ATP-Sensitive Potassium Channels in the Cerebral Circulation

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Background—In brain blood vessels, electrophysiological studies proving the existence of ATP-sensitive potassium channels (K_{ATP}) are scarce. However, numerous pharmacological studies establish the importance of K_{ATP} channels in these blood vessels. This review emphasizes the data supporting the importance of vascular K_{ATP} in the responses of brain blood vessels.

Summary of Review—Electrophysiological data show the existence of K_{ATP} in smooth muscle and endothelium of brain vessels. A much larger number of studies in virtually all experimental species have shown that classic openers of K_{ATP} dilate brain arteries and arterioles. This response can be blocked by glibenclamide, a selective inhibitor of K_{ATP} opening. Several physiological or pathophysiological responses are also blocked by glibenclamide. K_{ATP} contains a multiplicity of potential sites of interaction with drugs of diverse, sometimes unrelated, structures. Drugs with imidazole or guanidinium groups are particularly likely to have effects on K_{ATP}. This complicates interpretation of the actions of such drugs when used as supossedly selective pharmacological probes for other putative targets. A pH-sensitive site on the internal surface of cloned channels may explain the glibenclamide-inhibitable dilation produced by intracellular acidosis and perhaps by CO_{2}. In some situations K_{ATP} appears to be involved in either the synthesis/release or action of endothelium-derived mediators of cerebrovascular tone. The importance of K_{ATP} may be dependent on the portion of the cerebrovascular tree being studied and on diverse experimental conditions, age, species, and the presence of disease.

Conclusions—K_{ATP} have been shown to mediate a wide range of cerebrovascular response in physiologic or pathologic circumstances in a variety of experimental conditions. Their relevance to cerebrovascular responses in humans remains to be explored. (Stroke. 2003;34:1547-1552.)

Key Words: adenosine triphosphate ■ aging ■ autoregulation ■ biological factors ■ carbon dioxide ■ cerebrovascular resistance ■ diabetes mellitus ■ endothelium-derived relaxing factor ■ endothelium, vascular ■ enzyme inhibitors ■ hydrogen-ion concentration ■ hypertension ■ hypoxia ■ nitric oxide synthase ■ potassium channels ■ vasodilation

Previous reviews of potassium channels and cerebral circulation (eg, those by Faraci and Sobey\(^1\) and Kitazano et al\(^2\)) have not focused solely on the role of ATP-sensitive potassium channels (K_{ATP}). The present review considers only this class of channel, emphasizing pharmacological evidence for their existence and importance. This evidence includes but is not restricted to the effects of drugs whose specificity for K_{ATP} is well established in other vascular beds.\(^3,4\) Because of space limitations, in areas in which many articles are available for citation, only a few are given as examples of the group.

Electrophysiological Evidence

Single articles report patch clamp evidence for K_{ATP} in cerebrovascular smooth muscle\(^5\) and in cerebrovascular endothelium.\(^6\) Electric potential of cerebrovascular smooth muscle is affected\(^7-9\) in the appropriate direction by agents that are known to open or close K_{ATP}. For example,\(^8,9\) levromakalim, an opener of K_{ATP}, hyperpolarized rabbit brain arteries. Glibenclamide, an established blocker of K_{ATP}, inhibited the response. Glibenclamide also blocked the hyperpolarization of the rabbit middle cerebral artery produced by acetylcholine.\(^7\)

Dilation by Well-Established Openers of K_{ATP}

It has been established\(^3,4\) that cromakalim, levromakalim, aprikalim, and pinacidil dilate brain blood vessels in rabbits,\(^6,8,9\) cats,\(^10,11\) piglets,\(^12,13\) rats,\(^11,14,15\) sheep,\(^16\) and humans.\(^17\) Where tested, these dilations are blocked by glibenclamide. However, there is heterogeneity of response, affected by species and location within the cerebrovascular tree.\(^2,8,18,19\)

Channel Structure and Relation to Effects of Established Openers and Blockers

Channel structure and its relation to the effects of established openers and blockers have been well studied.\(^3,20-25\) K_{ATP} channels consist of 8 subunits (Table). Four have amino acid
sequences sufficiently similar to those of inward rectifying (IR) channels to warrant inclusion of $K_{\text{ATP}}$ in the IR superfamily. These IR units are named $I_{\text{K,S}}$ or $I_{\text{K,L}}$. However, $K_{\text{ATP}}$ have no significant inward rectifying properties. The IR directly affect pore size and contain sites with high affinity for ATP. Attachment of ATP causes reduction in open state probability of the channel. Other agents acting on the IR may also affect the open probability state of $K_{\text{ATP}}$. Opening small numbers can have significant effects.\(^3\)

Four additional subunits are attached to and interact with the IR to affect channel opening through mechanisms that are not fully understood. They are labeled SUR because they contain sites with high affinity for sulfonylurea compounds, whose binding causes an interaction between SUR and IR that inhibits channel opening. There may be 4 varieties\(^24,25\) of SUR, designated SUR1, SUR2A, 2B, and 2C. The type of SUR subunits in brain blood vessels has not been determined. Examination of $K_{\text{ATP}}$ elsewhere indicates that SUR2 bind classic openers like pinacidil, while SUR1 bind, with high affinity, the inhibiting sulfonylureas such as glibenclamide and tolbutamide. Glibenclamide may have a second, weaker binding site on SUR2. Unlike tolbutamide, the structure of glibenclamide includes a benzamido group that can bind to an additional site on SUR1. One may speculate that the absence of the additional binding sites for tolbutamide may explain the inability of tolbutamide to block dilation in situations\(^26,27\) of the additional binding sites for tolbutamide may explain.

### Other Drugs That Block $K_{\text{ATP}}$

The large number of sites affecting\(^22,24-28\) the open state of the channel may provide loci for binding and action of drugs with supposedly selective actions on other putative targets. Among such compounds are drugs containing imidazoline or guanidinium moieties.\(^29,30\) This may explain why tetrodotoxin, a guanidinium compound, and 7-nitroindazole, an imidazole compound, could block the dilating action of selective $K_{\text{ATP}}$ openers on rat pial arterioles.\(^15\)

Dimethyl sulfoxide and ethanol, 2 commonly used diluents of water-insoluble drugs, in commonly used final concentrations, could also prevent the dilation of pial arterioles by openers of $K_{\text{ATP}}$.\(^31,32\)

This effect was consistent with the observation that other antioxidants also inhibit dilation produced by openers of $K_{\text{ATP}}$.\(^32\) Conversely, some oxidants caused dilation that was blocked by glibenclamide.\(^33\) The effects of diverse oxidizing substances on $K_{\text{ATP}}$ and other $K^+$ channels have recently been reviewed.\(^34\)

Factors affecting the aforementioned results might include composition of irrigating fluid,\(^35\) the presence and type of paralytic drug in artificially respired animals, and the nature of the anesthetic.\(^36-38\)

### $K_{\text{ATP}},$ Nitric Oxide, and Endothelium-Derived Hyperpolarizing Factor

Endothelium-derived relaxing factor (EDRF/nitric oxide [NO])\(^39,40\) and endothelium-derived hyperpolarizing factor (EDHF)\(^41\) relax vascular smooth muscle (VSM). Acetylcholine releases both from endothelium. Most studies of $K_{\text{ATP}}$ in cerebral circulation have not distinguished between the role of $K_{\text{ATP}}$ in release of mediator(s) from endothelium versus their role in the action of the mediator(s) on VSM.

One study\(^42\) found that blockade of $K_{\text{ATP}}$ inhibited dilation by NO only if 2 other classes of $K^+$ channel were also blocked, concluding that one type of $K^+$ channel may exert effects that compensate for the loss of another type.

Dilation of piglet pial arterioles by an NO donor or by a cGMP analogue were partially inhibited by glibenclamide,\(^13\) the selective blocker of $K_{\text{ATP}}$. Since cGMP in VSM is the second messenger for NO, the $K_{\text{ATP}}$ linkage for response to NO may have been in the VSM.

Rabbit cerebral arteries\(^7,9,14\) responded to EDRF/NO when $K_{\text{ATP}}$ were blocked. Glibenclamide partly blocked dilation of rabbit middle cerebral artery while totally blocking hyperpolarization produced by acetylcholine. Dilation to a NO donor occurred without a change in membrane potential. These data suggested that response to acetylcholine had 1 component dependent on EDRF/NO but independent of $K_{\text{ATP}}$ and 1 component dependent on EDHF and $K_{\text{ATP}}$. However, in guinea pig middle cerebral artery, glibenclamide failed to impair the portion of dilation thought to be caused by EDHF.\(^43\)

### $K_{\text{ATP}}$ as Mediator of Other Endogenous Dilators

Dilation by calcitonin gene-related peptide (CGRP) and by vasointestinal peptide may be inhibited by glibenclamide. This may\(^2\) reflect the role of cAMP as second messenger for these dilators. When released by forskolin, cAMP dilated vessels, and the dilation could also be inhibited by glibenclamide.\(^2\) This has been contrasted\(^2\) with the failure of glibenclamide to impair the response to EDRF/NO, a cGMP-dependent mechanism. However, glibenclamide has been reported to inhibit dilation induced by cGMP in pial arterioles in piglets.\(^13\)
**K\textsubscript{ATP} and Response to Hypercapnia and pH**

Cerebral arteries and arterioles are dilated by increases in CO\textsubscript{2} and constricted by decreases in CO\textsubscript{2} with corresponding increases or decreases in cerebral blood flow. Because of the importance of the response to CO\textsubscript{2},\textsuperscript{44,45} it is of interest to see whether K\textsubscript{ATP} play a role in the response.

Glibenclamide inhibited all or part of the dilation of pial arterioles induced by hypercapnia\textsuperscript{11,46} in pentobarbital-aneSThetized, paralyzed, artificially respired rats and cats but failed in rats under other experimental conditions.\textsuperscript{47} In another study,\textsuperscript{48} hypercapnic dilation was not affected by glibenclamide unless the rats were first treated with a nitric oxide synthase (NOS) inhibitor and then repleted with a cGMP analogue, which was presumed to compensate for the decrease of cGMP brought about by NO depletion.

In humans,\textsuperscript{49} oral glibenclamide failed to inhibit increases in cerebral blood flow produced by hypercapnia. However, the effect of glibenclamide on forearm blood flow was used as a basis for concluding that an adequate dose of glibenclamide had been given, and there was no direct evidence that the glibenclamide reached the cerebral circulation in adequate doses.

Glibenclamide pretreatment was also reported to inhibit constriction produced by hypocapnia.\textsuperscript{50} However, the inhibiting effect of glibenclamide seemed somewhat paradoxical to the investigators\textsuperscript{50} because of the following: (1) hypocapnic constriction was thought to depend on closure of K\textsubscript{ATP} caused by increased pH; (2) glibenclamide was thought to prevent this constriction by closing K\textsubscript{ATP} so that there was no significant proportion of K\textsubscript{ATP} left open for hypocapnia to close; (3) however, this closure of channels by glibenclamide pretreatment should have constricted the vessels. The paradox resides in the absence of such constriction here and in other studies.\textsuperscript{1,8} The failure of glibenclamide to constrict had been explained by assuming\textsuperscript{1} that K\textsubscript{ATP} play no role in the maintenance of resting tone. It was assumed that, unless the vessel was dilated by a stimulus that opened K\textsubscript{ATP}, the open state probability of the channels was too small to affect tone. However, hypocapnic constriction then could not be explained by a further closing of the channels. To resolve the difficulties apparent in the attempt to explain all the results on the probability of K\textsubscript{ATP} opening, the authors suggested that there are redundant mechanisms for maintaining tone and that these are so rapidly brought into play that they prevent closure of the K\textsubscript{ATP} by glibenclamide from influencing the resting tone. If this is so, we must assume that in their experiments hypocapnia reduced the open state probability of K\textsubscript{ATP} even more than glibenclamide so that only hypocapnia successfully overrode the compensatory mechanisms acting to prevent a change in tone.

The response to CO\textsubscript{2} is actually mediated by the corresponding change of pH (reduced with hypercapnia and increased with hypocapnia) in blood and extracellular space or cerebrospinal fluid.\textsuperscript{51} Glibenclamide, the K\textsubscript{ATP} blocker, had no effect on dilation of penetrating arterioles of rat cerebellum and brain stem when external pH was decreased in vitro.\textsuperscript{52} However, glibenclamide inhibited dilation of penetrating arterioles of rat cerebrum, also caused by reduction of extravascular pH.\textsuperscript{53} The difference may reflect the regional differences in K\textsubscript{ATP} distribution. Dilation and constriction of penetrating arterioles of rat cerebrum were highly correlated with the resting potential, which was related linearly to the diameter.\textsuperscript{54} This is consistent with a role for K\textsubscript{ATP} since the change in the open state of the channels would alter the resting potential.

However, others\textsuperscript{55} found that hyperpolarization was not required for a decrease in pH to produce dilation. The same group\textsuperscript{55,56} showed that internal pH (pHi) paralleled the external pH in cerebrovascular smooth muscle. However, their data failed to support the belief that dilation occurred when pH, was reduced by increasing external CO\textsubscript{2}. Other investigators\textsuperscript{57–59} concluded that it was the external pH rather than internal pH that determined the dilation of the vessel. Despite the fact that several articles indicated that pHi did not control diameter, a diagram in a review\textsuperscript{1} of potassium channels and cerebral circulation appearing in 1998 indicated that decreases of pHi open K\textsubscript{ATP}.

This conclusion foreshadowed the more recent demonstration that cloned K\textsubscript{ATP} demonstrate a pH-sensitive site on the inside rather than the outside of the cell membrane.\textsuperscript{28} A decrease in pH led to an increase in the open state probability of K\textsubscript{ATP} over the range of pH encountered during hypocapnia, normocapnia, and hypercapnia. The pH-sensitive site was located on the IR\textsubscript{6.2} subunit rather than the SUR subunit.

Finding the pH-sensitive site on the inside of the cell membrane has yet to be reconciled with the studies that concluded that the response to hypercapnia is mediated by external pH. However in another setting, the weight of evidence has been judged to support control of diameter by pH. In this setting, CO\textsubscript{2} is elevated by acetazolamide, a carbonic anhydrase inhibitor. With the exception of 1 study,\textsuperscript{60} both hypercapnia and acetazolamide have been shown to reduce pHi in the brain. At a relatively early date, Severinghaus and Cote\textsuperscript{61} concluded that the preponderance of evidence supported the hypothesis that reduction of pHi caused vasodilation by acetazolamide.

Some data indicate that there may be 2 mechanisms for hypercapnic dilation, one of which is K\textsubscript{ATP} dependent and the other NO dependent.\textsuperscript{62} For example, a NOS inhibitor eliminated only a portion of the dilation of rat pial arterioles produced by acidosis, with the remaining portion eliminated by glibenclamide.\textsuperscript{62}

The existence of dual or alternative important mechanisms not involving NO is illustrated in knockout mice lacking the neuronal isoform of NOS,\textsuperscript{63} which is the isoform thought to mediate the response to hypercapnia in normal animals.\textsuperscript{64} Whether the NO-independent mechanism is K\textsubscript{ATP} dependent in the knockout mice remains to be determined. A further dissection of the relationship between NO, K\textsubscript{ATP}, and the response to hypercapnia requires that drugs that inhibit NOS do not have an effect on K\textsubscript{ATP}. This subject is discussed next.

**K\textsubscript{ATP} and Inhibitors of NOS**

The basis for the belief that NO mediates the response to hypercapnia resides largely\textsuperscript{64} in studies using NOS inhibitors. However, there are now reports\textsuperscript{11,15,46} that in rats and cats both NOS inhibitors and glibenclamide can block the dilation of pial arterioles produced by K\textsubscript{ATP} openers as well as by...
hypercapnia. The inhibiting action of the NOS inhibitors on K_ATP openers was not due to some (now eliminated) permissive action of NO because adding a NO donor failed to rescue the response to openers of K_ATP. Moreover, L-arginine, the substrate for NOS, was found necessary for the dilation produced by K_ATP openers. L-Lysine could substitute for L-arginine. Both amino acids may be transported by the same transport system. L-Lysine cannot be metabolized by NOS. Hence, its ability to substitute for L-arginine supports the interpretation that NO was not involved. These data indicate that under some circumstances K_ATP can mediate hypercapnic dilation in rats and cats. These data also suggest a need for caution in interpreting and designing studies using NOS inhibitors. It would appear advisable to first see whether these NOS inhibitors can block K_ATP under the proposed experimental conditions.

The cited studies employed pentobarbital anesthesia and paralyzed, artificially respired animals. No study of brain blood vessels has appeared in which either the effect of NOS inhibitors on K_ATP openers or the effect of glibenclamide on hypercapnic dilation has been systematically compared in the presence and absence of these other possibly pertinent experimental factors. However, there are a few studies, performed under other conditions, which, incidental to their primary purpose, describe the failure of NOS inhibitors to inhibit dilation by K_ATP openers.

On the other hand, the data in some studies are consistent with such an effect. For example, L-arginine (L-NAME), a NOS inhibitor, inhibited the dilation of piglet arteries by aprikalim, an opener of K_ATP. The possibility that L-NAME was inhibiting K_ATP was not considered. Instead, it was concluded that NO was involved in the dilation produced by aprikalim. This interpretation is consistent with the data. However, if so, L-NAME must have prevented either aprikalim or K_ATP from releasing NO from endothelium rather than blocking its effects, since glibenclamide, the bona fide blocker of K_ATP, had no effect on dilation produced by a NO donor. In another study, CGRP dilated the basilar artery. The dilation was partially blocked by a NOS inhibitor but was completely blocked by glibenclamide, the K_ATP blocker. This was interpreted as indicating 2 additive mechanisms for the dilation by CGRP. The possibility was not considered that L-NAME may have also been inhibiting K_ATP but with less efficacy, at the dose used, than glibenclamide.

Note added in proof: Following acceptance of this review, Santa et al. reported glibenclamide-inhibitable dilation of rat basilar artery in vivo by a drug selectively decreasing intracellular pH. Cultured basilar smooth muscle showed message for IR6.1 and for SUR-B but not for IR6.2. These data support studies cited in this review that indicate an intracellular pH-sensitive site on K_ATP. However, a NOS inhibitor, known to inhibit hypercapnic dilation, failed to inhibit dilation produced by the intracellular acidifier. Therefore, Santa et al restricted their conclusion to the suggestion of a role for K_ATP in mediating dilation produced by reduced pH but not in the context of hypercapnia.

Influence of Age, Diabetes, Hypertension, Hypotension, and Hypoxia

Maturation increased the responsiveness to a K_ATP opener in some branches of middle cerebral artery in sheep. However, there was no effect of aging on the response to K_ATP openers in Fischer rats.

Diabetes and some forms of hypertension may inhibit dilation by K_ATP openers. In stroke-prone spontaneously hypertensive rats, superimposition of subarachnoid hemorrhage greatly enhanced the previously diminished response. K_ATP may participate in the dilation produced by hypotension and hypoxia. Dilation during oxygen deprivation may result in part from decrease in ATP below the levels at which K_ATP opening is suppressed and in part from the acidosis that accompanies the hypoxia and can increase open state probability of K_ATP. In rats, the autoregulatory dilation in response to hypotension may be mediated by K_ATP in brain stem neurons. However, discussion of the role of K_ATP in brain stem control of cerebrovascular tone is beyond the scope of this review.

Summary

Patch clamp demonstrations of K_ATP in cerebrovascular smooth muscle and cerebrovascular endothelium are rare. However, most pharmacological data indicate that K_ATP exist in cerebral arteries and arterioles in virtually all species. The importance of K_ATP depends on the experimental conditions or pathological state. Multiple sites on the complex K_ATP structure provide loci for interaction with channel inhibitors like ATP and sulfonyleureas, with channel openers like pinacidil and cromakalim, and with hydrogen ions. In addition, structural complexity provides sites for interaction with a wide variety of other drugs, including imidazole or guanidinium derivatives. Therefore, many drugs, selectively interacting with other targets, may also inhibit K_ATP and thus have a previously unsuspected mechanism of action against a range of physiological or pathological responses. In some studies, the NOS substrate L-arginine and inhibitors of NOS interacted with K_ATP in pial arterioles. Therefore, under some circumstances the inhibition of responses by NOS inhibitors may not be proof of mediation by NO but instead may result from inhibition of K_ATP. Further studies are required to define conditions necessary for this effect. With respect to the role of K_ATP in endothelium-mediated dilation, further studies are necessary to distinguish between the role of K_ATP in the action of either EDRF/NO and/or endothelium-derived hyperpolarizing factor on VSM versus the role of K_ATP in the release of these dilators from endothelium. The possible role of K_ATP in mediating the response to pH (eg, in hypercapnia or hypoxia) is of particular interest because pH is an important modulator of cerebrovascular tone. Therefore, it is noteworthy that a pH-sensitive site on cloned K_ATP has been demonstrated. This site was expressed on the inside of the cell membrane, but several investigators believe that external pH rather than pHi is the determinant of hypercapnic dilation. Resolution of this conundrum awaits further investigations.

References

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