ATP-sensitive potassium channels may participate in the coupling of neuronal activity and cerebrovascular tone

THIEN-SON NGUYEN, H. RICHARD WINN, AND DAMIR JANIGRO

ATP-sensitive potassium channels may participate in the coupling of neuronal activity and cerebrovascular tone. Am. J. Physiol. Heart Circ. Physiol. 278: H878–H885, 2000.—K+ dilate and constrict cerebral vessels in a dose-dependent fashion. Modest elevations of abluminal K+ cause vasodilatation, whereas larger extracellular K+ concentration (\([K^+]_o\)) changes decrease cerebral blood flow. These dilations are believed to be mediated by opening of inward-rectifier potassium channels sensitive to Ba2+. Because BaCl2 also blocks ATP-sensitive K+ channels (KATP), we challenged K+ dilations in penetrating, resistance-size (<60 μm) rat neocortical vessels with the KATP channel blocker glibenclamide (1 μM). Glibenclamide reduced K+ responses from 138 ± 8 to 110 ± 0.8%. K+ constrictions were not affected by glibenclamide. The Na+\(\cdot\)K+\(\cdot\)pump inhibitor ouabain (200 μM) did not significantly change resting vessel diameter but decreased K+ dilations (from 153 ± 9 to 99 ± 2%). BaCl2 blocked K+ dilations with a half-maximal dissociation constant of 2.9 μM and reduced dilations to the specific KATP agonist pinacidil with equal potency. We conclude that, in resistance vessels, K+ dilations are mediated by KATP; we hypothesize that [K+]o causes activation of Na+\(\cdot\)K+ pumps, depletion of intracellular ATP concentration, and subsequent opening of KATP. This latter hypothesis is supported by the blocking effect of ouabain.

Cerebral blood flow; vascular smooth muscle; neuroimaging; endothelium; inward rectifier

PROXIMITY OF SITES of neurotransmitter release and neuronal electrical activity to the cerebrovasculature facilitates coupling of neuronal activity and cerebral blood flow (CBF). This communication depends on neuronal signals released synaptically [e.g., nitric oxide (14)] or directly linked to electric current flow through excitable membranes [e.g., K+ lost during action potential repolarization (23, 31)]. Because neuronal activity and synaptic transmission also cause immediate changes in tissue energy balance, coupling between CBF and neuronal activity may also depend on substances released in the abluminal space during periods of increased metabolic demand; the ATP-derived neurotransmitter and vasodilator adenosine has thus been referred to as a “metabolic” regulator of CBF (39). The resulting interactions of metabolic, synaptic, and ionic signals ensure local and dynamic regulation of CBF.

The anatomic relationship between cortical blood vessels and neurons suggests that small caliber arteries are the target for neuronal-vascular interactions. Because of technical limitations, however, studies of cerebrovascular regulation have been limited to observations on large-diameter or superficial vessels (>100 μm (8, 30)). Results obtained from these vessels suggested that K+‐mediated dilations are mediated by voltage-dependent, Ba2+-sensitive inward rectifier channels [KIR (30–32)]. Because Ba2+ also blocks metabolically regulated, ATP-sensitive channels expressed in vascular smooth muscle (VSM; see Ref. 31), we investigated the possible involvement of these ion channel mechanisms in K+‐mediated dilations.

In addition to standard patch-clamp recording and cell isolation methodology, we took advantage of the technique developed originally by Dacey and Duling (4) to obtain resistance-size arterioles from rat neocortex; these vessels are responsible for the transduction of neuronal signals into vascular changes and thus regulate metabolic supply and oxygenation of deep cortical layers. We have previously shown that these vessels express ATP-sensitive K+ (KATP) channels and respond to drastic intracellular ATP concentration ([ATP]) changes (in vitro ischemia) by activation of glibenclamide‐inhibitable dilations (17). It remains to be elucidated whether more physiological stimuli, such as modest elevation of parenchymal K+ comparable to those occurring during cortical activation (36), can cause similar coupling of metabolic changes to activation of K+ channels. Interestingly, McCarron and Halpern (26) reported two distinct mechanisms of cerebrovascular K+ dilations, one sensitive to ouabain, and presumably involving Na+\(\cdot\)K+\(\cdot\)ATPase activation by extracellular K+ concentration ([K+]o), and one with a Ba2+-sensitive component mediated by ion channels. It was concluded that a synergistic mechanism involving activation of pump activity and ion channels underlies K+‐induced dilations. However, a direct link between pump activity and K+ channels has only recently been demonstrated in cardiac and renal cells (20, 37).

The main goal of our study was to elucidate the mechanisms responsible for K+ dilations of resistance-size cerebral vessels. We tested the hypothesis that both KIR and KATP-dependent pathways are involved and that different VSM muscle ion channels mediate...
the dilations in response to prolonged or transient neuronal activity.

**METHOD**

Methodology for the in vitro isolation and cannulation of rat cerebral arterioles has been described in publications from this laboratory (17, 28, 29). Sprague-Dawley rats (300 g) were anesthetized with pentobarbital sodium (50 mg/kg ip) and decapitated. The brain was rapidly removed from the skull and immersed in cooled buffered saline solution (4°C) containing 1% dialyzed BSA and the following (in mM): 144 NaCl, 3.0 KCl, 2.5 CaCl2, 1.5 MgSO4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, 2.0 MOPS, and 1.21 NaH2PO4. A section of cerebral cortex ~2 mm thick and containing the first portion of the middle cerebral artery was dissected from the brain. The pia mater and its attached penetrating intracerebral arterioles were separated from the parenchyma, and an unbranched distal segment of a vessel, −0.5 mm in length, was severed from the pia. The vessel segment was then transferred to a temperature-controlled chamber (3.0 ml volume) mounted on the stage of an inverted microscope (Nikon). The isolated vessel was cannulated using a system of concentric glass pipettes (4) consisting of a perfusion pipette within a holding pipette. The pipettes were inserted into Plexiglas holders (White Instruments, Sultland, MD), mounted on micromanipulators, and attached to the microscope stage. Before the development of physiological tone, “passive” vessel diameter was measured. The bath solution was then changed to one without albumin, and bath temperature was raised to 37°C. The rate of intraluminal perfusion (4 µl/min) was chosen to avoid flow-mediated effects on vessel diameter (29). The extraluminal bath medium was continuously circulated with a roller pump at 1 ml/min. After an equilibration period of ~30 min, viable arterioles develop vasomotion and contract spontaneously. The vessels must constrict to <70% of the passive diameter to be usable. All drugs, including K+ (as KCl, isomolar substitution for NaCl), were applied extra- laminally. Glibenclamide was initially dissolved in DMSO and then was added to the perfusate.

VSM cells were isolated from the rat basilar artery as follows. Male Sprague-Dawley rats were killed, and the brain was removed. The basilar arteries were dissected in ice-cold, low-Ca2+/balanced salt solution (BSS). The BSS contained (in mM) 134 NaCl, 5.2 KCl, 1.2 MgSO4, 0.05 CaCl2, 10 HEPES, 11 glucose, 0.33 NaH2PO4, 4 dithiothreitol, and 0.06 papaverine hydrochloride, and 0.01% fatty acid-free BSA, pH 7.35. The enzymatic digestion protocol for the cerebral arteries was as follows. Vessels were transferred to a 15-ml centrifuge tube, and the following were added in 1 ml of BSS: collage-nase (type II, 2 mg/ml, 159 U/mg; Worthington), elastase (porcine pancreas, 0.5 mg/ml, 4.8 U/mg; Worthington), and soybean trypsin inhibitor (type I-S, 1 mg/ml; Sigma). The vessel was then incubated at 36°C on an orbital shaker (45 rpm) for 30 min. The enzyme solution was replaced with a new enzyme solution (0.4 mg/ml BSA, 0.4 mg/ml trypsin inhibitor, and 0.4 mg/ml protease), and incubation continued for another 10 min. With the use of an Eppendorf pipette with the tip cut to 2 mm in diameter, the vessel was then transferred to ice-cold BSS (1 ml, in microfuge tube) and mechanically dissociated to release VSM cells. After a wash (200 g), the cell pellet was resuspended in BSS and Ca2+ raised slowly by dilution with BSS containing 1.6 mM Ca2+ to a final Ca2+ concentration of 0.2 mM. Cells are normally refrigerated until use.

Patch-clamp recordings were performed as described previously by us for endothelial cells (16, 18) or following procedures specific for VSM (3, 30, 31). VSM cells were bathed in artificial cerebrospinal fluid (aCSF) composed of (in mM) 120 NaCl, 3.1 KCl, 1 MgCl2, 2 CaCl2, 5 MOPS, 26 NaHCO3, and 10 dextrose. Experiments were performed at room temperature (24–26°C). Experiments with high K+ were performed by adding potassium gluconate to aCSF; an isomolar concentration of NaCl was removed to maintain osmolarity. Patch-clamp recordings were obtained using an Axopatch 1C ampli- fier (Axon Instruments, Foster City, CA) in voltage- or current-clamp mode. Whole cell recordings were obtained with pipettes filled with (in mM) 140 potassium gluconate, 1 MgCl2, 2 Na2ATP, 0.3 NaGTP, 10 HEPES, and 0.5 EGTA, final pH of 7.2 (with NaOH). Pipettes had a resistance of 5–10 MΩ. Series resistance was monitored throughout the experiment and was usually around 15–30 MΩ. Series resistance compensation was routinely performed up to 70–80% (lag time 10 µs). Recordings were digitized at 48 kHz, filtered at 2–10 kHz, displayed on an oscilloscope, recorded on tape, and acquired on a Pentium 266 computer by pCLAMP6 (Axon Instruments). VSM were selected for recording under visual control with a Nikon microscope equipped with Hoffman optics at ×400 magnification. Cell membrane potential was corrected for the tip potential determined upon withdrawal of the pipette from the cell.

**RESULTS**

The vessels used for our experiments had a mean diameter of 48 ± 2.1 µm (range 45–66 µm). Isolated penetrating pial vessels developed spontaneous tone when cannulated and perfused intraluminally (4, 17). As shown in Fig. 1A, vessel diameter was measured approximately halfway through the length of the cannulated vessel, and these determinations were then repeated at the desired intervals at the same cursor location. Similar to in vivo arterioles, these vessels readily respond to pH and various vasodilators. Vessels exposed abluminally to modest K+ increases dilated promptly and reversibly (Fig. 1). Cerebrovascular responses to elevated K+ were characterized by steep concentration dependency, and small K+ elevations (5–10 mM) caused dilation while, at K+ = 47 mM, a statistically significant constriction occurred. K+-induced dilations were persistent in nature and lasted for the entire duration of K+ application (Fig. 1C). These results are in agreement with previous findings obtained from larger cortical vessels (21).

Consistent with the persistent nature of K+ dilations, sudden diameter increases elicited by 5 mM K+ were followed by a plateau. Both instantaneous and sustained dilations to K+ were greatly attenuated by low concentrations of the K+ channel blocker Ba2+ (Fig. 1C); Ba2+ itself, at concentrations of <100 µM, had little or no effect (Fig. 1D). At concentrations >100 µM, Ba2+ caused constriction.

Similar to Ba2+, the KATP channel blocker glibenclamide also dramatically reduced dilations induced by small elevations of K+ (5 mM above baseline; Fig. 2A); at the concentration used (1 µM), the effects of glibenclamide are specific for blockade of KATP (31). In agreement with data by others (26), dilations induced by larger (10 mM) elevations of extracellular K+ were insensitive to glibenclamide (114.4 ± 2.7 vs. 110.04 ± 0.81 with 1 µM glibenclamide; n = 6), suggesting that KATP involve-
ment is significant only for dilations induced by small increases in [K\textsuperscript{+}]\textsubscript{out}.

The effects of glibenclamide on the time course of K\textsuperscript{+}-induced dilation differed, however, from the effects of the mixed K\textsubscript{ATP}-K\textsubscript{IR} blocker Ba\textsubscript{2+}, because this specific K\textsubscript{ATP} channel blocker completely prevented steady-state dilations to [K\textsuperscript{+}]\textsubscript{out} but had little effect on the early dilatatory response (compare Fig. 2B with Fig. 1C). These results suggested that two separate ion channel mechanisms are responsible for the vasodilatory response to K\textsuperscript{+}. Because of its sensitivity to Ba\textsubscript{2+} and resistance to glibenclamide, the transient response appeared to be mediated almost exclusively by K\textsubscript{IR}, whereas glibenclamide blockade of the delayed response was consistent with involvement of K\textsubscript{ATP}.

We further tested whether the effects of Ba\textsubscript{2+} could be consistent with blockade of K\textsubscript{ATP} channels rather than an exclusive action on K\textsubscript{IR}. To this end, vasodilations induced by the K\textsubscript{ATP} agonist pinacidil (10 µM) were challenged with the same range of Ba\textsubscript{2+} concentrations that abolished steady-state K\textsuperscript{+} dilations. The profound inhibition of K\textsuperscript{+} dilations by Ba\textsubscript{2+} was paralleled by Ba\textsubscript{2+} potency to block pinacidil-induced, K\textsubscript{ATP}-mediated responses, demonstrating that Ba\textsubscript{2+} actions on the plateau response to K\textsuperscript{+} were consistent with an effect on K\textsubscript{ATP} channels.

Under physiological conditions, K\textsubscript{ATP} channels are tonically inhibited by intracellular ATP (18, 24). However, even modest decreases in the ATP to ADP ratio cause opening of K\textsubscript{ATP} (13). Tonic inhibition of K\textsubscript{ATP} in resting vessels was confirmed in this and other studies, and glibenclamide had little or no effect on vessel diameter at physiological K\textsuperscript{+} concentrations (e.g., Fig. 2A; see also Refs. 6, 8, 17, 31). However, previous results have shown that reduction of ATP by metabolic poisoning with cyanide promptly results in robust dilations sensitive to 1 µM glibenclamide and thus mediated by K\textsubscript{ATP} (17). Because high abluminal K\textsuperscript{+} and metabolic poisoning both cause comparable and glibenclamide-inhibitable opening of K\textsubscript{ATP}, what could constitute the link between elevated K\textsuperscript{+} and reduction of intracellular ATP sufficient to open K\textsubscript{ATP}?
In addition to effects on ion channel gating (21, 30), extracellular K⁺ acts as a powerful activator of Na⁺-K⁺-ATPase (7, 34). Because of the inherent dependency of the Na⁺-K⁺ pump on ATP hydrolysis, we speculated that extracellular K⁺-induced activation of the ATPase may have caused sufficient change in the ATP-to-ADP ratio to open K<sub>ATP</sub>. A prediction of this hypothesis is that blockade of the pump concomitant to application of high K⁺ may result in preservation of intracellular ATP, thus preventing K<sub>ATP</sub>-mediated vasodilations. This was directly tested by perfusion of the vessels with the pump inhibitor ouabain (0.2 mM) before and during application of K⁺ (Fig. 3A). Ouabain had per se little effect on vessel diameter but fully prevented the steady-state vasodilations induced by elevation of [K⁺]<sub>out</sub>. To assess the specificity of ouabain’s effects, and to rule out a direct effect on permeation through K<sub>ATP</sub>, vessels were exposed to pinacidil, and pinacidil-induced dilations were challenged with the same concentration of the Na⁺-K⁺-ATPase blocker (Fig. 3B). Although ouabain
fully prevented K\(^+\) dilations, responses elicited by the K\(_{\text{ATP}}\) opener pinacidil were unaffected.

Further direct evidence linking the actions of extracellular K\(^+\) to opening of K\(_{\text{ATP}}\) was obtained by patch-clamp experiments. These experiments were performed on VSM cells isolated from the basilar artery or from penetrating pial vessels (n = 16 and 3, respectively; since the results obtained were identical, findings from these two populations are pooled together). These cells express K\(_{\text{ATP}}\) and could therefore be used to determine physiological coupling between K\(_{\text{ATP}}\) channels and ATP hydrolysis induced by activation of the Na\(^+-\)K\(^+-\)ATPase. As shown in Fig. 4A, VSM from the basilar artery responded to application of elevated K\(^+\) (from 3 to 8 mM) with membrane hyperpolarization from cell resting potential (−43.5 ± 1.07 mV, n = 14). Furthermore, these membrane responses were attributable to opening of K\(_{\text{ATP}}\) since, at concentrations specific for blockade of these channels, glibenclamide greatly attenuated the membrane changes induced by elevated K\(^+\). Further increase in the concentration of glibenclamide (from 1 to 10 µM) did not significantly increase its blocking actions, suggesting that saturation of the effects was achieved at a concentration highly selective for K\(_{\text{ATP}}\). For these experiments, the whole cell recording pipette contained 3 mM ATP, a concentration that causes complete inhibition of K\(_{\text{ATP}}\). This was supported by the fact that glibenclamide per se did not cause any appreciable change in cell resting potential (n = 6).

When similar patch-clamp experiments were performed with 0 mM ATP in the pipette, recordings from basilar artery VSM yielded resting potential values that were significantly more negative than those recorded with 3 mM [ATP]\(_i\) (−64.5 mV, n = 4; Fig. 4C). This suggested that, under these recording conditions, increased conductance through K\(_{\text{ATP}}\) occurred. To test this hypothesis, cells were exposed to 1 µM glibenclamide. In contrast to what was observed in ATP-dialyzed cells, cells recorded with pipettes containing 0 mM ATP depolarized after exposure to the K\(_{\text{ATP}}\) blocker (+20 ± 5.2 mV on average, n = 4). If intracellular dialysis with 0 mM ATP promotes opening of K\(_{\text{ATP}}\) and if K\(^+\)-mediated hyperpolarizations are due to opening of previously inhibited channels, then exposure of these cells to increased K\(^+\) should not cause a significant change in resting membrane potential. This was di-

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**Fig. 4.** Patch-clamp recordings from VSM isolated from the basilar artery reveal K\(_{\text{ATP}}\)-mediated dilations to [K\(^+\)]\(_o\). A: recordings performed with 2 mM ATP in the pipette, standard intracellular solution, revealed small hyperpolarizations induced by addition of K\(^+\) (5 mM above baseline, to a final concentration of 8 mM). These responses were blocked or reduced by glibenclamide (1 µM) applied for >5 min before subsequent challenge with high [K\(^+\)]\(_o\). Glibenclamide per se had no effect on cell resting potential, in agreement with the hypothesis that K\(_{\text{ATP}}\) channels are tonically inhibited under these recording conditions. B: removal of ATP from the pipette solution caused disappearance of K\(^+\)-mediated responses but revealed large depolarizations induced by glibenclamide. Because resting membrane potential in these cells was significantly more negative than in cells dialyzed with 2 mM ATP (C), positive current was injected to offset this change in passive properties. Cumulative results of these experiments are shown in C. ATP, intracellular ATP. *P < 0.04.
rectly tested in three cells, and, as expected, addition of 
K\textsuperscript{+} to the recording medium did not cause any appreciable change of membrane potential. This was not solely due to the fact that these cells were more hyperpolarized than their ATP-containing correlates, since no response to elevated K\textsuperscript{+} could be recorded even at depolarized potentials (Fig. 4B).

### DISCUSSION

New concepts have emerged to explain neuronal regulation of CBF, but the original theory of metabolic regulation (33) is still experimentally supported. CBF and neuronal activity can be coupled by metabolic by-products (i.e., adenosine) with vasoactive properties; alternatively, K\textsuperscript{+} dissipated by neuronal firing may act as a CBF regulator. On the basis of present knowledge, K\textsuperscript{+}-mediated vasodilations occur exclusively by way of voltage-dependent K\textsubscript{IR} channels and are thus largely independent from metabolic changes. We report that, in small, resistance-size cortical arterioles and in the basilar artery, K\textsuperscript{+} dilations are also mediated by a mechanism linking K\textsubscript{ATP} channels to Na\textsuperscript{+}-K\textsuperscript{+}-pump activity. These results provide an additional mechanism for metabolic coupling of CBF to neuronal activity.

The coupling of brain cell function to the vascular system is the basis for a number of functional neuroimaging methods relevant for human studies. These methods map a specific localized brain activation through a vascular response, such as an increase in CBF or a change in blood oxygenation (38). Studies of the close interplay between neuronal activity and CBF have thus transcended basic science boundaries and have rapidly expanded into the field of clinical assessment of cerebral function. The exact mechanisms by which central nervous system neurons sense and regulate CBF have been elusive so far, but mechanisms involving K\textsuperscript{+} channels expressed in cerebral vasculature have recently received increasing attention (9, 22, 31). At least three temporally related events accom-

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**Fig. 5. Events underlying K\textsuperscript{+}-induced dilations in cerebral vessels and their relationship to neuronal activity.**

A: under physiological conditions, K\textsubscript{ATP} channels are inhibited by intracellular ATP. This is possible because metabolic demand is matched and is due to the low level of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity. Even small increases in extracellular K\textsuperscript{+} cause a significant activation of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, leading to increased hydrolysis of intracellular ATP. Although the decrease of [ATP] may not be sufficient to relieve the blockade of the channel, rising concentrations of intracellular ADP are sufficient to induce K\textsubscript{ATP} channel opening, leading to hyperpolarization and vasodilation. B: synergistic contribution of metabolic (K\textsubscript{ATP}) and purely voltage-dependent (K\textsubscript{IR}) K\textsuperscript{+} channels to the coupling of neuronal activity to cerebral blood flow. At low levels of above-baseline activity (and subsequent low increase in [K\textsuperscript{+}]\textsubscript{out}), transient responses are mediated predominantly by K\textsubscript{IR} channels, whereas sustained dilations are maintained by K\textsubscript{ATP} channels (our data, Fig. 2B). Hence, under conditions of phasic, brief activity, voltage-dependent mechanisms predominate, whereas tonic firing (as during sustained activity) leads to sufficient metabolic changes to allow for K\textsubscript{ATP}-mediated dilations. Large changes in [K\textsuperscript{+}]\textsubscript{out} lead to dilations (or constrictions) that are independent of K\textsubscript{ATP} channels and thus appear to be entirely mediated by K\textsubscript{IR} channels or other mechanisms.
pany neuronal activity: 1) rapid, transient changes in extracellular ion concentrations (e.g., \(K^+\)); 2) release of potentially vasoactive neurotransmitters (e.g., adenosine or nitric oxide); and 3) a transient tissue hypoxia derived from increased metabolic demand. Although evidence linking adenosine release to metabolic deprivation induced by neuronal firing has been long available (39), \(K^+\)-induced vasodilations have always been considered passive and dependent exclusively on \(K^+\) redistribution through voltage-dependent channels and subsequent changes in VSM resting potential.

The handling of extracellular \(K^+\) by glia and by cerebrovascular smooth muscle has been studied extensively (5, 15, 21, 25, 27, 30–32), but studies from relatively large cerebral vessels have failed to unmask a possible link between metabolic cytosolic changes in parenchymal vessels and \(K^+\) channel activity. This is somehow surprising since VSM cells are endowed with \(K^+\) channels regulated by subtle changes in cellular ATP content (1). \(K_{\text{ATP}}\) channels have been shown to mediate anoxic/ischemic vasodilations and may participate in cerebral autoregulation (12). The results presented herein expose an additional cerebrovascular regulatory mechanism propitiated by opening of \(K_{\text{ATP}}\), i.e., coupling of brain activity to CBF by \(K^+\) lost during neuronal firing.

Aboluminal application of \(K^+\) to isolated vessels may cause changes in resting potential in both endothelial and VSM cells. Furthermore, both \(K_{\text{ATP}}\) and \(K_{\text{IR}}\) are expressed in both cell types (18, 19, 21). It is thus possible that involvement of endothelial channels underlies some of the response to aboluminal \(K^+\). Several considerations and experimental results rule against this hypothesis. 1) If \(K^+\) dilations are a mechanism designed to link CBF and net loss of \(K^+\) from parenchymal neurons, it is important that commonly occurring changes in blood \(K^+\) concentrations do not affect CBF, whereas even small changes in extravascular \([K^+]_{\text{out}}\) participate in the regulation of CBF. 2) Results from other laboratories (e.g., Ref. 21) clearly demonstrated that vessels with denuded endothelium still undergo \(K^+\) mediated dilations. 3) We have recently estimated the “tightness” of the transendothelial barrier of the penetrating pial vessels used for the experiments presented herein (10, 11). These experiments demonstrated the existence of a tight transendothelial barrier. Therefore, it is unlikely that spillover of \(K^+\) into the intraluminal compartment may underlie the observed responses, since the highly blood-brain barrier-impermeant ion, \(Ba^{2+}\), was capable of abolishing this response when applied abuminally.

It has to be noted that the sensitivity of \(K^+\)-mediated dilations to ouabain and \(Ba^{2+}\) was first described by McCarron and Halpern (26). The novel finding in this study is twofold. 1) The blockade of dilations induced by \(K^+\) by the \(K^+\)-channel blocker is not exclusively due to an effect on voltage-dependent channels (\(K_{\text{IR}}\)) but rather on a metabolically regulated type (\(K_{\text{ATP}}\)). 2) The effects of ouabain are not directly related to the inhibition of the \(Na^–K^+\)-ATPase but rather to the subsequent hydrolysis of energy substrates.

Two questions remain. 1) How does extracellular \(K^+\) cause opening of \(K_{\text{ATP}}\), the “metabolic sensors” of VSM? 2) Do voltage-dependent \(K_{\text{IR}}\) channels play any role in the regulation of cerebrovascular tone in resistance-size vessels exposed to elevated \([K^+]_{\text{out}}\)? As shown in Fig. 2B, glibenclamide’s effects on \(K^+\)-induced dilations are more pronounced after steady-state \(K^+\) dilations; in contrast, \(Ba^{2+}\) abolished both the initial and plateau components. Thus it appears that \(Ba^{2+}\) acted by simultaneously blocking an early dilatory response, insensitive to \(K_{\text{ATP}}\) blockers and in all likelihood mediated by \(K_{\text{IR}}\), whereas the effects on the plateau could be mimicked by glibenclamide and hence appeared to be \(K_{\text{ATP}}\) mediated. This delayed sensitivity to \(K_{\text{ATP}}\) blockers suggested that the metabolic changes leading to opening of \(K_{\text{ATP}}\) channels required prolonged exposure to \([K^+]_{\text{out}}\). Because the effects of \(Na^–K^+\)-ATPase blockade by ouabain also overlapped with the effects of glibenclamide, we suggest that activation of the \(Na^–K^+\)-ATPase acts as a link between changes in extracellular \(K^+\) and VSM ATP content (Fig. 5A).

Recent insights into the intracellular mechanisms regulating \(K_{\text{ATP}}\) conductance have demonstrated that, in intact cells, even small decreases in intracellular ATP can lead to \(K^+\) flux through \(K_{\text{ATP}}\) (2, 35). Furthermore, in isolated membrane patches, activation of \(Na^–K^+\)-ATPase causes sufficient ATP hydrolysis to cause activation of the pump inhibitor ouabain on \(K_{\text{ATP}}\)-mediated currents (20). The latter effect could also be noticed in cell-free membrane patches, suggesting that nucleotide concentrations relevant to ion channel gating are closely associated with the plasma membrane, further strengthening the notion that localized changes in metabolic activity may be adequate signals for activation of a \(K_{\text{ATP}}\) conductance. Taken together, these results support a mechanism linking extracellular \(K^+\) increases, \(Na^–K^+\)-ATPase, and \(K_{\text{ATP}}\). Interestingly, \(K_{\text{ATP}}\)-mediated, glibenclamide-inhibitable dilations were observed in our study only after modest (5 mM above baseline) changes in \([K^+]_{\text{out}}\); at \([K^+]_{\text{out}}\) > 10 mM, \(Ba^{2+}\) sensitivity could be completely accounted for by a mechanism involving \(K_{\text{IR}}\). Hence, a cooperation of two molecularly and biophysically distinct \(K^+\) channels seems to be involved.

In conclusion, our results demonstrate that, in addition to voltage-dependent \(K_{\text{IR}}\) channels, VSM respond to \([K^+]_{\text{out}}\) by activation of a \(K_{\text{ATP}}\) current. The implications of this finding warrant a reinterpretation of the mechanisms linking neuronal activation to the regulation of CBF.

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Address for reprint requests and other correspondence: D. J. anigro, Cleveland Clinic Foundation NB-20, Neurosurgery, 9500 Euclid Ave., Cleveland, OH 44195 (E-mail: janigrd@ccf.org).

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