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Short communication

Regulation of intracellular Ca^{2+} by 8-bromoguanosine 3′:5′-cyclic monophosphate, felodipine and ryanodine in rat caudal artery

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Abstract

$^{45}Ca^{2+}$ efflux in isolated rat caudal artery was measured in the absence and presence of 8-bromoguanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), felodipine or ryanodine after stimulation of α_1-adrenoceptors. The objectives of this study were to identify the mechanisms of action of 8-Br-cGMP, felodipine and ryanodine in a vascular resistance vessel. 8-Br-cGMP and ryanodine but not felodipine increased basal $^{45}Ca^{2+}$ efflux. Phenylephrine-induced $^{45}Ca^{2+}$ efflux was reduced by all three antagonists. The results of this study demonstrate that, (1) 8-Br-cGMP-mediated relaxation is affected in part by an increased extrusion of intracellular Ca^{2+} and/or inhibition of intracellular Ca^{2+} release, (2) the Ca^{2+}-channel antagonist, felodipine, impairs intracellular Ca^{2+} release and (3) ryanodine reduced phenylephrine-induced Ca^{2+} efflux by depleting intracellular Ca^{2+} stores.

Keywords: Ca^{2+} efflux; Smooth muscle; Ca^{2+}, intracellular; α_1-Adrenoceptor

1. Introduction

A number of mechanisms have been proposed to explain the vasodilating effect of guanosine 3′:5′-cyclic monophosphate (cGMP) since the relationship between endothelium-derived relaxing factor and cGMP was established. These have include (a) reduction in intracellular Ca^{2+} induced by decreased influx (Collins et al., 1986; Chen and Rembold, 1992), (b) increased Ca^{2+} extrusion (Collins et al., 1985, 1986), (c) impaired intracellular Ca^{2+} release (Collins et al., 1986) and (d) reduced intracellular Ca^{2+} sensitivity of force without changes in the concentration of intracellular Ca^{2+} (Chen and Rembold, 1992). In a recent study, we had demonstrated that felodipine but not 8-bromoguanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), reduced phenylephrine-induced production of inositol 1,4,5-trisphosphate (IP_3) in the rat caudal artery (Lum Min and Tabrizchi, 1995). In addition, we reported that 8-Br-cGMP and felodipine together were unable to produce additive inhibition of phenylephrine-induced contractions in the rat caudal artery. We speculated that this may have been due to the fact that 8-Br-cGMP was affecting a felodipine-sensitive pathway in inhibiting intracellular Ca^{2+} release subsequent to the production of IP_3 in the rat caudal artery (Lum Min and Tabrizchi, 1995). It is believed that IP_3 is a second messenger responsible for intracellular Ca^{2+} release (Berridge, 1993).

Therefore, the main objective of the present study was to compare the effects of three agents, 8-Br-cGMP, felodipine and ryanodine, on phenylephrine-induced $^{45}Ca^{2+}$ efflux in rat caudal artery.

2. Materials and methods

Male Sprague-Dawley rats (300–400 g) were anaesthetized with sodium pentobarbital (65 mg/kg) i.p. and the caudal artery was removed and placed in Krebs-bicarbonate buffer of the following composition (in mM): NaCl, 120; KCl, 4.6; glucose, 11; $MgCl_2$, 1.2; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.3. The pH of the buffer following saturation with a 95% O_2/5% CO_2 gas mixture was 7.4. Arteries were dissected free and cleaned of connective tissue, and the endothelial cell layer was removed from cleaned arteries by inserting a wire through the lumen and gently rubbing (Lum Min and Tabrizchi, 1995). Arteries (1.0 cm length) were then split open.

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The tissues were allowed to equilibrate for 120 min in Krebs buffer at 37°C. Following equilibration, tissues were twice transferred to fresh Krebs (6.0 ml) containing 25 μCi $^{45}Ca^{2+}$ to load for 60 min. Loaded tissues were washed in Krebs (50 ml) for 30 min in the absence or presence of prazosin (300 nM), 8-Br-cGMP (10 μM), felodipine (10 nM) or ryanodine (3.0 μM). The tissues were then passed, at 3 min intervals, through a series of vials containing Krebs (3.0 ml) in the absence or presence of antagonists and/or phenylephrine. Scinti-Safe 30% scintillation fluid (3.0 ml) was added to each vial and the radioactivity counted in a Packard 1600TR liquid scintillation counter. The efficiency of the counter was 80%. Each tissue was blotted, weighed and dissolved in hydrogen peroxide/perchloric acid (1.0:1.0) (200 μM). Scintillation fluid was added to the dissolved tissues and the radioactivity in each tissue counted.

2.1. Data and statistical analysis

For each experiment, the amount of $^{45}Ca^{2+}$ released as a percent of the total $^{45}Ca^{2+}$ loaded was plotted over time, where the total $^{45}Ca^{2+}$ loaded was determined by summing the radioactive counts per min per vial and the radioactive counts per min remaining in the tissue. The resulting curve was fitted to the equation $C = C_0 e^{-kt}$, where $C = {}^{45}Ca^{2+}$ concentration at time t in min, $C_0 =$ initial $^{45}Ca^{2+}$ concentration at $t = 0$ min and $k =$ the elimination constant per min. Average k values were compared using an unpaired Student's t-test; a probability of error of less than 0.05 was selected as the criterion for statistical significance.

2.2. Chemicals

8-Bromoguanosine 3′:5′-cyclic monophosphate sodium salt (8-Br-cGMP) and L-phenylephrine HCl were purchased from Sigma. Ryanodine was purchased from Calbiochem and felodipine was a gift from Hässle. $^{45}Ca^{2+}$ was purchased from New England Nuclear. With the exception of felodipine, all drug solutions were prepared in double distilled water. A 10 mM felodipine stock solution was made in 80% ethanol; dilutions were made with double distilled water.

3. Results

The parameter measured in this study, $^{45}Ca^{2+}$ efflux, is well characterized by a simple elimination model; consequently, the resulting efflux curves were fitted with the elimination rate constant equation $C = C_0 e^{-kt}$ as defined in Section 2.1.

As a positive control $^{45}Ca^{2+}$ efflux was measured in the presence of the selective α_1-adrenoceptor antagonist, prazosin (300 nM). The presence of prazosin did not alter basal $^{45}Ca^{2+}$ efflux (Table 1). However, prazosin was able to significantly ($P < 0.05$; $n = 6$) impair the phenylephrine-induced (3.0 and 10 μM) $^{45}Ca^{2+}$ efflux by 58% and 70%, respectively (Table 2). In contrast to prazosin, both 8-Br-cGMP (10 μM) and ryanodine (3.0 μM) significantly ($P < 0.05$; $n = 6$) increased basal $^{45}Ca^{2+}$ efflux (Table 1). In fact, ryanodine was able to significantly increase basal $^{45}Ca^{2+}$ efflux above that of 8-Br-cGMP (Table 1).

Phenylephrine induced a concentration-dependent increase in the rate of $^{45}Ca^{2+}$ efflux (Table 2). 8-Br-cGMP was able to significantly ($P < 0.05$; $n = 6$) reduce phenylephrine-induced $^{45}Ca^{2+}$ efflux at 10 μM but not 3.0 μM (Table 2). Phenylephrine-induced (10 μM) $^{45}Ca^{2+}$ efflux was reduced by 29% in the presence of 8-Br cGMP (Table 2). Felodipine (10 nM) like praozsin did not affect basal $^{45}Ca^{2+}$ efflux (Table 1) but it did significantly ($P < 0.05$; $n = 6$) reduce $^{45}Ca^{2+}$ efflux in the presence of 10 but not

Table 1
Basal $^{45}Ca^{2+}$ efflux from rat caudal artery in the absence of phenylephrine

Groups	k (min)
Control	0.020 ± 0.008
Prazosin (300 nM)	0.028 ± 0.003
8-Br-cGMP (10 μM)	0.041 ± 0.004[a]
Felodipine (10 nM)	0.024 ± 0.005
Ryanodine (10 μM)	0.069 ± 0.008[a,b]

Elimination rate constant values (k) were obtained from individual exponential decay curves. Each value represents the mean of six experiments ± S.E.
[a]Significantly different from control, $P < 0.05$.
[b]Significantly different from 8-Br-cGMP, $P < 0.05$.

Table 2
Phenylephrine-induced $^{45}Ca^{2\pm}$ efflux from rat caudal artery

Groups	k (min)
After addition of 3 μM phenylephrine	
Control	0.079 ± 0.009
Prazosin (300 nM)	0.033 ± 0.004[a]
8-Br-cGMP (10 μM)	0.074 ± 0.010
Felodipine (10 nM)	0.073 ± 0.008
Ryanodine (10 μM)	0.032 ± 0.013[a]
After addition of 10 μM phenylephrine	
Control	0.115 ± 0.010[a]
Prazosin (300 nM)	0.035 ± 0.003[b]
8-Br-cGMP (10 μM)	0.082 ± 0.007[b]
Felodipine (10 nM)	0.066 ± 0.010[b]
Ryanodine (10 μM)	0.040 ± 0.008[b]

Elimination rate constant values (k), were obtained from individual exponential decay curves. Each value represents the mean of six experiments ± S.E.
[a]Significantly different from 3 μM phenylephrine control, $P < 0.05$.
[b]Significantly different from 10 μM phenylephrine control, $P < 0.05$.

3.0 μM phenylephrine (Table 2). Felodipine was able to reduce phenylephrine-induced (10 μM) $^{45}Ca^{2+}$ efflux by 43% (Table 2). On the other hand, ryanodine was able to significantly ($P < 0.05$; $n = 6$) inhibit phenylephrine-induced $^{45}Ca^{2+}$ efflux at both 3.0 μM and 10 μM phenylephrine (Table 2), lowering the elimination rate constant by 59% and 65%, respectively.

4. Discussion

This study demonstrates that α_1-adrenoceptor agonist-induced intracellular $^{45}Ca^{2+}$ release in rat caudal artery is impaired by prazosin, 8-Br-cGMP, felodipine and ryanodine. Furthermore, 8-Br-cGMP and ryanodine can induce $^{45}Ca^{2+}$ efflux in the absence of the agonist. These observations support the view that cGMP-mediated vasodilatation is, in part, through inhibition of intracellular Ca^{2+} release subsequent to IP_3 production, and additionally by the stimulation of Ca^{2+} extrusion. Furthermore, by blocking Ca^{2+} influx through voltage-sensitive channels, the dihydropyridine Ca^{2+}-channel antagonist felodipine impairs intracellular Ca^{2+} release. Finally, ryanodine impairs phenylephrine-induced $^{45}Ca^{2+}$ efflux by exhausting intracellular Ca^{2+} stores.

Although 8-Br-cGMP and atriopeptin II, a synthetic atrial peptide believed to stimulate production of cGMP, have been shown to block agonist-induced $^{45}Ca^{2+}$ efflux in rat and rabbit aorta (Collins et al., 1986; Meisheri et al., 1986), the present study is the first report of such an affect in a vascular resistance vessel. However, it is unlikely that cGMP acts solely through inhibition of intracellular Ca^{2+} release. Therefore, it is not perhaps surprising that 8-Br-cGMP was able to increase basal $^{45}Ca^{2+}$ efflux since it has previously been reported that 8-Br-cGMP was capable of stimulating the activity of plasmalemmal Ca^{2+}-ATPase in purified preparations (Popescu et al., 1985; Furukawa et al., 1988; Rashatwar et al., 1987). Depletion of phenylephrine-sensitive intracellular Ca^{2+} pools may, in part, account for reduced Ca^{2+} efflux that was observed in the presence of 8-Br-cGMP. Certainly such a view is consistent with the idea that nitrovasodilators produce relaxation of arterial smooth muscle by reducing the concentration of intracellular Ca^{2+} (McDaniel et al., 1992).

However surprisingly, ionomycin-induced $^{45}Ca^{2+}$ efflux in aortic smooth muscle cells was reportedly increased by 0.5 mM 8-Br-cGMP (Furukawa et al., 1991), and Ca^{2+} release from the sarcoplasmic reticulum in response to IP_3 and caffeine was not affected by 10 μM cGMP in saponin skinned primary cultures of rat aortic smooth muscle cells (Twort and Van Breemen, 1988). It is most likely that such differences as agonist concentrations and/or tissue preparations could account for these discrepancies.

We had previously reported that 8-Br-cGMP and felodipine did not additively impair phenylephrine-induced contractions in the rat caudal artery (Lum Min and Tabrizchi, 1995), indicating that ultimately the two agents impaired excitation-contraction coupling by similar mode. In the present study, felodipine, like 8-Br-cGMP, reduced phenylephrine-induced $^{45}Ca^{2+}$ efflux thus indicating they each, in part, could impair phenylephrine-induced contractions by reducing Ca^{2+} release at intracellular levels. We had reported that felodipine but not 8-Br-cGMP could inhibit phenylephrine-induced phosphatidylinositol turnover in the caudal artery (Lum Min and Tabrizchi, 1995). Therefore, the felodipine-sensitive IP_3 pathway responsible for intracellular Ca^{2+} release is likely susceptible to attack due to reduce production of IP_3. The IP_3 receptor has been shown to be phosphorylated by cGMP-dependent kinase in intact vascular smooth muscle (Komalavilas and Lincoln, 1994); this could be the mechanism by which 8-Br-cGMP inhibited Ca^{2+} release.

Ryanodine is believed to inhibit smooth muscle contraction by depleting intracellular Ca^{2+} stores (Kanmura et al., 1988; Hwang and Van Breemen, 1987; Julou-Schaeffer and Freslon, 1988). In rat caudal artery ryanodine inhibited phenylephrine-induced contractions (Lum Min and Tabrizchi, 1995), and as demonstrated in this study, phenylephrine-induced $^{45}Ca^{2+}$ efflux. We found that basal $^{45}Ca^{2+}$ efflux was increased in the presence of this antagonist. This may indicate that ryanodine depleted intracellular Ca^{2+} store(s) that is/are normally utilized subsequent to α_1-adrenoceptor activation. Although both 8-Br-cGMP and ryanodine increased basal $^{45}Ca^{2+}$ extrusion and impaired phenylephrine-induced $^{45}Ca^{2+}$ efflux, their mechanisms of action are probably different. We had found that 8-Br-cGMP and ryanodine produced additive inhibition of phenylephrine-induced contractions in rat caudal artery (Lum Min and Tabrizchi, 1995). In the present study, ryanodine reduced $^{45}Ca^{2+}$ efflux at both phenylephrine concentrations, while 8-Br-cGMP only reduced efflux at the higher phenylephrine concentration. Moreover, ryanodine was able to significantly increase basal Ca^{2+} efflux when compared to 8-Br cGMP. Thus, it seems possible that 8-Br-cGMP may have reduced intracellular Ca^{2+} release by inhibiting the action of IP_3, while ryanodine depleted the intracellular Ca^{2+} stores. In addition, it is possible that ryanodine was able to reduce phenylephrine-stimulated ^{45}Ca efflux in vascular muscle by desensitizing sites at intracellular level that are responsible for calcium release.

In summary, 8-Br-cGMP can influence vascular tone, in part, by increased extrusion of intracellular Ca^{2+} and inhibition of intracellular Ca^{2+} release, where as the Ca^{2+}-channel antagonist, felodipine, impairs intracellular Ca^{2+} release. Most likely, ryanodine affects smooth muscle contraction by depleting intracellular Ca^{2+} stores as well as inhibiting release. Taken together, our results indicate that α_1-adrenoceptor-induced mobilization of intracellular Ca^{2+} in rat caudal artery is regulated through cGMP-, felodipine- and ryanodine-sensitive pathways that may or may not be unique unto themselves.

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