Appendix

We calculated changes in concentrations of phosphate compounds using the model described by Allen and Orchard (31). The model included ATP, ADP, AMP, Creatine phosphate (PCr), creatine (Cr) and P_i under thermodynamic equilibrium.

$$\mathrm{ATP} \longrightarrow \mathrm{ADP+P_i} \tag{A1}$$

$$\mathrm{PCr+ADP} \Leftrightarrow \mathrm{ATP+Cr} \tag{A2}$$

$$\mathrm{2ADP} \longleftrightarrow \mathrm{ATP+AMP} \tag{A3}$$

With starting concentrations of ATP ($[ATP_{tot}]$=5 mM), ADP ($[ADP_{tot}]$=0 mM), AMP ($[AMP_{tot}]$=0 mM), P_i ($[P_{i\ tot}]$=1 mM), PCr ($[PCr_{tot}]$=12 mM) and Cr ($[Cr_{tot}]$=0 mM), the following simultaneous equations (A4-A8) were solved at any given $[ATP_{tot}]$.

$$\text{Total adenosine nucleotide}=[ATP_{tot}]+[ADP_{tot}]+[AMP_{tot}]=5\text{ mM} \tag{A4}$$

$$\text{Total creatine compound}=[PCr_{tot}]+[Cr_{tot}]=12\text{ mM} \tag{A5}$$

$$\text{Total phosphate}=[PCr_{tot}]+[P_{i\ tot}]+3[ATP_{tot}]+2[ADP_{tot}]+[AMP_{tot}]=28\text{ mM} \tag{A6}$$

$$([ATP_{tot}]\,[Cr_{tot}])/([ADP_{tot}]\,[PCr_{tot}])=200 \tag{A7}$$

$$[ADP_{tot}]^2/([ATP_{tot}]\,[AMP_{tot}])=1 \tag{A8}$$

Mg^{2+} binding to ATP, ADP, AMP, PCr and P_i was calculated with the following apparent dissociation constants for Mg^{2+} ($K_D{}'$) assumed for pH 6.8, 150 mM K^+, 10 nM Ca^{2+} and 25°C: ATP 0.191 mM (32), ADP 2.25 mM (32), AMP 16.7 mM (33), PCr 50.5 mM (32), P_i 33.5 mM (32). If a rise of $[Mg^{2+}]_i$ from 2.44 mM to 2.80 mM is associated with a decrease in $[ATP_{tot}]$ from x mM to 0 mM (see text), changes in a Mg^{2+}-bound buffer concentration (Δ[MgB], where B stands for one of 5 Mg^{2+} buffers) can be calculated by the following equation.

$$\Delta[\mathrm{MgB}] = \frac{2.80\,[B_{tot}]_{ATP=0}}{K_D{}' + 2.80} - \frac{2.44\,[B_{tot}]_{ATP=x}}{K_D{}' + 2.44}\quad , \tag{A9}$$

where $[B_{tot}]_{ATP=0}$ and $[B_{tot}]_{ATP=x}$ denote the total concentration of buffer B at $[ATP_{tot}]$=0 mM and x mM, respectively. Note that Δ[MgB] (or $-\Delta$[MgB]) represents a concentration of Mg^{2+} that is removed from (or added to) the free cytoplasmic pool by binding to (or dissociation from) the buffer B. In the absence of other buffers and transport for Mg^{2+}, a decrease in the sum of Δ[MgB] should be equal to an increase in $[Mg^{2+}]_i$.

Table S1. Major constituents of the superfusion solutions

(mM)	NaCl	NMDG	KCl	KMS	$MgCl_2$	$MgMs_2$	$[Mg^{2+}]$	$[Na^+]$	$[K^+]$
Ca-free Tyrode's	135		5.4	0	1.0	0	1	140	5.4
Na-depleting	0	135	5.4	0	1.0	0	1	0.3	5.4
Mg-loading	0	0	5.4	0	68.5	24	93	5.2	5.4
Hypertonic 70Na	64.7	0	0	140	1.0	0	1	70	140
Hypertonic 0Na	0	69.7	0	140	1.0	0	1	0.3	140

Ms, methanesulfonate; NMDG, n-methyl-D-glucamine. The pH of the isotonic solutions was adjusted to 7.40 with NaOH (for Ca-free Tyrode’s solution, and Mg-loading solution) or HCl (for Na-depleting solution). The pH of the hypertonic solutions was set to 7.15 with NaOH (hypertonic 70Na solution) or HCl (hypertonic 0Na solution). These hypertonic solutions had ~40% higher osmolality than other solutions. All solutions contained 0.1 mM K_2EGTA, 0.33 mM NaH_2PO_4 and 10 mM HEPES, and were essentially free of Ca^{2+}. For superfusion of normally-energized cells, 5 mM glucose was usually included (see text for exceptions). Final concentrations of Mg^{2+}, Na^+ and K^+ are shown in the right-most three columns.

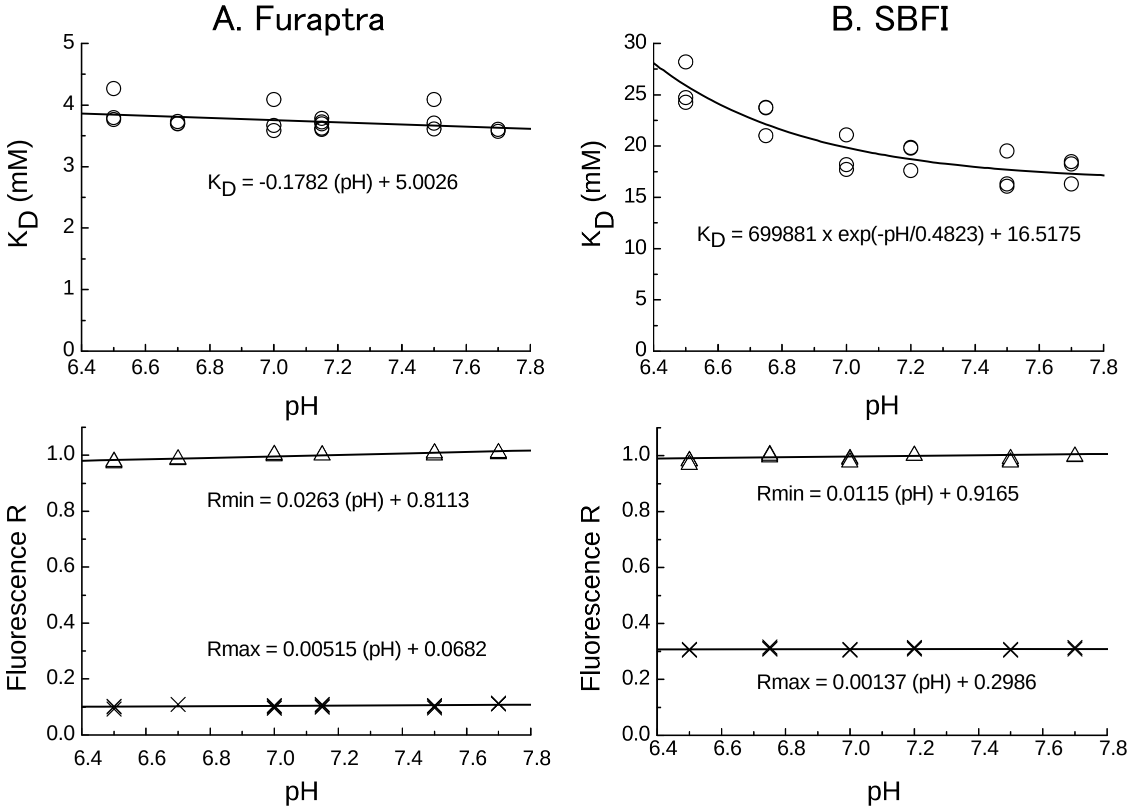

Figure S1

Effects of pH on K_D, R_{min} and R_{max} of furaptra (A) and SBFI (B). Spectrofluorometry measurements were made in a 1-cm quartz cell with the pH of the solution varying between 6.5 and 7.7 at 25°C, using PIPES or MOPS as a buffer. (A) The solutions contained 150-0 mM KCl, 0-50 mM $MgCl_2$, 0.1 mM EGTA, 0.5 μM furaptra and 10 mM MOPS (or PIPES), and pH was adjusted by KOH. From a set of furaptra R values obtained at 0 mM, 0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM, 20 mM and 50 mM Mg^{2+} concentrations, estimates of K_D, R_{min} and R_{max} were obtained by the least-squares fitting with Eq. 1. (B) The solutions contained 150-0 mM KCl, 0-150 mM NaCl, 0.1 mM EGTA, 5 μM SBFI and 5 mM MOPS (or PIPES), and pH was adjusted by KOH. A set of SBFI R values obtained at 0 mM, 10 mM, 20 mM, 50 mM, 100 mM and 150 mM Na^+ concentrations were least-squares fitted with an equation analogous to Eq. 1, and the best fitted K_D, R_{min} and R_{max} were obtained. In A and B, the upper panels plot the fitted K_D values (circles), and the lower panels plot values of R_{min} (triangles) and R_{max} (crosses) relative to R_{min} at pH 7.15-7.2. For pooled data sets of each symbol type, the regression line was drawn by least-squares fitting with a function of the form indicated in the graphs (solid lines).

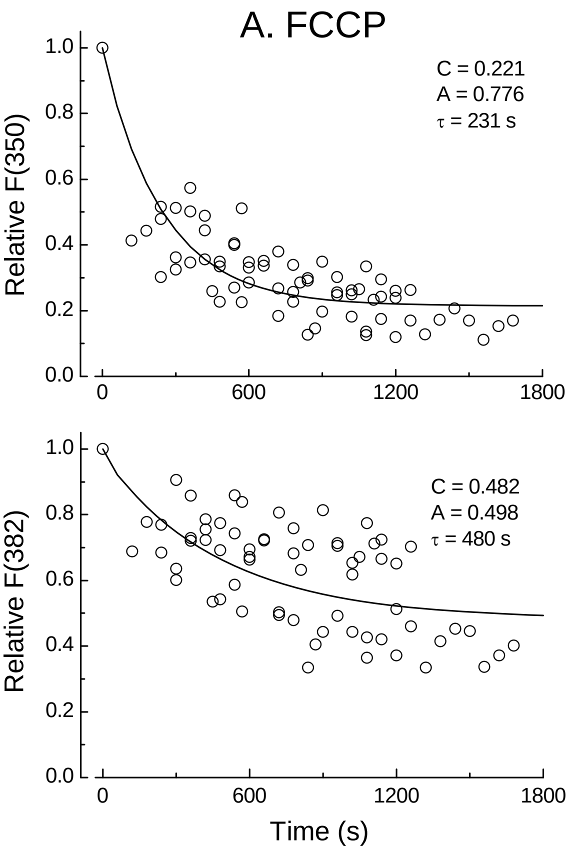

Figure S2

Effects of FCCP (A) and KCN (B) on the cell autofluorescence excited at 350 nm (upper) and 382 nm (lower) without indicator loading. Intensities of the autofluorescence relative to the values measured just before the drug application (the first data points in A and B) are plotted as a function of time. Pooled data from 11 cells (A) and 9 cells (B) were fitted by an exponential decay function of the form: $F(\lambda)=C+A\times\exp(-t/\tau)$, where t is time after the drug application, and τ is a time constant. Constants C and A give, respectively, a nadir and a scaling factor. For B, the first data points at time 0 were excluded from the fit. The least-squares fitted curves with the parameter values shown in the panels are indicated by solid lines.