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PREFERENTIAL ACCUMULATION OF LITHIUM IN THE DENSE BODIES OF HUMAN PLATELETS

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Abstract—From 50 to 73% of the lithium contained in platelets of patients receiving oral therapy with lithium carbonate was released by brief thrombin treatment. Similarly, about 50% of the lithium in platelets of normal volunteers incubated with lithium chloride was thrombin-releasable. The data indicate that an amount of lithium approximately equal to 10% of the calcium content was sequestered in the dense bodies (amine storage organelles) of human platelets. Electron microprobe analysis of dense bodies suggests that the addition of lithium did not change the phosphorus content but produced a loss of about 10% of the dense-body calcium. Nevertheless, synthetic solid analogues of the dense-body core incubated with lithium chloride did not sequester lithium preferentially over potassium and failed to exchange calcium for lithium. Thus, the mechanism responsible for the observed changes in platelet dense bodies may be related to selective membrane permeability properties rather than to binding of lithium to nucleotides or pyrophosphate in the dense-body core.

Because of its utility in the treatment of manic-depressive illness, lithium has been studied in a variety of cellular and subcellular systems, and a number of hypotheses have been proposed to account for its therapeutic efficacy [1, 2]. For example, lithium may bind intracellularly at sites normally occupied by calcium [3] or by magnesium [4, 5]. Lithium might also be expected to accumulate in nucleotide-rich environments (such as those inside the amine-storing vesicles of adrenergic nerves, chromaffin cells, and platelets), since it displays a relatively high affinity for both ATP and ADP [4, 5]. To investigate this possibility, we have examined the lithium content and thrombin-induced release of lithium in intact human platelets. Regardless of whether platelets are obtained from patients receiving long-term lithium carbonate therapy or are loaded with lithium by incubation with exogenous lithium, the bulk of the intra-platelet lithium appears to be contained in the nucleotide-rich dense bodies.

MATERIALS AND METHODS

Collection of platelets from patients receiving lithium carbonate therapy. Whole blood (100 ml) was collected using ACD-A anticoagulant, either from normal donors or from psychiatric patients receiving oral lithium carbonate (average daily dose, 1 g) continuously for at least 6 weeks prior to donation. Platelet-rich plasma (PRP) was prepared by serial differential centrifugation [6] and was diluted 1:1 with a buffer at pH 7.4 containing 116 mM NaCl, 4 mM KCl, 1.1 mM $MgSO_4$, 1.8 mM KH_2PO_4, 25 mM Tris, 10 mM citrate, and 2 mM EDTA [7]. The diluted PRP was centrifuged at 1000 *g* for 20 min, an aliquot of the supernatant fraction was saved for lithium analysis, and the platelets were resuspended in 50 ml of the same buffer containing no EDTA, 5.9 mM glucose, and 0.35% bovine serum albumin (crystallized and lyophilized; obtained from the Sigma Chemical Co., St. Louis, MO). The resuspended platelets were divided into two equal aliquots. One aliquot was warmed to 37° and fixed with formaldehyde (final concentration 1.5%) [8]; the other aliquot was warmed to 37°, incubated with 4 units/ml of human thrombin for 1 min, and then fixed with formaldehyde. Platelets in both aliquots were pelleted, resuspended in 1 ml of distilled water, and sonicated for 1 min at maximum power (Branson model W-140 sonifier with a ¼-inch tip). The sonicates and aliquots of the platelet-poor plasma were frozen and stored at −80° until they could be assayed. Platelet counts were performed with an Electrozone Celloscope with a 19 μm orifice and a logarithmic amplifier (Particle Data Corp., Elmhurst, IL).

Platelets from six patients receiving chronic lithium therapy were labeled in PRP by incubation with 10^{-8} M [^{3}H]-5-hydroxytryptamine (28.2 Ci/mmole, New England Nuclear Corp., Boston, MA) for 30 min at 37°. Platelets were then pelleted, resuspended, and treated with thrombin as described above.

Incubation of platelets with exogenous lithium. Blood was collected as described above from normal volunteers with no history of hematologic disorders or drug ingestion for 10 days prior to donation. PRP was incubated for 90 min at 37° with 10 mM LiCl, following which the platelets were resuspended in buffer and processed as described above.

Measurement of platelet lithium. Lithium was measured using an atomic absorption spectrophotometer (Perkin–Elmer model 603, Perkin–Elmer Corp., Norwalk, CT) operated in the emission-chopped mode at a wavelength of 670.8 nm. Values

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given for each point are the means of two measurements.

Electron microprobe analysis of platelet dense bodies. Air-dried platelet whole mounts were prepared as described elsewhere [9] from the PRP of eleven normal donors and six patients receiving lithium maintenance therapy. Dense-body calcium and phosphorus contents were evaluated as described previously [10–13] utilizing an Hitachi H-500 electron microscope operated in the scanning-transmission mode with an acceleration voltage of 75 kV, a beam diameter of approximately 100 nm, and a final magnification of 20,000 ×. X-Ray spectra were recorded for 100 sec (live-time counting) with a Kevex Mark IV energy-dispersive detector (30 mm^2 area) and a model 5100 data acquisition and analysis system. Total gross counts in windows set for phosphorus (1.9 to 2.10 keV) and for calcium (3.60 to 3.80 keV) were recorded and corrected for the background contribution by a programmable background subtraction routine.

Preparation and examination of amorphous solids. Precipitates were prepared as described elsewhere [14] by rapid mixing of a solution containing 201 mM $Ca(NO_3)_2$ with a solution containing 50 mM sodium ATP, 54 mM sodium ADP, and 20 mM sodium pyrophosphate. Precipitate was formed immediately (45 mg of solid per ml of slurry); the final solution pH was 7.4, and the supernatant fraction contained 51 mM Ca^{2+}, 15 mM ATP, 13 mM ADP, and 0.1 mM pyrophosphate. In some cases, 4 ml of the reaction slurry was mixed with either 10 or 50 mM LiCl or KCl. Solids were separated from the supernatant fraction by filtration [14]; calcium, sodium, and potassium were analyzed by atomic absorption spectroscopy, inorganic phosphate and pyrophosphate by the method of Putnins and Yamada [15], and ATP, ADP, and AMP by enzymatic methods [16, 17].

RESULTS

Platelets from six patients receiving long-term therapy with lithium carbonate contained appreciable amounts of lithium, with a mean value of 2.9×10^{-18} moles/platelet (Table 1). From 50 to 73% of the total platelet lithium was released by brief thrombin treatment.

As reported previously [18], incubation of platelets from normal donors with 10 mM exogenous lithium permitted the cells to accumulate about ten times more lithium than the amount seen in platelets from patients (average 3.9×10^{-17} moles/platelet; Table 2). Approximately 50% of this lithium was released following brief thrombin treatment. Several previous studies have shown that brief treatment with thrombin (1 min at 37°) releases from 65 to 95% of the total content of dense bodies in a normal platelet population [7, 19]. The release can be monitored by counting the dense bodies present in air-dried whole mounts [9] or by measuring the percentage release of radiolabeled 5-hydroxytryptamine (5-HT) [7]. When platelets from six patients receiving chronic lithium therapy were labeled with 10^{-8} M [^{3}H]-5-HT, from 70 to 90% of the total [^{3}H]-5-HT present was released by thrombin treatment.

Table 2. Lithium concentrations in normal platelets following incubation with 10 mM LiCl for 90 min at 37°

Experiment number	Platelet lithium concentration (moles/platelet)	Percent release of platelet lithium with thrombin
1	2.87×10^{-17}	48.3
2	4.88×10^{-17}	49.2

Dense bodies in platelets from eleven donors with no history of hematologic disorders averaged 2988 net X-ray counts for phosphorus (range 1764 to 4331) and 1807 net X-ray counts for calcium (range 930 to 3305), with an average phosphorus/calcium ratio of 1.78 (range 1.31 to 2.18) (Table 3). These ranges include values similar to those reported previously for other normal donors [10–13]. When probed under essentially identical microscope operating conditions, dense bodies in platelets from six patients receiving lithium carbonate therapy averaged 3124 for phosphorus, 1520 for calcium, and 2.23 for the phosphorus/calcium ratio. Although the combined mean for calcium in the lithium group was approximately 16% lower than the combined mean for the control group, the difference was not statistically significant ($t = 1.00$, with 15 degrees of freedom). The phosphorus/calcium ratio for the lithium group was 25% higher than that for the control group, and this difference was statistically significant at the 0.05 level ($t = 2.20$ with 15 degrees of freedom).

Synthetic solids prepared in the presence of 10 or 50 mM lithium chloride contained 0.10 μmole of lithium per mg of dried solid (Table 4). Nevertheless, the calcium content, as well as the phosphate-residue

Table 1. Measurements of lithium concentrations in platelets and plasma from patients receiving maintenance therapy with lithium carbonate

Patient number	Plasma lithium concentration (m-equiv./liter)	Platelet lithium concentration (moles/platelet)	Percent release of platelet lithium with thrombin
1	0.400	1.62×10^{-18}	72.7
2	0.373	2.28×10^{-18}	68.8
3	1.466	6.13×10^{-18}	64.2
4	0.827	1.74×10^{-18}	66.7
5	1.012	3.17×10^{-18}	55.5
6	0.488	2.30×10^{-18}	50.0

Table 3. Comparison of phosphorus and calcium contents in platelet dense bodies from normal volunteers and patients on long-term lithium therapy

	Number of dense bodies probed	Phosphorus (net count, mean ± S.E.M)	Calcium (net count, mean ± S.E.M.)	Ratio P/Ca (mean ± S.E.M.)
Normal				
1	20	3555 ± 436	2250 ± 316	1.72 ± 0.10
2	29	2199 ± 176	1362 ± 112	1.63 ± 0.05
3	20	4331 ± 347	3305 ± 263	1.31 ± 0.02
4	25	3365 ± 177	2289 ± 157	1.51 ± 0.04
5	23	3016 ± 218	1435 ± 119	2.18 ± 0.09
6	25	2130 ± 160	1276 ± 107	1.72 ± 0.06
7	26	3074 ± 375	2233 ± 316	1.49 ± 0.12
8	25	2506 ± 231	1371 ± 140	1.94 ± 0.12
9	25	3805 ± 269	1872 ± 123	2.04 ± 0.05
10	25	1764 ± 120	930 ± 77	1.97 ± 0.06
11	25	3128 ± 165	1552 ± 105	2.11 ± 0.08
Combined mean ± S.E.M.		2988 ± 234	1807 ± 202	1.78 ± 0.09
Lithium-treated				
1	30	3378 ± 272	1855 ± 147	1.86 ± 0.06
2	25	2291 ± 169	1509 ± 96	1.58 ± 0.11
3	25	3162 ± 247	1759 ± 120	1.90 ± 0.16
4	25	3257 ± 179	1389 ± 91	2.41 ± 0.08
5	25	3298 ± 243	1459 ± 148	2.50 ± 0.17
6	25	3363 ± 153	1146 ± 86	3.12 ± 0.14
Combined mean ± S.E.M.		3124 ± 170	1520 ± 105	2.23 ± 0.23*

* Significantly different from control, $P < 0.05$ (Student's t-test, two-tailed, unpaired).

composition, was unchanged from that of control solids or from that of solids prepared with 10 or 50 mM potassium chloride.

DISCUSSION

Several workers have demonstrated that human platelets accumulate lithium, both following chronic lithium ingestion and after *in vitro* incubation of platelets with extracellular lithium [1–3, 6, 18]. It has usually been assumed that the process occurs by equilibration of lithium across the plasma membrane, since the accumulation is approximately linear with respect to time of incubation and initial lithium concentration, and since the estimated levels of lithium in platelet cytoplasm approximate those in the surrounding medium. Our data indicate that about two-thirds of the total intra-platelet lithium, and on the average 80% of the total dense bodies, were released following brief treatment with thrombin. Brief thrombin treatment releases dense bodies but does not appear to release an appreciable amount of the potassium present in platelet cytoplasm [20]. It therefore seems unlikely that the released lithium had been cytoplasmic in location, and one may conclude that approximately 65% of the intra-platelet lithium was sequestered in dense bodies.

Lithium added to dense bodies *in vivo* did not appear to produce significant changes in the total phosphorus content. Although the apparent 16% reduction in total calcium content was not statistically significant, the ratio of phosphorus to calcium was increased by 25% (a statistically significant increase). There may therefore have been a reduction in dense-body calcium content, but it seems unlikely to have been the result of direct displacement of

Table 4. Comparison of the compositions of synthetic analogues of dense bodies in aqueous solutions before and after the addition of lithium

Solution conditions	Amount of material present (μmoles/mg dried solid, mean ± S.E.M.)								
	Calcium	ATP	ADP	AMP	P_2O_7	PO_4	Sodium	Potassium	Lithium
Aqueous slurry	3.01 ±0.04	0.575 ±0.027	0.842 ±0.015	0.025 ±0.009	0.375 ±0.024	0.040 ±0.017	0.52 ±0.01	0.56 ±0.01	0
Aqueous slurry plus 10 mM LiCl	2.96	0.525	0.819	0.013	0.335	0.058	0.53	0.57	0.102
Aqueous slurry plus 50 mM LiCl	3.00	0.553	0.825	0.021	0.424	0.045	0.56	0.57	0.105

phosphate-associated calcium by lithium. Assuming eight dense bodies per platelet, a typical dense body may contain 3.6×10^{-19} moles of lithium in the presence of an extracellular lithium concentration of 1 mM, and 4.9×10^{-18} moles of lithium following incubation at an initial extracellular concentration of 10 mM. These amounts of lithium represent from 2 to 20% of the amount of calcium present in a typical dense body [19, 21], which would suggest stoiochiometric exchange of lithium for calcium. However, such exchange would not provide charge equivalence, and is not in fact observed to occur in synthetic analogues of the dense-body core.

In a dense body with an estimated volume of 7×10^{-18} liters [9], the intra-vesicular lithium concentration would range between 50 and 700 mM, and the dense body: extracellular medium lithium gradient would exceed 50:1. The mechanism responsible for lithium uptake and sequestration against an apparent concentration gradient is unclear. The dense-body membrane apparently does not permit the entry of calcium, since dense-body calcium exchanges little, or not at all, with intracellular or extracellular calcium [22–24]. Furthermore, synthetic analogues of the dense-body core show an equal but low affinity for the monovalent cations—sodium, potassium, and lithium (unpublished data). Specific or high-affinity binding to intra-vesicular nucleotides thus does not appear to account for the high lithium content of dense bodies observed here.

The dense-body core is believed to exist as an amorphous solid composed of nucleotide–pyrophosphate–calcium precipitates [10, 14]. Examination of lithium binding in dense bodies, therefore, does not provide an unambiguous test of the hypothesis [4, 5] that lithium in other cells with different types of storage sites can compete successfully with divalent cations for binding to stored nucleotides. A better test might be obtained by examination of lithium uptake into mobile systems such as nucleotide–cation mixtures, chromaffin vesicles, or the dense bodies of pig platelets. The binding characteristics of the nucleotides in such systems could also be evaluated more critically by nuclear magnetic resonance spectroscopy of their phosphorous resonances, since the contents are in a sufficiently fluid state to permit such observation.

It would be interesting to determine whether or not lithium accumulates in other nucleotide-rich vesicles, regardless of the mechanism, because the storage vesicles of aminergic nerves are believed to contain appreciable amounts of ATP [25]. If lithium were to accumulate in nerve vesicles, terminal deplorization could lead to the appearance of high local concentrations of lithium at the synaptic cleft—a phenomenon possibly related to the psychotropic actions of lithium therapy.

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