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Adenosine decreases intracellular free calcium concentrations in cultured vascular smooth muscle cells from rat aorta

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Using an intracellularly trapped dye, quin 2, effects of adenosine on intracellular free calcium concentrations ($[Ca^{2+}]_i$) were recorded, microfluorometrically, using rat aortic medial vascular smooth muscle cells (VSMCs) in primary culture. Regardless of whether cells were at rest (in 5 mM K^+), at K^+-depolarization (in 55 mM K^+) or at Ca^{2+} depletion (in Ca^{2+}-free media), adenosine induced a rapid reduction of $[Ca^{2+}]_i$, following which there was a gradual increase to pre-exposure levels, in cells at rest and in the case of Ca^{2+} depletion. Only when the cells were depolarized (55 mM K^+) did adenosine induce a new steady $[Ca^{2+}]_i$ level, lower than the pre-exposure value. These findings indicate that decrease in $[Ca^{2+}]_i$ by adenosine is one possible mechanism involved in the adenosine-mediated vasodilatation, and that adenosine decreases $[Ca^{2+}]_i$ by direct extrusion, by sequestration, or by inhibiting the influx of Ca^{2+} into VSMCs.

Adenosine; Quin 2; (Vascular smooth muscle cell, Rat aorta)

1. INTRODUCTION

Adenosine is a putative vasodilator [1], but the mechanisms whereby it elicits vascular smooth muscle relaxation are not well understood. Two main and alternative mechanisms have received serious consideration: (i) Adenosine may decrease the availability of Ca^{2+} for the contractile process. It has been suggested that adenosine decreases sarcolemmal permeability to Ca^{2+} [2,3], and also alters intracellular Ca^{2+} release or sequestration [4–6]. (ii) Adenosine increases intracellular adenosine 3′,5′-cyclic monophosphate levels, which in turn may decrease sensitivity of the myofilament to the Ca^{2+} in smooth muscle [7,8].

Using microfluorometry of the $[Ca^{2+}]_i$-sensitive dye quin2, we recorded Ca^{2+} transients induced when cultured VSMCs were exposed to adenosine. This seems to be the first evidence that adenosine actively and transiently decreases $[Ca^{2+}]_i$, regardless of the level of $[Ca^{2+}]_i$ at the time of application.

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Abbreviations: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; PSS, physiological saline solution; VSMCs, vascular smooth muscle cells

2. MATERIALS AND METHODS

Quin2/AM was purchased from Dotite (Japan) and adenosine from Sigma (USA).

2.1. *Cell culture and loading cells with quin2*

Rat aortic medial smooth muscle cells were cultured as described [9]. On days 5 or 6, just before reaching confluency, the cultured cells on

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Lux chamber slides were loaded with quin2, as the acetoxy methyl ester (quin2/AM) [10] as described [11,12]. Unless otherwise indicated, the measurements of $[Ca^{2+}]_i$ transients were performed in PSS at 25°C. The millimolar composition of normal PSS (pH 7.4 at 25°C) was: NaCl, 135; KCL, 5; $CaCl_2$, 1; $MgCl_2$, 1; glucose, 5.5; Hepes, 10. High K^+ (55 mM) PSS was prepared by the equimolar replacement of NaCl with KCl. The composition of Ca^{2+}-free PSS was the same as in normal PSS, except that it contained 2 mM EGTA instead of 1 mM $CaCl_2$. We used primary cell cultures throughout.

2.2. *Microfluorometry of quin2*

The fluorescence intensity in a spot ($<1\ \mu m^2$) of the 3 μm apart from the nucleus was measured microfluorometrically, using our method [11,12]. The changes in $[Ca^{2+}]_i$ were expressed in arbitrary units. $[Ca^{2+}]_i$ of VSMCs in normal PSS (5 mM K^+), high K^+ (55 mM K^+) and Ca^{2+}-free PSS (2 mM EGTA) for 10 min were 104 ± 6 nM, 348 ± 45 nM and 47 ± 15 nM, respectively (n = 4), as determined by the method of Tsien et al. [10], using suspended VSMCs after trypsinization [9].

3. RESULTS AND DISCUSSION

Fig.1 demonstrates typical examples of the time course of the response of fluorescence levels of cytosolic spots observed when cultured VSMCs were exposed to 10^{-6} M adenosine, either in normal PSS or in Ca^{2+}-free PSS containing 2 mM EGTA. In normal PSS containing 5 mM K^+, adenosine induced a transient decrease in fluorescence levels, namely, a transient decrease in $[Ca^{2+}]_i$ (fig.1A). $[Ca^{2+}]_i$ decreased rapidly, reaching the lowest level at 2 min after the application of adenosine, and then gradually increased and returned to the pre-exposure level within 8 min, despite the continuous application of adenosine. When VSMCs were exposed to Ca^{2+}-free PSS, $[Ca^{2+}]_i$ decreased within 6 min to reach the steady-state level (fig.1B). When these VSMCs with low $[Ca^{2+}]_i$ due to the absence of extracellular Ca^{2+} were exposed to adenosine, a transient and further decrease in $[Ca^{2+}]_i$ occurred and the time course was similar to that observed in the presence of extracellular Ca^{2+}.

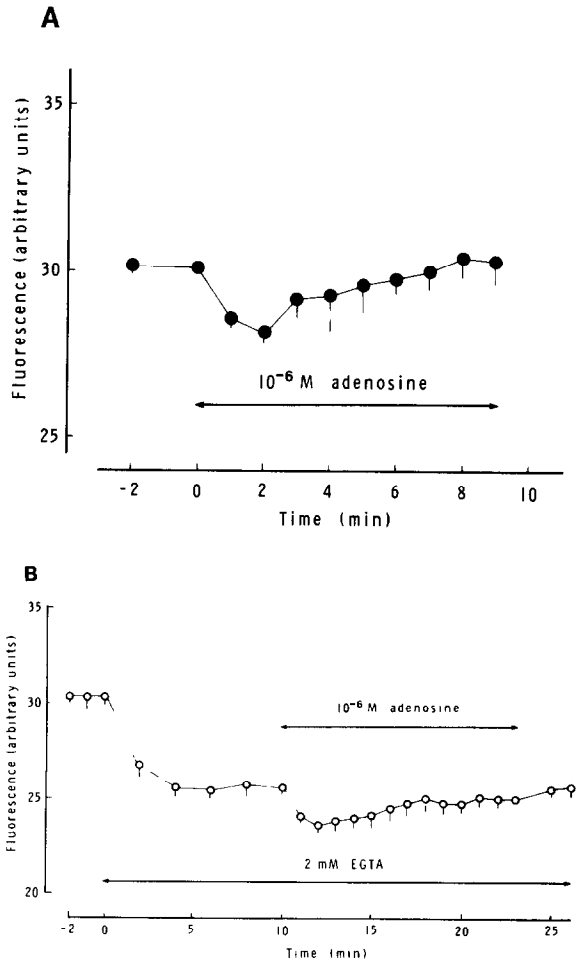

Fig.1. (A) A typical time course of the effect of 10^{-6} M adenosine on fluorescence signals in the cytosol of VSMCs in normal PSS (1 mM Ca^{2+}, 5 mM K^+) and (B) in Ca^{2+}-free PSS containing 2 mM EGTA. Data are mean ± SD of 5 experiments, and the number of cells counted in each plot was 8.

When VSMCs were incubated with high K^+ (55 mM) PSS, $[Ca^{2+}]_i$ increased rapidly, reached a steady state in 2 min, then remained unchanged (fig.2). Previous work demonstrated that 55 mM is the minimum concentration of KCl required to induce the maximal $[Ca^{2+}]_i$ increase by K^+-depolarization [11]. As shown in fig.2, adenosine (10^{-6} M) rapidly and markedly reduced the increased $[Ca^{2+}]_i$, as induced by 55 mM extracellular K^+. $[Ca^{2+}]_i$ reached the lowest level at 2 min (the early effect), and then, despite the continuous application of adenosine, increased to

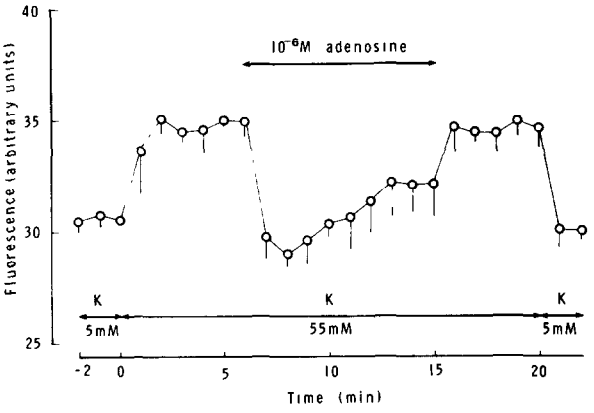

Fig.2. A typical time course of the effect of 10^{-6} M adenosine on fluorescence signals during 55 mM K^+-depolarization. Data are mean ± SD of 5 experiments.

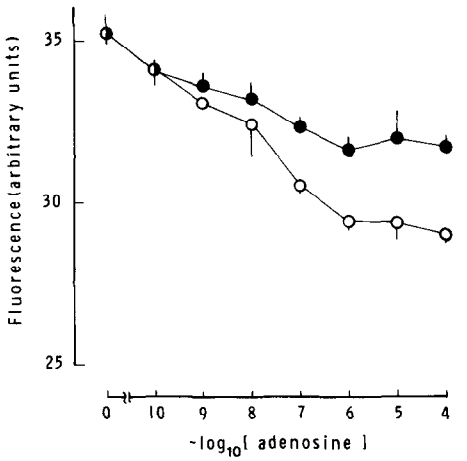

Fig.3. Dose-dependent effect of adenosine on fluorescence signals during 55 mM K^+-depolarization. The effects on the lowest level at 2 min (○) and the steady-state level at 8 min (●) are plotted separately. Data are mean ± SD of 4 experiments.

reach a higher steady-state level, within 8 min (the late effect). This new level was significantly ($p < 0.01$, analysis of variance) lower than the value observed at 55 mM K^+-depolarization, and significantly ($p < 0.01$) higher than that observed in normal PSS (5 mM K^+). As shown in fig.3, changes in the concentration of adenosine induced a concentration-dependent reduction in the level of $[Ca^{2+}]_i$ increased by high extracellular K^+, both at the early reduction phase (2 min) and the steady state (8 min; $p < 0.05$). The minimum concentration of adenosine required to induce the maximum reduction of $[Ca^{2+}]_i$ at 2 and 8 min of application was 10^{-6} M.

These findings suggest at least two potential effects of adenosine on Ca^{2+} homeostasis of VSMCs. (i) Adenosine may actively decrease $[Ca^{2+}]_i$ of VSMCs during the early phase of application. Since this early and transient effect was observed not only when VSMCs were in the resting state (5 mM K^+) or at high K^+-depolarization but also when they were exposed to Ca^{2+}-free PSS, the effect may be not due to alteration in the sarcolemmal permeability to Ca^{2+} or in the Ca^{2+} influx through Ca^{2+} channels, rather, it may be related to the acceleration of Ca^{2+} extrusion from the cell or to intracellular sequestration. (ii) Adenosine produces, as the late effect, a new steady state in $[Ca^{2+}]_i$ during application, particularly when VSMCs are depolarized by high extracellular K^+. $[Ca^{2+}]_i$ in the case of this steady state is lower than that seen with pre-exposure to adenosine. Since the extent of $[Ca^{2+}]_i$ elevation induced by K^+-depolarization depends on the extracellular K^+ concentration which may determine the Ca^{2+} influx through the sarcolemma [11], and since the present study indicated that the extent of $[Ca^{2+}]_i$ reduction as the late effect depends on adenosine concentration, it is plausible that this late effect may be due to the adenosine-mediated inhibition of the Ca^{2+} influx through the sarcolemma. The source of Ca^{2+} during a gradual rise of $[Ca^{2+}]_i$ after 2 min of adenosine application may be the intracellular store site, because this phenomenon was observed both in the presence and absence of extracellular Ca^{2+}.

It was reported that adenosine depressed the uptake of $^{45}Ca^{2+}$ caused by K^+-depolarization in cultured VSMCs of the rat aorta [2]. This observation is in good agreement with that of the present study that adenosine decreases $[Ca^{2+}]_i$, as the late effect, during K^+-depolarization. Using the chemically loaded biofluorescent protein aequorin, the effect of adenosine on $[Ca^{2+}]_i$ during 33 mM K^+-depolarization has been recorded, using the ferret portal vein [13]. At low doses, adenosine was found to decrease $[Ca^{2+}]_i$ but at high concentrations (over 3.7×10^{-6} M) it increased $[Ca^{2+}]_i$ and relaxed the vein. These findings apparently con-

tradict our observation that adenosine dose-dependently reduces $[Ca^{2+}]_i$ during K^+-depolarization. Whether or not this discrepancy in the effect of adenosine on $[Ca^{2+}]_i$ of VSMCs is due to species differences, tissues used or the characteristics of Ca^{2+} indicators and their loading conditions, remains to be elucidated.

These present findings indicate that the decrease in $[Ca^{2+}]_i$ is one important mechanism related to adenosine-mediated vasodilation.

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