Cromakalim Dilates Rat Cerebral Arteries In Vitro

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Using an in vitro perfusion method, we examined the effects of cromakalim, a potassium channel opener, on the superior cerebellar arteries of 24 rats. Cromakalim had no effect on contractions induced by 129 mM K⁺ until a concentration of 10⁻¹ M was reached. Contractions evoked by 10⁻⁸ M serotonin were attenuated by cromakalim in a concentration-dependent manner (p<0.05). The diameter of untreated superior cerebellar arteries remained almost constant with increasing perfusion pressure. However, in the presence of cromakalim, vessel diameter increased with increasing perfusion pressure. At concentrations of 3x10⁻⁸ M, cromakalim also inhibited basal myogenic tone and dilated unstimulated arteries (p<0.01). These results suggest that cromakalim is a cerebrovascular dilator acting on both receptor-mediated and myogenic contractions. (Stroke 1991;22:221-224)

Potassium channel openers such as cromakalim, nicorandil, pinacidil, or minoxidil are a novel group of antihypertensive agents that facilitate the opening of potassium channels in vascular smooth muscle, hyperpolarize the membrane, and lead to vasorelaxation.¹,² Cerebral arteries are more dependent on extracellular Ca²⁺ influx for their contractions³⁻⁵ than are systemic arteries. Thus, potassium channel openers, which hyperpolarize the membrane and block voltage-dependent Ca²⁺ influx, may have a higher selectivity for cerebral arteries. Such agents may also be of use in the treatment of cerebral vasospasm since membrane depolarization by blockade of potassium channels reportedly occurs in experimental subarachnoid hemorrhage.⁶ Despite the potentially beneficial effects of potassium channel openers, little is known about their action on cerebral blood vessels. We designed the present experiments to determine the basic effects of cromakalim on the superior cerebellar arteries of rats.

Materials and Methods

Twenty-four male Wistar-Kyoto rats weighing 350–400 g were anesthetized with ether and decapitated. The superior cerebellar arteries were perfused by a method adapted from Osol and Halpem.⁷ We used superior cerebellar arteries because they are suitable for cannulation and usually have few side branches. Briefly, the arteries were quickly excised and placed in Krebs' solution at room temperature. The surrounding connective tissue was removed from the vessel with great care to avoid mechanical damage, and all side branches were ligated with silk thread. The vessels were then cannulated at both ends with glass micropipettes and fixed with suture on the pipettes, then superfused (extraluminal, 2.0 ml/min) and perfused (intraluminal, 0.25 ml/min) independently with warmed (36°C) Krebs' solution in an organ bath (2 ml capacity). The vessels were confirmed to be free of leaks by the maintenance of high perfusion pressure (>80 mm Hg). Perfusion pressure was monitored upstream of the vessels and set at the desired level by changing the resistance of the outflow tract with a screw that squeezed a silicone tube connected to the pipette. The outer diameter of the vessels was continuously monitored with a video camera system (CCTV Camera Model KP-130, Hitachi, Tokyo, Japan; ×100 magnification).

The vessels were equilibrated for 60 minutes at 5–20 mm Hg perfusion pressure and then pressurized to the desired level. At the beginning of the experiment, vascular reactivity was tested by applying 10⁻⁵ M serotonin (5-HT).

We used Krebs' solution of the following millimolar composition: Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.6, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 137, and glucose 11.5. High-K⁺ solution (129 mM) was made by isotonic substitution of Na⁺ by K⁺. The solutions were continuously aerated with 84% N₂, 10% CO₂, and 6% O₂. The pH of the extraluminal solution was kept between 7.35 and 7.45, and PO₂ was kept between 130 and 145 mm Hg.

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The high-K⁺ solution and drugs were applied on the external surface of the vessels. In preliminary experiments, it was confirmed that vascular responses were almost the same to drugs applied extraluminally or intraluminally and extraluminally. The drugs used were 5-HT (Sigma Chemical Co., St. Louis, Mo.) and BRL 34915 (cromakalim, Beecham Pharmaceutical Co., Harlow, UK).

The results are expressed as median±SD or mean±SD. Statistical significance was tested by the Mann-Whitney test (for those values expressed as a percentage of the control, comparison of medians) and Student’s paired t test; p<0.05 was considered significant.

**Results**

The mean±SD diameter of the superior cerebellar arteries was 240±14 μm (n=16) immediately after vessel cannulation under 5–20 mm Hg perfusion pressure. Perfusion pressure was determined by the resistance that the vessels generated. The arteries developed spontaneous tone during the 60 minutes’ incubation, and vessel diameter was 200±13 μm (n=16) at the same perfusion pressure. After increasing the pressure to 80 mm Hg, vessel diameter was 203±11 mm Hg (n=14).

The arteries were contracted with the high-K⁺ solution at a perfusion pressure of 80 mm Hg, and then cromakalim was applied in a cumulative fashion at concentrations of 10⁻⁸ to 10⁻⁵ M at 5-minute intervals (Figure 1). In the absence of cromakalim, the contractions reached a maximum within 5 minutes and were maintained for up to 35 minutes with a slight decline. At concentrations up to 3×10⁻⁶ M cromakalim had no inhibitory effect on contractions induced by the high-K⁺ solution. However, at a higher concentration (10⁻⁵ M), cromakalim reduced the contractions significantly (control: 75±5%, 10⁻⁵ M cromakalim: 100±8%, p<0.05 between medians, diameter of unstimulated arteries 100%).

Superior cerebellar arteries were contracted with 10⁻⁵ M 5-HT, and the effect of 10⁻⁸ to 3×10⁻⁶ M cromakalim (cumulative application) was tested (Figure 2). Perfusion pressure was kept at 80 mm Hg. In the absence of cromakalim, 5-HT maintained maximal contraction for 30 minutes without a significant decline. However, the 5-HT-induced contraction was reduced by 10⁻⁸ to 3×10⁻⁶ M cromakalim in a concentration-dependent manner.

Superior cerebellar arteries developed myogenic tone during the incubation period. The effect of cromakalim on this tone was examined under 80 mm Hg perfusion pressure (Figure 3). Cromakalim (10⁻⁷ to 10⁻⁵ M) was applied in a cumulative fashion at 5-minute intervals, and concentrations of ≥3×10⁻⁶ M induced concentration-dependent dilation.

When perfusion pressure was elevated in 20-mm Hg steps from 0 (no flow) to 100 mm Hg, superior cerebellar arteries dilated in a pressure-dependent manner.
Cromakalim is a selective and potent potassium channel opener and may be of use in the treatment of hypertension. Our results indicate that cromakalim inhibits not only receptor-mediated contractions, but also myogenic vascular tone and the vascular response to a change in perfusion pressure in rat cerebral arteries. Since cromakalim is lipophilic, it will cross the blood-brain barrier and will reach the vascular smooth muscle and neuronal cells under in vivo conditions.

Cromakalim will be very effective against those contractions mediated by voltage-dependent Ca\(^{2+}\) influx (i.e., that due to membrane depolarization or action potentials) in terms of its mechanism of action. Calcium antagonists are reported to be more effective in cerebral arteries than in systemic arteries, suggesting that voltage-dependent Ca\(^{2+}\) influx plays an important role in contractions of the cerebral arteries. Furthermore, action potentials can be evoked easily in cerebral arteries by electrical stimulation, stretching, or outward current injection. Thus, cromakalim may dilate cerebral arteries preferentially compared with systemic arteries.

On the other hand, receptor-mediated contractions of vascular smooth muscle could be more resistant to cromakalim since these contractions are not necessarily dependent on membrane depolarization (pharmacomechanical coupling). However, beginning at low concentrations, cromakalim inhibited the contractions evoked by 5-HT. One reason for this may be that membrane depolarization plays an important role in addition to a receptor-mediated mechanism (i.e., Ca\(^{2+}\) release from intracellular stores or receptor-operated Ca\(^{2+}\) influx) in 5-HT-induced contractions of cerebral arteries. Indeed, in rabbit basilar artery, 10\(^{-6}\) M 5-HT depolarizes the membrane by approximately 20 mV.

The effects of an antihypertensive agent on cerebral autoregulation are important aspects to be studied. In hypertensive patients or animals, the cerebral autoregulation curve shifts to the right, elevating both the lower and upper limits of autoregulation. Hence, rapid and excessive antihypertensive treatment can cause cerebral hypoperfusion by lowering the blood pressure to below critical levels. Thus, an antihypertensive agent that does not impair cerebral autoregulation will be a better choice for those patients whose cerebral circulation is compromised by events such as transient ischemic attacks or previous strokes. In our experiment, the outer diameter of superior cerebellar arteries remained almost the same in spite of elevations in perfusion pressure between 40 and 100 mm Hg. Similar in vitro observations have been reported in cerebral arteries of rats, cats, and calves, and this property partly accounts for in vivo cerebral autoregulation. Cromakalim impairs this in vitro autoregulatory mechanism in a concentration-dependent manner, suggesting that cromakalim interferes with in vivo cerebral autoregulation. However, whether cromakalim actually impairs cerebral autoregulation must be determined by further in vivo studies.

Myogenic tone is commonly seen in cerebral arteries but is usually absent in mesenteric arteries of...
similar size (80–320 \(\mu\)m) in rats.\textsuperscript{20} Therefore, it is of interest to determine the effects of a vasodilator on myogenic tone in cerebral arteries. Cromakalim at >3\(\times\)10\(^{-6}\) M suppressed the tone and dilated unstimulated arteries in a concentration-dependent manner. Thus, cromakalim inhibited receptor-mediated contractions at low concentrations and myogenic tone at high concentrations.

Potassium channel openers are thought to have no effect on contractions induced by >80 mM K\(^+\) because under these conditions the membrane potential is almost the same as the equilibrium potential for K\(^+\) and because voltage-dependent calcium channels remain open even after the opening of potassium channels.\textsuperscript{21} Cromakalim at up to 3\(\times\)10\(^{-6}\) M did not inhibit contractions induced by 129 mM K\(^+\), suggesting that cromakalim is a relatively specific potassium channel opener. However, 10\(^{-5}\) M cromakalim inhibited contractions induced by 129 mM K\(^+\), suggesting that at high concentrations cromakalim acts through mechanism(s) other than the opening of potassium channels. Although the mechanism remains unknown, cromakalim does not increase the concentrations of either cyclic adenosine monophosphate or cyclic guanosine monophosphate.\textsuperscript{22,23}

In rat mesenteric arteries, action potentials evoked by outward current pulses were inhibited by cromakalim in the absence of membrane hyperpolarization. Such a result implies a calcium-entry-blocking action of the compound.\textsuperscript{24}

In conclusion, cromakalim inhibited contractions evoked by 5-HT and the myogenic response to elevated perfusion pressure in rat cerebral arteries. At high concentrations, cromakalim suppressed the myogenic tone of unstimulated arteries and contractions at high concentrations of K\(^+\). This latter effect may be caused by mechanisms other than the opening of potassium channels.

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References


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