A comparative study of calcium homeostasis and function following inhibition of calcium channels in human platelets

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Intracellular calcium is tightly regulated by various calcium pumps in platelets and this regulation in essential for platelet activation. We investigated the effects of three calcium ATPase inhibitors: Carboxyeosin (acts at the Plasma membrane Ca^{2+}ATPase (PMCA)), 2,5-Di(t-butyl)1,4-benzohydroquinone (BHQ) (acts at the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA)) and Bepridil (acts at the Na^{2+}/Ca^{2+} exchanger (NCX)). Fura-2 loaded platelets were used to measure changes in basal and activated cytosolic calcium within platelets. The study was then extended to observe the functional consequences of these inhibitors using a variety of functional assays. Al experiments were conducted at 37˚C.CE (10-40µM) and Bepridil (10-100µM) concentration-dependently, increased cytosolic basal calcium levels in resting platelets while BHQ (0.1-10µM) had no effect. Calcium extrusion following treatment with Thapsigargin (1µM) was inhibited in the presence of CE, whereas BHQ and Bepridil had no effect. All three agents inhibited thrombin and collagen mediated platelet aggregation and all three enhanced clot retraction. CE and BHQ increased adhesion to fibrinogen/thrombin (r*efer to Table 1).*

In conclusion, all three calcium pumps play a pivotal but contrasting role in calcium homeostasis. PMCA and the NCX appear to regulate resting $[Ca^{2+}]_i$ in resting platelets whereas SERCA appears to have a negligible role. All three pumps positively regulate platelet aggregation and regulate the early and later stages of platelet activation i.e. clot retraction to different extents. Thus the various Ca^{2+} pumps responsible for Ca^{2+} removal from platelets differentially regulate platelet homeostasis and function and may have potential in various platelet-mediated disorders.

Table: Effects of Ca channel inhibitors on calcium regulation and function

Data shown as % cf. control.

Study	PMCA	SERCA	NCX
Basal calcium levels (resting platelets)	39%±9.6 ** increase	No significance	159%±8.2 ** increase
Calcium extrusion (stimulated platelets)	2%±4.5** decrease	100%±6.7** decrease	100%±8.1** decrease
Aggregation (Thrombin)	84%±9.7** decrease	82%±11** decrease	76±9.3% ** decrease
Adhesion	40%±2.5** increase	317%±4.5** increase	-
Clot retraction	40%±3.8** decrease in clot size	69%±4.1** decease in clot size	92%±5.9** decrease in clot size

***P<0.1 n=4*