Inward rectifier potassium channels in the rat middle cerebral artery

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Physiol. 43): R541–R547, 1998.—Inward rectifier K⁺ increases in K₀, which neurons communicate with adjacent vascular tissue. These neurons may be a physiologically relevant mechanism by which neurons communicate with adjacent vascular tissue. 

Kᵢₛ are characterized as voltage-gated K⁺ channels that close with depolarization and have open-state probabilities increased with increased K⁺ (7,8). The open-state probabilities may be altered with pressure. 

METHODS

Animals and harvesting arteries. The experimental protocol was approved by the Animal Protocol Review Committee at Baylor College of Medicine. Male Long-Evans rats weighing between 275 and 350 g were anesthetized with isoflurane. After loss of the righting reflex, each rat was decapitated, and the brain was removed from the cranium and placed in cold physiological salt solution (see below for composition). The left and right MCAs were carefully removed beginning at the circle of Willis and continuing distally for ~5-6 mm. 

Mounting arteries. Arteries were placed in an arteriograph (Living Systems, Burlington, VT), and a micropipette was inserted into the proximal end of each artery and secured with a 10-0 nylon suture. The lumen was gently perfused with physiological salt solution to remove blood and other contents and the distal end was cannulated with a second glass micropipette and secured. A segment of each artery ~1 mm in length and lying between branch points was positioned in vitro; dilation; vascular smooth muscle
between the tips of the two micropipettes. Each artery was bathed in physiological salt solution that was continually circulated from a reservoir in which it was equilibrated with a gas consisting of 20% O₂-5% CO₂-balance N₂. The physiological salt solution in the bath was maintained at 37°C using a circulating water bath and heat-exchange column (2).

Luminal or transmural pressure was created by raising reservoirs, connected to the micropipettes by Tygon tubing, to the appropriate height above each artery. Transmural pressure was adjusted to 40, 85, or 100 mmHg, depending on the protocol used. Luminal perfusion was adjusted to 100 µl/min by setting the inflow and outflow reservoirs at different heights. Pressure transducers between the micropipettes and the reservoirs provided a measure of perfusion pressure. A flowmeter (model 11, Gilmont Instruments, Barrington, IL), connected to the tubing leading to the output reservoir, measured luminal flow. The luminal perfusate was gassed in the input reservoir. In addition, the luminal perfusate traveled through gas-permeable Silastic tubing in the bath before perfusing the lumen of each artery to ensure that it was properly gassed and equilibrated to 37°C. Samples of the physiological salt solution were analyzed for PO₂, Pco₂, and pH using a Corning model 280 analyzer (Medfield, MA).

The arteries were allowed at least 1 h to stabilize before any experiments were conducted. During this time period, the diameters decreased to ~75% of the initial diameter after pressurization. This development of spontaneous tone was indicative of a viable artery.

General experimental outline and design. For KCl concentration-effect experiments on the rat MCA, KCl was added to the extraluminal bath (which already contained 4.7 mM KCl). Each KCl concentration was allowed to equilibrate for 5 min, and the diameter was measured before the next concentration was added. In subsequent experiments, only one concentration of KCl (15 mM hypertonic) was added extraluminally to induce the response (final KCl concn 19.7 mM). This concentration was allowed to equilibrate for 5 min before a diameter measurement was recorded. Unless stated otherwise, all experiments were done at 85 mmHg. For other experiments, tissues were randomly pressurized to 40, 85, or 100 mmHg. In some studies (described in Fig. 2), repeated KCl-induced dilations were conducted on a single vessel. After an initial control response to the addition of 15 mM KCl, the MCA was washed with fresh physiological salt solution and allowed 10 min to stabilize, and a second KCl response was conducted in the presence of a pharmacological agent or after removal of the endothelium. To control for these multiple measurements on a single MCA, two KCl-induced dilations were conducted on a single vessel without the addition of any drug or manipulation.

In some studies, KCl was added to the luminal perfusate instead of the extraluminal bath to observe its effects on the endothelium (intact vessels) or smooth muscle (denuded vessels). The endothelium was removed in some experiments by passing 15 ml of air through the lumen of the MCAs 30–45 min before the addition of KCl (9). Removal of the endothelium was confirmed using ATP, a purinoreceptor agonist that requires intact endothelium for dilation (31).

Reagents and drugs. BaCl₂, CsCl, 4-aminopyridine (4-AP), tetraethylammonium (TEA), ouabain, glibenclamide, and N⁶-nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma (St. Louis, MO).

The physiological salt solution consisted of the following (27): 119 mM NaCl, 24 mM NaHCO₃, 4.7 mM KCl, 1.18 mM KH₂PO₄, 1.17 mM MgSO₄, 1.6 mM CaCl₂, 5.5 mM glucose, and 0.026 mM EDTA.

Statistics. Data are expressed as means ± SE. Paired Student’s t-tests were used for comparison of groups in studies shown in Figs. 2 and 5B. Repeated-measures analysis of variance with a post hoc Student-Newman-Keuls test, when appropriate, was used for all other studies. The acceptable level of significance was defined as P < 0.05.

RESULTS

Are Kᵢ₅s on rat MCA? After the development of spontaneous tone, the MCA diameter in the first study was 231 ± 14 µm (n = 7). Figure 1 shows a concentration-effect curve to the addition of KCl to the extraluminal bath in the MCAs. Increasing the concentration of KCl dilated the MCAs in a concentration-dependent manner up to the concentration of 15 mM KCl. For the study shown in Fig. 1, the MCA dilated 21.6% (P < 0.001, n = 7) after the addition of 15 mM KCl (from 231 ± 14 to 281 ± 16 µm).

Figure 2 shows responses to various K⁺ channel inhibitors on the dilation induced by the addition of 15 mM KCl. Time controls demonstrated that a second dilation produced by KCl was very similar to the first. Thus each MCA could act as its own control, where the first dilation induced by KCl was control and the second experimental. The ATP-sensitive K⁺ channel antagonist, glibenclamide (10 µM), and the concentration-selective Ca²⁺-sensitive K⁺ channel antagonist, TEA (1 mM), had no significant effects on the KCl-induced dilations. The selective antagonist for delayed-rectifier K⁺ channels, 4-AP (1 mM), significantly potentiated the response (30 vs. 22.6%, n = 6, P < 0.001). BaCl₂ (40 µM), the concentration-selective inhibitor of Kᵢ₅s,
significantly attenuated the KCl-induced dilation by 86% (n = 8, P < 0.001). Figure 2 also shows that dilations produced by the addition of 15 mM KCl were not significantly affected by inhibition of the Na+-K+-adenosinetriphosphatase (ATPase) pump with ouabain (1 mM).

Figure 3 shows cumulative concentration response curves to BaCl2 on tissues that were dilated with 15 mM KCl as previously described. All experiments were done in the presence of 4-AP (1 mM) to selectively eliminate the delayed-rectifier K+ channels component of voltage-mediated K+ fluxes during membrane potential changes. The addition of KCl to the extraluminal bath produced a dilation of 32 ± 3% (P < 0.001, from 203 ± 12 to 268 ± 15 µm, n = 7). BaCl2 significantly inhibited the KCl-induced dilation in a dose-dependent manner (Fig. 3). At 60 µM BaCl2, the MCA diameter had returned to the original baseline before the addition of KCl; i.e., the dilation had been completely inhibited (P < 0.001). Higher BaCl2 concentrations (80–160 µM) constricted the vessels below the initial resting basal diameter (pre-KCl-induced dilation). The maximal constriction (12 ± 3% below original baseline, n = 7) occurred at 120 µM BaCl2 (P < 0.001, compared with initial resting basal diameter). All changes due to BaCl2 could be reversed by washing the tissue with fresh physiological salt solution.

The physiological salt solution was kept isotonic by reducing the concentration of NaCl to offset the increase in CsCl concentration. CsCl inhibited the dilation in a dose-dependent manner. As with BaCl2, a constriction was observed at the highest CsCl dose (50 mM). CsCl concentrations >50 mM were not used because of precipitate formation in the physiological salt solution. In tissues already exposed to 50 mM CsCl, addition of 120 µM BaCl2 produced a further constriction not different from that with BaCl2 alone (12 ± 3 vs. 16 ± 2%, Figs. 3 and 4B, respectively).
Do dilations produced by activation of Kir's on rat MCA involve endothelium? The addition of 15 mM KCl to the luminal perfusate in intact vessels (with endothelium) did not dilate MCAs as it did when added to the extraluminal bath (Fig. 5A). KCl in the luminal perfusate was in direct contact with the endothelium and was denied access to the vascular smooth muscle by the endothelial barrier (tight junctions). However, when the endothelium was removed, the luminal perfusate had direct access to the vascular smooth muscle. In this latter case, the addition of KCl to the lumen dilated the MCAs (Fig. 5A).

Not only did removal of the endothelium allow dilations to occur when luminal KCl was increased, but it did not affect the dilations produced by the addition of 15 mM KCl to the extraluminal bath (Fig. 5B). Furthermore, the addition of L-NAME (10 µM), an inhibitor of nitric oxide synthase, to the luminal perfusate and extraluminal bath in sufficient concentrations to block nitric oxide production by the endothelium (31) did not affect the dilation to the addition of 15 mM KCl (Fig. 5B).

Do Kir's contribute to resting tone of rat MCA? Figure 6 shows the results of increasing concentrations of BaCl2 to the resting diameter of the MCA. All concentrations of BaCl2 (20–160 µM) produced a significant decrease in vessel diameter. The maximal constriction (8%) occurred at 120 µM BaCl2 (P < 0.001, from resting diameter of 208 ± 7 to 191 ± 19 µm, n = 6). There was no significant difference between the maximal constrictions produced by BaCl2 in MCAs diluted with KCl (Figs. 3 and 4) vs. MCAs at resting tone (nondilated, Fig. 6). The constrictions produced by BaCl2 were not affected after blocking Ca2+-sensitive, ATP-sensitive, or delayed-rectifier K+ channels with TEA (1 mM, n = 8), glibenclamide (10 µM, n = 7), or 4-AP (1 mM, n = 7), respectively (data not shown).

Is Kir function altered with changes in transmural pressure? MCAs were pressurized to 40, 85, and 100 mmHg in a random order. With increased pressures, there was a significant decrease in diameter. At 40, 85, and 100 mmHg, the mean diameters were 210 ± 8, 198 ± 6, and 188 ± 6 µm, respectively. There were no significant differences in the dilations produced by the addition of 15 mM KCl to the extraluminal bath at 40 (20 ± 2%, n = 5), 85 (21 ± 4%, n = 5), or 100 mmHg (22 ± 3%, n = 5) (Fig. 7, top). In the same MCAs, BaCl2 (120 µM)-induced constrictions were compared at the different pressures (Fig. 7, bottom). There were no significant differences between constrictions at 40 (8 ± 2%, n = 5), 85 (6.5 ± 5%, n = 5), or 100 mmHg (5.5 ± 3%, n = 5).

DISCUSSION

We report four findings regarding Kir's in the cerebral circulation. 1) Kir's are located on the MCA of the rat and are associated with dilation when activated. 2) Dilations produced by activation of the Kir's involve only the vascular smooth muscle. There is no involvement of the endothelium in the dilation. 3) Kir's contribute to the resting tone of the rat MCA. 4) The Kir function is independent of pressure in the rat MCA.

Kir's are located on MCA of the rat and are associated with dilation when activated. One of the characteristics of Kir's is that their open-state probability increases with increased extracellular K+ (16, 27). It therefore follows that the addition of KCl (10–15 mM) to the bath would open Kir's present on MCAs. Indeed, we found that MCAs diluted in a concentration-dependent manner with the addition of KCl to the extracellular bath (Fig. 1). Furthermore, these dilations were not affected...
We conclude that the endothelium is not involved with the KCl-induced dilations in the rat MCA. In MCAs in which the endothelium had been removed, dilations produced by the extraluminal application of KCl were no different from control MCAs with intact endothelium (Fig. 5B). Second, the direct application of KCl to the endothelial surface (luminal administration) did not dilate the MCAs (Fig. 5A). However, after removal of the endothelium, the luminal administration of KCl did dilate the MCAs as did the extraluminal application (Fig. 5A). These results conclusively demonstrate that the endothelium was not involved in the response to increased KCl.

Kir's contribute to resting tone of rat MCA. BaCl_2 constricted MCAs at resting tone (nondilated, Fig. 6), suggesting that some Kir's are already open during resting conditions. This constriction in response to BaCl_2 appears to be due to the selective closure of Kir's, since the response was not affected by blocking of other K^+ channels (ATP-sensitive, Ca^{2+}-sensitive, and delayed-rectifier K^+ channels). The open Kir's in MCAs maintained the resting diameter 8–12% more dilated than if these channels were closed or not present (Fig. 6). Support for this notion comes from in vitro electrophysiological studies in branches of the rat MCA (7). Although contractile state of the MCA branches was not measured in these studies, Edwards et al. (7) demonstrated that BaCl_2 shifted the resting membrane potential toward depolarization. We propose that Kir's that are open during the resting condition maintain cerebral vessels at a more hyperpolarized state; this more hyperpolarized state renders the arteries in a more dilated state. Thus the Kir's contribute to the resting tone of the cerebral arteries.

Kir function is independent of pressure in rat MCA. In many arteries, including the rat MCA, the diameter is inversely related to the transmural pressure; i.e., the artery constricts when the pressure is increased (27). This phenomenon is referred to as the myogenic response (13). The mechanism involves a complicated signal transduction, which ultimately depolarizes and constricts the vascular smooth muscle (10, 11).

Given that Kir's are voltage sensitive and close with depolarization, increasing transmural pressure (inducing depolarization of vascular smooth muscle) might lead to closure of Kir's. Thus the ratio of open to closed Kir channels could be a function of the transmural pressure of the MCA. We used KCl-induced dilations as a means of opening closed Kir's and thus a measure of dilating capacity by opening Kir channels. Alternatively, we used BaCl_2-induced constrictions in resting MCAs to close those Kir channels that were open.

In MCAs, the resting diameter was inversely related to the transmural pressure; the diameters were 210, 198, and 188 µm (P < 0.05) at 40, 85, and 100 mmHg, respectively. The transmural pressure, and presumably the membrane potential, had no significant effects on dilations in response to the addition of 15 mM KCl or constrictions in response to BaCl_2. At first glance, our results appear to indicate that the Kir's are not sensitive to changes in membrane potential in the MCA. How-
ever, over the range of pressures in our study, the membrane potential may not exceed the working range of channel function (12). In addition, the gating properties of the Kir channels are modulated by intracellular polyamines (14, 26). The general understanding is that decreased polyamine levels result in a greater open probability of these channels at depolarized conditions. If endogenous polyamine concentrations, primarily spermine, were to decrease with increased pressure or depolarization, then open probability for the Kir channels would be expected to shift to more positive potentials (21). Regardless, it is concluded from our results that Kir channels are fully functional between 40 and 100 mmHg, a pressure range that brackets normal physiological pressures.

During pathological conditions such as hypertension, in which vessels would be exposed to elevated pressure (>120 mmHg), the membrane potential might become more positive and exceed the range for the open probability of Kir channels. The net result might be inactivation or reduction of function of these channels. Consistent with this idea, McCarron and Halpern (22) demonstrated that Kir function in posterior cerebral arteries is abolished in chronically hypertensive rats. Furthermore, Harder et al. (12) reported that vascular smooth muscle cells in MCA s isolated from chronically hypertensive rats were more depolarized for a given pressure than the same arteries isolated from normotensive rats.

In summary, we find that Kir channels are located on the vascular smooth muscle of the rat MCA similar to the posterior cerebral artery. When activated, these Kir channels produce a dilation of the artery and are therefore probably an important control mechanism for regulation of cerebral blood flow. These channels are functional over a wide pressure range, allowing for tight regulation during different physiological conditions. Finally, during resting conditions, there are Kir channels that can be opened to increase flow or closed to decrease flow. Thus Kir channels are important in setting the resting tone of the MCA.

Perspectives

For the past 25–30 years, it has been thought that increased Kir densities due to increased activity of the tissue (e.g., contractile activity in skeletal muscle or neuronal activity in brain) is a mechanism for dilating vessels and increasing blood flow (3, 5, 18, 19). In the brain, Kir increases to ~12 mM when neurons are activated (30). Local increases in Kir in the brain can either diffuse to nearby arteries and arterioles or can be aided by astrocytic glia through a proposed mechanism termed K⁺ siphoning (25, 29). This latter mechanism depends on the uptake of Kir in the extracellular fluid by astrocytes (spatial buffering) and the subsequent release of K⁺ at the astrocytic endfeet surrounding cerebral vessels. K⁺ siphoning can raise the Kir concentration at the resistance arteries quicker and to a higher level than simple diffusion. Although Kir appeared to be a link between function and flow, the mechanism as to how K⁺ dilated arteries and arterioles in selective vascular beds remained elusive for a number of years. With the recent identification of Kir channels and their existence on selected vascular smooth muscle cells, a possible mechanism has been discovered.

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