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THE EFFECTS OF ALTERATIONS OF TRANSMEMBRANE Na^+ AND K^+ GRADIENTS BY IONOPHORES (NIGERICIN, MONENSIN) ON SEROTONIN TRANSPORT IN HUMAN BLOOD PLATELETS

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Summary

Ionophores (monensin, nigericin) capable of transporting both Na^+ and K^+ across cell membranes down their concentration gradients reduce the rate and total magnitude of serotonin uptake by platelets. The effect of the ionophores was time dependent, so that inhibition increased progressively until eventually uptake ceased entirely. Nigericin and monensin produced loss of platelet K^+ and an equivalent molar uptake of Na^+ thereby abolishing the normal transmembrane Na^+ and K^+ gradients. The time course of these ionophore-induced cation shifts at 37° C corresponded to the rate at which inhibition of serotonin transport developed. The ionophores did not affect total ATP concentration of platelets nor the metabolic pool of ATP formed from [^{14}C]adenine. Nigericin and monensin released about 80% of platelet ^{14}C and endogenous serotonin over a 30 min period, without release of platelet adenine nucleotides, calcium or β-glucuronidase. The ionophores did not elicit platelet aggregation nor did they prevent maximal aggregation brought about by ADP, collagen or A23187. Replacement of Na^+ in the medium by K^+ abolished serotonin uptake but only 10—20% of endogenous serotonin was released. In KCl medium the Na^+ gradient was initially reversed, but quickly dissipated as Na^+ reequilibrated with the extracellular fluid. At 37° C the ionophores did not affect either the rate of Na^+ reequilibration or the efflux of [^{14}C]serotonin. Na^+ reequilibration was slower at 20° C and the ionophores significantly increased platelet Na^+ loss and strongly inhibited the efflux of [^{14}C]serotonin. The data support a mechanism of serotonin transport due to a Na^+-dependent carrier-mediated process which need not be directly dependent on metabolic energy, but which does require metabolic energy to maintain normal $Na^+ + K^+$ gradients.

Introduction

The uptake of serotonin by platelets is presumed to be mediated by a process requiring metabolic energy since it occurs against what appear to be

very large concentration gradients [1], exhibits a high temperature coefficient [2], requires glucose in the medium [3] and is inhibited by metabolic poisons [3–5]. Furthermore, the initial rate of uptake at 37° C as a function of extracellular serotonin concentration fits the Michaelis-Menten equation [1,4,6], suggesting that it may be carrier mediated. Serotonin uptake by platelets has been shown to be dependent upon extracellular Na^+ [7,9] and chloride [7,8]. The affinity of a postulated carrier for serotonin is presumably influenced by the relative amounts of Na^+ and K^+ at the transporting surface. The asymmetry of ion distribution across the cell membrane would drive the substrate-carrier (C) complex (serotonin-C-Na^+Cl^-) towards the region of low Na^+ inside the cell. Na^+ appears to control the affinity of the carrier for serotonin [7,9] whereas Cl^- primarily affects the rate of translocation of the carrier-serotonin complex [8]. Serotonin uptake was shown to vary inversely with the intracellular concentration of sodium ($[Na^+]_i$) [10]. Though these experiments substantiated the concept that amine uptake was linked to the transmembrane Na^+ gradient they were carried out with platelets treated with metabolic inhibitors (dinitrophenol, fluoride) or ouabain in order to manipulate the internal Na^+ content. Under these conditions the maximum accumulation of serotonin in poisoned cells, under the influence of the Na^+ gradient, was only 20% of that occurring in normal platelets. One is left to conclude that most of the serotonin uptake is directly dependent upon metabolic energy, even with the existence of a favorable Na^+ gradient. Another theory [11] proposed that platelet serotonin is freely diffusable down its concentration gradient, and almost completely exchangeable with extracellular serotonin. This "pump-leak" theory attributed the maintenance of high concentrations of the amine within the platelet to an active serotonin pump directly dependent upon metabolic energy.

In the experiments reported in this paper we studied the transport of serotonin in platelets which had been treated with certain ionophores (i.e. nigericin and monensin) that are able to transport Na^+ and K^+ across cellular membranes. In this way the intracellular Na^+ and K^+ concentrations were selectively modified without significantly affecting the extracellular cation or anion environment. It was therefore possible to assess the influence of the transmembrane ionic gradients upon serotonin uptake without significant interference with energy-generating processes. The results are consistent with the predominant role of transmembrane cation gradients in the regulation of platelet serotonin transport.

Methods and Materials

Platelet preparations. Platelet-rich plasma was prepared from freshly drawn human blood anticoagulated with the acid/citrate/dextrose (1 : 6, v/v) of Aster and Jandl [13]. Centrifugation for 15 min at room temperature at 120 × *g* yielded platelet-rich plasma. Human platelet concentrates were generously supplied by the Connecticut Red Cross Blood Center. Platelet concentrates in plasma were employed after dilution with a physiological salt solution, designated as NaCl-suspension medium, containing 137 mM NaCl, 5.4 mM KCl, 0.2% (w/v) dextrose, 4 mg/ml of bovine serum albumin (Cohn fraction V, Sigma Chemical Co., St. Louis, Mo.), 25 mM Tris · HCl, pH 7.4 or 6.5. Washed

platelets were prepared from platelet concentrates [12], and were suspended in NaCl-suspension medium. In some experiments the platelet concentrates were either washed with, or diluted into, a solution designated KCl-suspension medium in which KCl replaced all the NaCl. Protein was determined in 0.1% deoxycholate-lysed cells by a biuret method [14]. Platelet counts with a Technicon Platelet Counting System (Technicon Instruments Corp., Tarrytown, N.Y.) yielded a value of 10^9 platelets per 1.8 ± 0.2 (mean ± S.E.) mg of protein.

Serotonin uptake and release. Serotonin uptake by platelets was studied as previously described [12] utilizing [^{14}C]serotonin creatinine sulfate (Amersham/Searle Corp., Arlington Heights, Ill.) with a specific activity of 57 Ci/mol. Uptake was measured at both pH 6.5 and 7.4 since Weissbach and Redfield [5] noted that at the lower pH the uptake saturated at about 20—30 μM whereas at high pH a significant amount of uptake appeared to be due to simple diffusion, at concentrations of serotonin above 0.28 mM. In our experiments the serotonin concentration was usually kept below 20 μM and no important differences in the effect of ionophores on serotonin uptake were noted at the two pH values.

[^{14}C]Serotonin (5—50 μl, 10 μCi/ml) was added to stirred platelet suspensions at 37° C to give a total serotonin concentration of 0.35—3.5 μM. Additional non-radioactive serotonin was added when required to increase the total concentration of the amine. Ionophores dissolved in ethanol were added in 5—20 μl aliquots of varying concentrations of stock solutions. Equivalent amounts of ethanol added alone to the platelet suspensions had no significant effect.

The release of total serotonin and [^{14}C]serotonin was studied in platelet concentrates which had been initially washed in NaCl-suspension medium and concentrated to about 30 mg protein/ml. After incubation with [^{14}C]serotonin for 30 min at 37 or 21° C, 0.2-ml aliquots were added to 4.8 ml of NaCl- or KCl-suspension medium (± ionophore) at 37 or 21° C and stirred continuously. Aliquots of the incubation mixture were removed periodically and after centrifugation (8000 × *g* for 30 s) the supernatant fluid was removed and the radioactivity measured in a liquid scintillation counter. The total serotonin content in platelet pellets was assayed by the fluorimetric method of Udenfriend et al. [15].

Platelet Na^+, K^+ and Ca^{2+} measurements. Platelets were separated from the extracellular fluid by centrifugation at 8000 × *g* through 0.25 M sucrose solution. The pellets were resuspended in 1.0 ml 0.35 M $HClO_4$, sonicated for 30 s and protein was removed by centrifugation to obtain extracts for analysis. Na^+ and K^+ were measured by flame photometry. Calcium was determined by atomic absorbance spectrophotometry [12]. Values for intracellular Na^+ and K^+ were corrected for the volume of incubation medium trapped in the platelet pellets as determined with [^{14}C]inulin. Intracellular cation concentrations were calculated using the data of Born and Bricknell [2] for cell volume and water content.

The effect of the transmembrane Na^+ gradient on [^{14}C]serotonin release from platelets in KCl-medium was investigated at 37 and 21° C by the following procedure. Washed platelets which had accumulated [^{14}C]serotonin in NaCl-suspension medium were pelleted by centrifugation at 1500 × *g* for 10 min and

then quickly resuspended in KCl-suspension medium. Aliquots of these suspensions were centrifuged (2 min, 8000 × *g*) at intervals over a 20 min period and the supernatants and pellets were analyzed for released [^{14}C] serotonin and Na^+. These experiments were performed with and without the presence of 27 μM nigericin or monensin.

^{42}K efflux. Washed platelets were incubated for 60 min at 37° C with NaCl-suspension medium containing ^{42}KCl and then washed twice. Nigericin (27 μM) was added to one aliquot of cells and samples of control and nigericin-treated cells taken at various time intervals thereafter were layered onto 0.25 M sucrose solution and centrifuged (8000 × *g*/2 min) in plastic tubes. The pellets were dissolved in 1 ml 1% Triton X-100 and the radioactivity of the extracts was measured in a gamma spectrometer. The efflux of ^{42}K was calculated as described previously by Sjodin and Henderson [16].

Adenine nucleotide determinations. Total adenine nucleotide content of platelet pellets and the supernatant medium was determined spectrophotometrically [12,17]. ATP was determined by the firefly luminescence method in ethanol/EDTA extracts of platelets [18]. The effect of ionophores on adenine nucleotide metabolism was also assessed by labelling the metabolic pool of nucleotides with [^{14}C] adenine [19]. [8-^{14}C] Adenine, 0.2 μCi/ml, at a total concentration of 0.67 μM was incubated with washed platelets for 2 h at 37° C [19]. Nigericin (27 μM) was added to one-half of the platelet suspension. Platelets separated by centrifugation from the incubation medium at 5 and 10 min were extracted with EDTA/ethanol [18]. The nucleotides in the extract plus carrier ATP, ADP and AMP were separated on cellulose MN-300 (Analabs) thin-layer chromatography plates [20]. Nucleotide spots located with an ultraviolet lamp were scraped from the plates and extracted for scintillation counting.

Enzyme assays. β-Glucuronidase was assayed in the platelet-suspension medium and in platelet lysates by the method of Fishman [21] with phenolphthalein glucuronide as the substrate. Lactate dehydrogenase was similarly measured by the method of Bergmeyer et al. [22].

Platelet aggregation. Platelet aggregation was measured turbidimetrically as previously described by Miller et al. [12].

Chemicals. [^{14}C] Serotonin (5-hydroxy[2-^{14}C] tryptamine creatinine sulphate) and [8-^{14}C] adenine were obtained from Amersham/Searle Corp. Ionophores monensin, nigericin and A23187 were generously supplied by Eli Lilly and Co., and dicyclohexyl crown 18 was a gift of E.I. Dupont de Nemours and Co., Inc. Collagen was prepared from bovine Achilles tendon (Sigma) by extraction with acetic acid as described by Miller [23]. Adenine nucleotides, serotonin (5-hydroxytryptamine creatinine sulphate), valinomycin and thrombin were obtained from Sigma Chemical Co.

Results

In our experiments uptake of [^{14}C] serotonin by platelets exhibited the characteristics previously described namely, saturable kinetics, an apparent K_m of 0.18 μM, and a dependence upon the presence of extracellular Na^+. [^{14}C]-Serotonin uptake was abolished in washed platelets suspended in a medium in

which all Na^+ was replaced by K^+, but subsequent washing and resuspension of the same platelets in normal NaCl-suspension medium restored serotonin uptake to normal.

Effects of ionophores on serotonin uptake

Preincubation of platelet-rich plasma, washed platelets, or diluted platelet concentrates with nigericin or monensin at 37° C resulted in a rapid inhibition of the rate of serotonin uptake, as well as a reduction in total amount of amine accumulated (Fig. 1). Neither valinomycin nor the synthetic ionophore dicyclohexyl crown 18 affected serotonin transport under the same conditions. In normal platelets repeated uptake of low concentrations of serotonin could be demonstrated by periodic sequential additions of [^{14}C]serotonin to the incubation medium. After addition of monensin or nigericin no additional uptake of the amine could be demonstrated after the initial control uptake (Fig. 2). Serotonin uptake was also inhibited by ionophores at 20° C. The presence of plasma proteins or bovine serum albumin increased the concentration of the ionophores needed to inhibit serotonin uptake (Fig. 1), due to the binding of the ionophores to albumin. The magnitude of the inhibition of serotonin uptake was also a function of the time of exposure to the ionophores. When [^{14}C]serotonin was added soon after the ionophores (i.e. within 1—3 min) amine uptake took place at a much reduced rate for several minutes, but subsequently ceased entirely. Upon longer periods of preincubation with ionophores (i.e., 5—10 min) the initial rate and total magnitude of serotonin uptake was progressively reduced (Fig. 3). When uptake was completely arrested the inhibition was not reversed by increasing extracellular serotonin 10-fold. Thus, the ionophores not only decreased the initial rate of serotonin transport, but in addition produced some progressive effect which led inexorably, to a complete loss of the capacity of platelets to accumulate the amine. For this reason it is not possible to express the effect of any particular

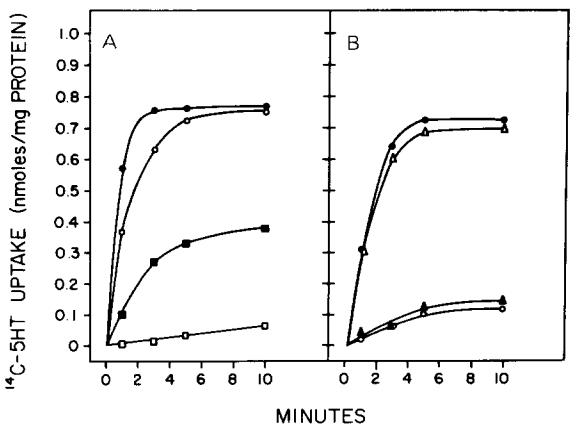

Fig. 1. Effect of nigericin, monensin and valinomycin on [^{14}C]serotonin uptake by platelets. (A) Platelets (2 mg protein/ml) in plasma incubated with 1.6 μM [^{14}C]serotonin at 37° C (●). Platelets preincubated (prior to addition of [^{14}C]serotonin) for 3 min with nigericin: 27 μM (□), 2.7 μM (■) and 0.27 μM (○). (B) Washed platelets in NaCl medium. No added serum albumin. Nigericin (0.27 μM, ○), monensin (0.27 μm, ▲) and valinomycin (0.27 μM, △) added 3 min prior to 1.6 μ M [^{14}C]serotonin. ●, control platelets.

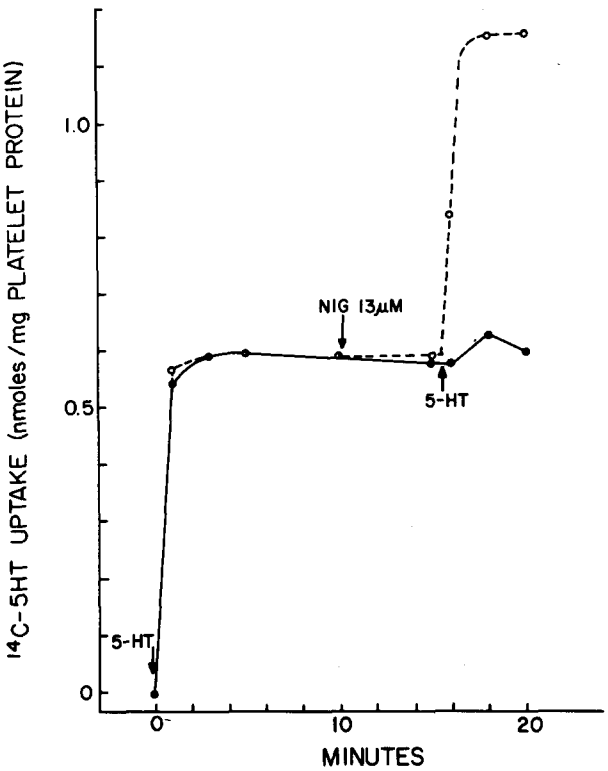

Fig. 2. Effect of nigericin on [^{14}C] serotonin uptake. [^{14}C] Serotonin (0.1 μCi/ml) was added to 5.0-ml aliquots of diluted platelet concentrates (2.0 mg protein/ml) at 37° C. At the times indicated the radioactivity in the supernatant was measured. At 10 min nigericin (13 μM) was added to only one platelet group (•). A second aliquot of [^{14}C] serotonin was added to both groups at 15 min.

concentration of ionophore in terms of its effect on the K_m or V for serotonin transport.

Effects of ionophores on platelet Na^+ and K^+

As shown in Fig. 4 nigericin and monensin drastically altered the platelet cation composition. The ionophores rapidly produced a nearly total loss of platelet K^+ and an equivalent molar uptake of Na^+ until the intracellular cation concentrations were essentially at an equilibrium with those in the very much larger extracellular space. The time course of these cation shifts corresponded to the period over which serotonin uptake came to a halt. Addition of 27 μM nigericin to washed platelets in NaCl-suspension medium produced an exponential fall in the cellular K^+ concentration ($[K_i^+]$) with a half-time of 1 min, from an initial level of 87.5 mM to 6 mM after 5 min (Fig. 4). At the same time intracellular Na^+ rose from an average value of 41 to 119 mM. Monensin initially induced the same rate of loss of platelet K_i^+, but after 1 min K_i^+ loss slowed to an exponential rate with a half-time of 3 min or more. Since monensin exhibits a preference in affinity for Na^+ compared to K^+ [24] it is likely that the concurrent rise in $[Na_i^+]$ resulted in competition with $[K_i^+]$ and thus tended to inhibit the ionophore-mediated K_i^+ efflux. The undirectional

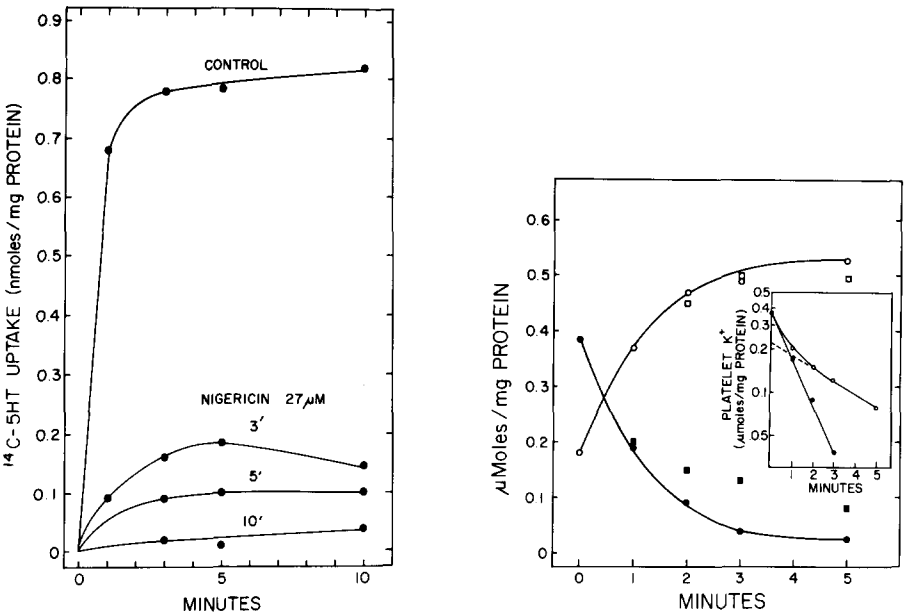

Fig. 3. Uptake of [^{14}C]serotonin by platelets in NaCl-suspension medium (+ bovine serum albumin) at 37° C. Platelets (3 mg protein/ml) were preincubated with 27 μM nigericin for 3, 5 or 10 min, as indicated, prior to addition of [^{14}C]serotonin (2.8 μM).

Fig. 4. Effect of nigericin and monensin on the sodium and potassium content of washed platelets at 37° C in NaCl-suspension medium. 27 μM nigericin (○,●) or monensin (□,■) were added and platelet pellets were analyzed for sodium (○,□) and potassium (●,■) at the times indicated. Each point is the mean of six separate experiments. The standard errors were less than 10% of the mean in all cases. Insert: Semi-logarithmic plot of potassium loss from platelets in the presence of nigericin (●) or monensin (○).

efflux of $^{42}K_i$ from platelets increased 11—18-fold in the presence of 27 μM monensin (Fig. 5).

The loss of K_i^+ from platelets resulted in an increase of $[K_0^+]$ from 5.4 to 6.6 mM, whereas the fall in $[Na_0^+]$ was only about 1 mM. Such small changes in extracellular K^+ and Na^+ have no inhibitory effect on serotonin transport [9]. As a result of these shifts in platelet cations the initial sodium gradient across the cell membrane, i.e. $[Na_0^+]/[Na_i^+]$ fell from 3.4 to 1.1 and the potassium gradient $[K_i^+]/[K_0^+]$ collapsed from an initial value of 16 to nearly 1.0. In the presence of 27 μM valinomycin the platelet K_i^+ fell less than 10% in 15 min. Valinomycin unlike nigericin and monensin has a negligible affinity for Na^+ as compared to K^+ [24] and its complex with K^+ bears a net positive charge. In order to preserve electroneutrality either a cation (i.e. Na^+, H^+) must exchange for K^+, or an anion must accompany the valinomycin · K^+ complex. The movement of these other ions can therefore be rate limiting with respect to valinomycin-induced K^+ transport. The mobility of valinomycin · K^+ complexes within the membrane may also be influenced by the membrane potential.

Adenine nucleotides in ionophore-treated platelets

Under the specified conditions monensin and nigericin did not appear to affect energy-conserving reactions in the platelet. Incubation of washed

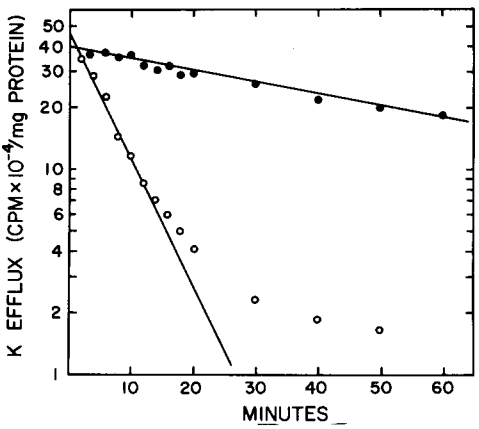

Fig. 5. Effect of monensin on ^{42}K efflux from platelets. Washed platelets were allowed to take up ^{42}K for 1 h in NaCl-suspension medium and ^{42}K efflux was measured as described under Methods and Materials. •, controls; ○, plus 27 μM monensin.

platelets with nigericin or monensin for 5—10 min, did not reduce the concentrations of total platelet adenine nucleotides, total platelet ATP, or the metabolic pool of ATP labelled by exogenous [^{14}C]adenine (Table I).

Serotonin release by ionophores

When ionophores were added to platelets after they had already completely taken up [^{14}C]serotonin under normal conditions substantial loss of radioactive amine was observed. The maximum rate of release was only about 1/100 that of the initial rate of [^{14}C]serotonin uptake. In addition, about 80% of the total

TABLE I

EFFECTS OF NIGERICIN ON PLATELET ADENINE NUCLEOTIDES AND THE RELEASE OF ADENINE NUCLEOTIDES, CALCIUM AND β-GLUCURONIDASE

See Methods and Materials for experimental details. All values are means ± S.E. for 4—10 experiments

	Controls		+ Nigericin [a]	
Total platelet adenine nucleotide content (nmol/mg protein)	48.1	(±0.8)	49.2	(±1.1)
Total platelet ATP (nmol/mg protein)	23.7	(±2.4)	24.0	(±1.5) [b]
Radioactivity of adenine nucleotide pool labelled with [8-^{14}C]adenine (cpm/mg protein)				
ATP	36 525	(±2325)	41 520	(±2486)
ADP	10 550	(±550)	10 713	(±549)
Adenine nucleotide released (nmol/mg protein)	0.49	(±0.05) [c]	±0.62	(±0.05)
Calcium released (nmol/mg protein)	—		0.90	(±0.26) [d]
β-Glucuronidase released (μmol/mg protein per 3 h)	0.045	(±0.005) [e]	0.045	(±0.002)

[a] Samples were taken for analysis after 10 min incubation of platelets with 27 μM nigericin.
[b] After incubation with monensin (27 μM) for 10 min ATP was 23.0 (±0.8).
[c] Thrombin (0.5 unit/ml) released 35.6 nmol/mg protein.
[d] The total cell calcium was 78 nmol/mg protein. Thrombin (0.5 unit/ml) and A23187 (5 μM) released 46.7 and 51.6 nmol/mg protein, respectively.
[e] The platelet lysate hydrolyzed 2.37 μmol phenolphthalein glucuronide/mg protein in 3 h.

cellular serotonin (non-radioactive) content was released by the ionophores over a 20—30 min period (Fig. 6). Ionophore-induced release of serotonin was not the result of the release reaction since its time course was much slower and it was not accompanied by release of platelet adenine nucleotides, β-glucuronidase or calcium (Table I). Furthermore, lactate dehydrogenase was not released into the medium so that it is reasonable to conclude that the ionophores did not produce significant damage to membrane integrity. Platelets were not aggregated by monensin or nigericin, but they were capable of aggregating maximally upon the subsequent addition of ADP, collagen or the calcium ionophore, A23187. Ionophore-induced release of serotonin from platelets was not due to the transport of ionophore-serotonin complexes, because unlike the ionophore X-537A, nigericin and monensin have little affinity for amines [24]. Moreover, we found that the meager serotonin transport by these ionophores in a two phase system (toluene/butanol/water) was completely abolished in the presence of physiological concentrations of KCl or NaCl.

Serotonin release in KCl medium

When platelets which had accumulated [^{14}C]serotonin in NaCl-medium were resuspended in KCl-medium at 37° C the accumulated radioactive amine was released to the extent of about 27% over a 20—30 min period, but the loss of endogenous total serotonin amounted to only 11—20% and was nearly over during the first 5 min; thereafter total platelet serotonin remained essentially constant in the KCl medium (Fig. 6). Nigericin had no effect on the loss of [^{14}C]serotonin from platelets in KCl-medium at 37° C, but markedly reduced it at 20° C (Fig. 7). In KCl-medium the intracellular platelet sodium fell very rapidly at 37° C and was not effected by nigericin. However, at 20° C the rate of Na^+ loss was slower, and it was significantly increased by ionophore (Fig. 7). Thus, at the lower temperature the initially reversed Na^+ gradient (i.e. $[Na_i^+] > [Na_o^+]$) was more quickly dissipated in the presence of the ionophore.

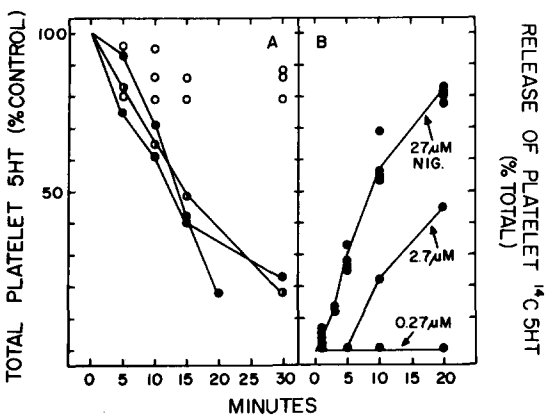

Fig. 6. (A) Release of endogenous platelet serotonin by ionophores or high K^+. Washed platelets (1 mg protein/ml) at 37° C incubated in KCl medium (○); NaCl medium plus nigericin (◑); or NaCl medium plus monensin (●) at 27 μM. (B) Release of [^{14}C]serotonin from platelets by nigericin at concentrations of 0.27, 2.7 and 27 μM. Platelets were originally exposed (prior to nigericin) to 2.8 μM [^{14}C]serotonin for 15 min at 37° C during which time 96% of the amine was taken up.

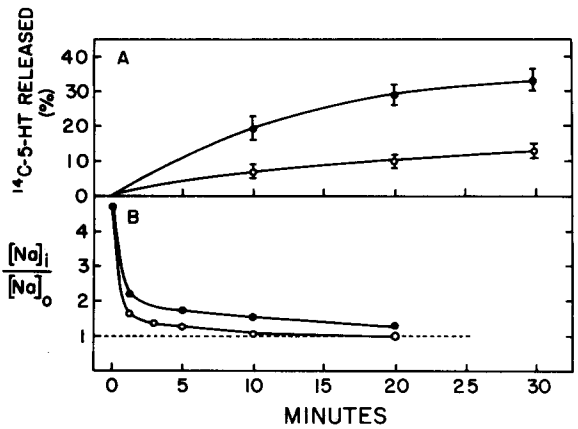

Fig. 7. Release of [^{14}C]serotonin from washed platelets in KCl medium at 20° C. Concentrated washed platelets (30 mg protein/ml) in NaCl medium were incubated with [^{14}C]serotonin (1.8 μM) for 30 min at 20° C. Aliquots (0.2 ml) were then added to 4.8 ml of KCl medium in the absence (•) or presence of 27 μM monensin (○). At the times indicated ^{14}C radioactivity and sodium in the supernatant, and the sodium in the pellet, were measured. Each point in A is the mean of four experiments (± S.E.). [^{14}C]-Serotonin release is expressed as a percentage of the total ^{14}C originally present in the cells. (B) A plot of the ratio of the intracellular Na^+ to the extracellular Na^+ concentration.

Discussion

Serotonin transport has previously been shown to be inhibited by metabolic poisons, glucose deprivation, lack of K^+ in the extracellular fluid and antagonism of the $Na^+ + K^+$ pump. In each case marked loss of platelet K^+ occurs, which in order to maintain electroneutrality is compensated for by uptake of Na^+, thereby resulting in a collapse of the normal transmembrane ionic gradients [10]. Ionophores (i.e. monensin, nigericin) capable of transporting both Na^+ and K^+ across cell membranes also completely dissipated the platelet transmembrane Na^+ and K^+ gradients. The time course of ionophore-induced inhibition of serotonin uptake coincided with the ionophore-induced changes in cellular K^+ and Na^+ content. The effects of the ionophores on serotonin uptake are particularly noteworthy because they occurred despite the existence of a normal extracellular ionic environment and the lack of interference with energy-producing metabolic processes since the total cellular ATP and the metabolic ATP pool, labelled by exogenous [^{14}C]adenine, were not diminished. The sufficiency of energy stores is further indicated by the ability of platelets to undergo the more demanding energy-consuming processes [19] of aggregation and the release reaction after exposure to nigericin or monensin. This evidence all leads to the conclusion that the presence of sufficient transmembrane gradients for Na^+ and K^+ are a precondition for serotonin transport. On the other hand, these experiments do not rule out involvement of ATP in serotonin transport, possibly through an energy transduction step directly linked to carrier translocation involving phosphorylated protein intermediates such as are involved in other transport processes [25,26].

The model of solute transport linked to ion gradients also predicts symmetry in the system [26]; i.e. if a Na^+ gradient is imposed in the opposite sense, then enhanced extrusion of substrate should be observed. It is interesting therefore

to note that in high KCl medium there was a transient efflux of serotonin from platelets which was greatest initially, at the moment of highest $[Na_i^+]/[Na_o^+]$, but rapidly slowed as Na^+ reequilibrated. Moreover, this serotonin efflux was markedly reduced when the addition of $Na^+ + K^+$ ionophores significantly hastened the dissipation of the reversed Na^+ gradient. This result, although consistent with the ion gradient model, by no means establishes its verity.

If the major proportion of intracellular serotonin were freely diffusible in the cytoplasm [11] then the removal of serotonin from the surrounding medium, or prevention of serotonin uptake, should lead to a release of the diffusible serotonin pool into the large extracellular space. In fact, in serotonin-free media or in the presence of inhibitors of uptake, platelets maintain their serotonin stores intact for rather long periods in vitro [27]. In high KCl media we observed that only a small fraction of platelet serotonin was released, despite the complete arrest of serotonin uptake. This strongly argues for a compartmentalization of the major fraction of serotonin within the platelet in a non-diffusible state. The magnitude of the serotonin fraction released in KCl may correspond to the free cytoplasmic pool calculated by Reimers et al. [28] utilizing a method of compartmental analysis of serotonin fluxes. Nigericin and monensin caused a much greater release of platelet serotonin than that produced by replacement of extracellular Na^+ with K^+. The most obvious difference between these two situations was that the cytoplasmic Na^+ and K^+ contents were strikingly different. Platelets in NaCl medium plus ionophore attained a high concentration of Na^+ and very low K^+, whereas in KCl medium platelets had a high K^+ content and very little Na^+. Possibly the stability of serotonin storage within secretory granules can be adversely affected by a high cytoplasmic Na^+/K^+ ratio, thus accounting for the greater loss of amine under such conditions.

The importance of the dense granules to the serotonin uptake process is substantiated by the observations that in storage pool disease [29] and Chediak-Higashi syndrome [30] the markedly reduced ability of platelets to accumulate serotonin was associated with a striking reduction in the storage pool of adenine nucleotides, and in the latter case the abundance of dense granules as well [31]. Similarly, platelets degranulated by thrombin exhibited apparently normal initial uptake rates for serotonin but much diminished total accumulation because of the loss of amine storage capacity [28].

From the existing data a model for serotonin transport in platelets may be deduced with the following characteristics: (1) serotonin uptake occurs by facilitated diffusion through the membrane via a mobile Na^+-dependent carrier, along the direction of the Na^+ gradient, and probably with an accompanying anion (Cl^-); (2) serotonin dissociation from the carrier is increased when Na^+ is displaced by K^+ at the cytoplasmic surface of the membrane and the K^+-carrier complex then diffuses to the outer surface of the membrane in the direction of the K^+ gradient; (3) metabolic energy must be expended in order to maintain the normal transmembrane Na^+/K^+ gradients; (4) cytoplasmic serotonin is normally maintained quite low, favoring serotonin influx, as a result of sequestration of amine largely within secretory granules. The latter process is not well understood but may involve ATP in the formation of insoluble complexes with serotonin within the granules [4,27,32].

References

1 Hughes, F.B. and Brodie, B.B. (1959) J. Pharmacol. Exp. Ther. 127, 96
2 Born, G.V.R. and Bricknell, J. (1959) J. Physiol. Lond. 147, 153
3 Sano, I., Kakimoto, J. and Taniguchi, K. (1958) Am. J. Physiol. 195, 495
4 Born, G.V.R. and Gillson, R.E. (1959) J. Physiol. Lond. 146, 472
5 Weissbach, H. and Redfield, B.G. (1960) J. Biol. Chem. 235, 3287
6 Humphrey, J.H. and Toh, C.C. (1954) J. Physiol. Lond. 124, 300
7 Lingjaerde, Jr., O. (1969) FEBS Lett. 3, 103
8 Lingjaerde, Jr., O. (1971) Acta Physiol. Scand. 81, 75
9 Sneddon, J.M. (1969) Br. J. Pharmacol. 37, 680
10 Sneddon, J.M. (1971) Br. J. Pharmacol. 43, 834
11 Okuda, M. and Nemerson, Y. (1971) Am. J. Physiol. 220, 283
12 Miller, J.L., Katz, A.J. and Feinstein, M.B. (1975) Thromb. Diath. Haemorrh. 33, 286
13 Aster, R.H. and Jandl, J.H. (1964) J. Clin. Invest. 43, 843
14 Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751
15 Udenfriend, S., Weissbach, H. and Clark, C.T. (1955) J. Biol. Chem. 215, 337
16 Sjodin, R.A. and Henderson, E.G. (1964) J. Gen. Physiol. 47, 605
17 Murer, E.H. (1968) Biochim. Biophys. Acta 162, 320
18 Holmsen, H., Storm, E. and Day, H.J. (1972) Anal. Biochem. 46, 489
19 Holmsen, H., Day, H.J. and Setkowsky, C.A. (1972) Biochem. J. 129, 67
20 Randerath, J. (1966) Thin Layer Chromatography, Academic Press, New York
21 Fishman, A. (1965) in Methods of Enzymatic Analysis (Bergemeyer, H., ed.), p. 869, Academic Press, New York
22 Bergmeyer, H., Bernt, E. and Hess, B. (1965) in Methods of Enzymatic Analysis (Bergmeyer, H., ed.), p. 736, Academic Press, New York
23 Miller, J.L. (1974) Ph.D. Thesis, University of Connecticut
24 Pressman, B.C. (1976) Annu. Rev. Biochem. 501
25 Fahn, S., Koval, G.J. and Albers, R.W. (1966) J. Biol. Chem. 241, 1882
26 Kimmich, G., Tucker, A.M., Barrett, E. and Randles, J. (1972) in The Role of Membranes in Metabolic Regulation (Mehlman, M.A. and Hanson, R.W., eds.), p. 287, Academic Press, New York
27 Pletscher, A., DaPrada, M., Berneis, K.H. and Tranzer, J.P. (1971) Experientia 27, 993
28 Reimers, H.J., Allen, D.J., Feuerstein, I.A. and Mustard, J.F. (1975) J. Cell Biol. 65, 359
29 Holmsen, H. and Weiss, H.I. (1970) Br. J. Haematol. 19, 643
30 Buchanan, G.R. and Handin, R.I. (1976) Blood 47, 944
31 Costa, J.L., Fanci, A.S. and Wolff, S.M. (1976) Blood 48, 517
32 DaPrada, M., Tranzer, J.P. and Pletscher, A. (1972) Experientia 28, 1328