Upregulation of Cytosolic Ca\textsuperscript{2+} Increases by Cyclic ADP-ribose in NG108-15 Neuronal Cells: In Comparison with Inositol Tetrakisphosphate in Fibroblast Cells

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It has been reported that the depolarization-induced increase in [Ca\textsuperscript{2+}]\textsubscript{i} is potentiated by cyclic ADP-ribose (cADPR) or caffeine in neurons (Hua et al. 1994; Kano et al. 1995). Modulation of L-type voltage-activated Ca\textsuperscript{2+} channels (VACCs) by caffeine and ryanodine has also been shown in cerebellar neurons (Chavis et al. 1996). However, mechanisms whereby ryanodine receptors, cADPR, and L-type VACCs interact have not been established yet. Thus, we examined the effects of cADPR on a [Ca\textsuperscript{2+}]\textsubscript{i} rise in NG108-15 cells. NG108-15 cells were pre-loaded with fura-2 and whole-cell patch-clamped (Hashii et al. 1996). Application of 10 μM cADPR itself did not trigger any [Ca\textsuperscript{2+}]\textsubscript{i} rise at the resting membrane potential of -45 mV. However, cADPR augmented the increased [Ca\textsuperscript{2+}]\textsubscript{i} resulting from Ca\textsuperscript{2+} mobilization. Extracellular applications of bradykinin (20 μl of 2.5 μM, final 100 nM) to NG108-15 cells evoked an immediate and transient [Ca\textsuperscript{2+}]\textsubscript{i} rise in the presence of 2 mM [Ca\textsuperscript{2+}]. The [Ca\textsuperscript{2+}]\textsubscript{i} level at the peak and 3 min after the bradykinin application was about 280% and 110% of the concentration (73 nM) just before bradykinin applications. Usually, after cADPR infusion the peak bradykinin response was significantly larger (4-fold) than the control (2.8-fold). The [Ca\textsuperscript{2+}]\textsubscript{i} level after 3 min of bradykinin application returned to the control level, at which time the effect of cADPR disappeared. Intracellular applications of 10 μM Ins(1,4,5)P\textsubscript{3} into NG108-15 cells elicited a transient [Ca\textsuperscript{2+}]\textsubscript{i} rise by 1.7-fold at the peak of the preinjection level. When Ins(1,4,5)P\textsubscript{3} and cADPR were simultaneously infused, the peak [Ca\textsuperscript{2+}]\textsubscript{i} was significantly increased to 2.2-fold of the control. The higher [Ca\textsuperscript{2+}]\textsubscript{i} level was maintained longer by cADPR. This result suggests that cADPR can further...
potentiate the \([Ca^{2+}]_i\) elevation that resulted from InsP3-induced \(Ca^{2+}\) mobilization, probably by stimulating \(CICR\). Successive extracellular applications of 10 \(\mu\)M ryanodine (at the final concentration) produced transient \([Ca^{2+}]_i\) rises of up to 130\% of the \([Ca^{2+}]_i\) level just before the first ryanodine application in control cells. In cells infused with 10 \(\mu\)M cADPR, the average peak value of \(Ca^{2+}\) transients in response to ryanodine applications increased up to 180\% of the control level. The enhancement by cADPR is evidenced by the larger peak response, showing that cADPR can modify ryanodine-sensitive CICR channels in NG108-15 cells.

cADPR and \(\beta-NAD^+\), a precursor of cADPR, amplified the \([Ca^{2+}]_i\) rise via \(Ca^{2+}\) influx through VACCs. The average \([Ca^{2+}]_i\) increase at 5 min after holding at -30 mV was about 140\% in cADPR- and \(\beta-NAD^+\)-treated cells, while it was 110\% in control cells. The facilitating effect of \(\beta-NAD^+\) was abolished by addition of 8-bromo-cADPR, indicating that the \(\beta-NAD^+\) effect may be mediated by cADPR. Membrane potential dependency of cADPR and \(\beta-NAD^+\) potentiation corresponded to L-type VACCs.

These results, together with our previous data (Higashida et al. 1995), suggest that cADPR functions as an endogenous regulator for neuronal signaling by amplifying \([Ca^{2+}]_i\) increases resulting from \(Ca^{2+}\) influx through L-type VACCs and from \(Ca^{2+}\) mobilization. The possible role of cADPR in the neuronal G108-15 cells is summarized in Fig. 1A, in which cADPR is seen to play an important role.