, Schönhöfer, Bogdanski & Brodie, 1969) but differs from noradrenaline transport in synaptosomes which, although Na^+ dependent (Colburn, Goodwin, Murphy, Bunney & Davis, 1968), does not appear to be linked to Na^+ gradients (White & Keen, 1970, 1971).

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