Alterations in $K_{ATP}$ and $K_{Ca}$ channel function in cerebral arteries of insulin-resistant rats

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Erdős, Benedek, Allison W. Miller, and David W. Busija. Alterations in $K_{ATP}$ and $K_{Ca}$ channel function in cerebral arteries of insulin-resistant rats. Am J Physiol Heart Circ Physiol 283: H2472–H2477, 2002.—We examined whether insulin resistance alters the function of ATP-dependent and $Ca^{2+}$-activated $K^+$ channels ($K_{ATP}$ and $K_{Ca}$ channels, respectively) in pressurized isolated middle cerebral arteries (MCAs) from fructose-fed insulin-resistant (IR) and control rats. Blockade of $K_{Ca}$ channels with tetraethylammonium chloride (TEA, 2.5 mM) or iberiotoxin (IBTX, 0.1 $\mu$M) increased the spontaneously developed tone in control MCAs by 10.5 ± 1.3% ($n=10$) and 13.3 ± 2.3% ($n=6$), respectively. In the IR arteries, TEA induced similar constrictions (8.0 ± 1.1%, $n=10$), but IBTX constricted the IR arteries by only 3.1 ± 0.9% ($n=8$; $P<0.01$). Bradykinin (BK)-induced endothelium-mediated relaxation was reduced in IR MCAs. Maximum relaxation to BK ($10^{-6}$ M) was 42 ± 4% in control ($n=9$) and 19 ± 2% in IR ($n=10$; $P<0.01$) arteries. Pretreatment with TEA, IBTX, or the $K_{ATP}$ channel blocker glibenclamide (10 $\mu$M) inhibited relaxation to BK in control MCAs but did not alter dilation in IR arteries. Relaxation to the $K_{ATP}$ channel opener cromakalim was also diminished in IR MCAs. Maximum relaxation to cromakalim ($10^{-5}$ M) was 48 ± 3% in control ($n=6$) and 19 ± 2% in IR arteries ($n=6$; $P<0.01$). These findings demonstrate that insulin resistance alters the function of $K_{ATP}$ and $K_{Ca}$ channels in isolated MCAs and affects the control of resting vascular tone and the mediation of dilator stimuli.

middle cerebral artery; bradykinin; tetraethylammonium chloride; iberiotoxin; glibenclamide

INSULIN RESISTANCE IS a risk factor for stroke in humans (21, 27); however, the underlying mechanisms have not yet been clarified. One possible factor in the elevated risk of stroke may be the impairment of endothelium-mediated relaxation in cerebral arteries (5). In our previous study (5), we demonstrated that responses to endothelium-dependent dilator agents are reduced in the middle cerebral arteries (MCAs) of insulin-resistant (IR) animals as a result of impaired cyclooxygenase (COX)-dependent relaxation, whereas nitric oxide (NO)-mediated responses remain intact.

ATP-dependent and $Ca^{2+}$-activated $K^+$ channels ($K_{ATP}$ and $K_{Ca}$ channels, respectively) are important targets of mediators released from the endothelium including those that are produced by COX (8, 11, 25, 29). In addition, it has been shown previously that insulin resistance alters the function of these ion channels in mesenteric and coronary arteries and leads to diminished endothelium-dependent relaxation (13, 15, 22, 23). Therefore, we hypothesized that insulin resistance affects the $K_{ATP}$ and $K_{Ca}$ channel functions in cerebral arteries and ultimately leads to altered vascular regulation. No previous studies have addressed this issue with regard to the cerebral circulation.

In this study, the functions of $K_{ATP}$ and $K_{Ca}$ channels were examined in isolated MCAs of control and IR rats. We studied the roles of these ion channels in the regulation of spontaneous vascular tone as well as in endothelium-dependent dilation induced by bradykinin (BK). In addition, we assessed the function of $K_{ATP}$ channels with the subtype-specific channel opener cromakalim. We used a well-established model in which insulin resistance was induced by the feeding of a high-fructose diet. Fructose-fed rats within the conditions of the current study are characterized as having hyperinsulinemia and dyslipidemia (i.e., hypertriglyceridermia and decreased levels of high-density lipoprotein) yet are normotensive and normoglycemic (14, 23).

METHODS

The experimental protocol was approved by the Animal Care and Use Committee at Wake Forest University School of Medicine. Male Sprague-Dawley rats were obtained at 6 wk of age and randomized into one of two groups: control ($n=35$) and IR ($n=33$). Animals in the IR group were fed a fructose-rich diet that contained 66% fructose, 22% casein, and 12% lard plus essential vitamins and minerals (Teklad Labs; Madison, WI), whereas control animals received standard rat chow. After a 4-wk diet treatment, the rats (in a fasting state) were anesthetized with pentobarbital sodium (50 mg/kg ip) and anticoagulated with heparin sodium (500 U ip).

Biochemical measurements. To confirm that fructose-fed rats were indeed insulin resistant, we measured plasma

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insulin and glucose levels after 12 h of fasting using a rat-insulin ELISA kit (Crystal Chem; Chicago, IL) and Trinder reagent (Sigma; St. Louis, MO), respectively. Fasting glucose levels were similar between the two groups (control rats, 117 ± 2; IR rats, 134 ± 3 mg/dl), whereas insulin was markedly increased in the IR rats (control rats, 649 ± 87; IR rats, 1,781 ± 331 pg/ml; $P < 0.05$). The mean body weight was similar in the two groups of animals (control, 311 ± 7; IR rats, 313 ± 5; $P$ not significant).

**Determination of vascular reactivity.** Methods have been described previously in detail (5). Briefly, after each rat was decapitated, the brain was immediately removed and placed in cold, oxygenated modified Krebs-Ringer bicarbonate solution [that contained (in mM) 119 NaCl, 4.7 KCl, 24 NaHCO$_3$, 1.18 KH$_2$PO$_4$, 1.17 MgSO$_4$, 0.026 EDTA, 1.6 CaCl$_2$, and 5.5 glucose)]. Both MCAs were carefully harvested, and a section of MCA (∼2 mm in length) was transferred to a vessel chamber and mounted and secured between two glass micropipettes. The vessel chamber was transferred to an inverted light-microscope stage, and a video dimension analyzer (Living Systems Instrumentation; Burlington, VT) was used to measure the intraluminal diameter. Oxygenated Krebs solution maintained at 37°C was continuously circulated through the vessel bath, and the lumen of the artery was also filled with Krebs solution through the micropipettes. The outflow cannula was clamped off while the inflow cannula was connected to an elevated reservoir to maintain a constant intraluminal pressure of 80 mmHg. After the MCAs were mounted and pressurized, the MCAs from either control or IR rats developed spontaneous tone by constricting to ∼70% of the initial diameter over the course of 1 h. Drugs were added abuminally into the bath solution, and only one concentration-response experiment was performed per artery.

Function of the K$_{Ca}$ channels was studied with the non-selective K$_{Ca}$ channel blocker tetraethylammonium chloride (TEA, 2.5 mM) and the large-conductance, Ca$^{2+}$-activated K$^+$ channel (BK$_{Ca}$) blocker iberiotoxin (IBTX, 0.1 μM). K$_{ATP}$ channels were examined with glibenclamide (10 μM) and cromakalim (10$^{-9}$ to 10$^{-6}$ M). Endothelium-dependent relaxation was induced by BK (10$^{-9}$ to 10$^{-6}$ M), and the role of NO in the BK-induced responses was evaluated with N$^{\text{m}}$-nitro-l-arginine methyl ester (l-NAME, 10 μM). The doses of these drugs have been shown previously to be effective on isolated arteries from rats (15, 30).

Blockade of the K$_{Ca}$ channels induced vasoconstriction in the MCAs. Therefore, in separate experiments, we examined whether the changes in BK-induced relaxation after the application of TEA or IBTX were a consequence of the increased vascular tone rather than the inhibition of these ion channels. Dilation to BK was assessed after the MCAs were constricted to a similar level with the application of endothelin-1 (ET-1).

**Chemicals.** BK, l-NAME, TEA, IBTX, glibenclamide, and ET-1 were obtained from Sigma, whereas cromakalim was obtained from Tocris Cookson. All drugs were dissolved in Krebs solution except for cromakalim, which was dissolved in DMSO and Krebs solution. The same concentration of DMSO alone had no effect on vessel diameter.

**Data analysis.** All data are expressed as means ± SE. The magnitudes of spontaneous vascular tone and treatment-induced vasoconstriction were calculated as percentages of maximal intraluminal diameter, whereas vascular relaxations were calculated as percentages of preconstriction. Maximal diameter was measured as the initial diameter immediately after pressurization; for control, maximal diameter was also measured at the end of the experiments after complete relaxation with sodium nitroprusside (3 × 10$^{-4}$ M). It was shown previously in the same experimental model that the initial diameter of the arteries is the same as the maximal diameter that was obtained in Ca$^{2+}$-free solution (30). The concentration-response curves were evaluated at each concentration for differences between treated and untreated arteries from control and IR groups using ANOVA and subsequent Tukey's post hoc test. Changes in spontaneous vascular tone in control and IR MCAs after administration of specific K$^+$ channel blockers were compared using Student's $t$-test. The criterion for significance was $P < 0.05$.

**RESULTS**

The maximal intraluminal diameter of the MCAs did not differ between the two groups (control, 227 ± 2; n = 58; IR, 223 ± 2 μm, n = 54). The spontaneously developed tone was also similar in that the MCAs constricted to 72 ± 1% and 74 ± 1% of the initial diameter in the control and IR groups, respectively.

Blockade of the K$_{Ca}$ channels with TEA significantly augmented the spontaneous vascular tone of the arteries from both control and IR rats by an additional 10.5 ± 1.3% (n = 10) and 8.0 ± 1.1% (n = 10), respectively (Fig. 1; difference not significant). The selective BK$_{Ca}$-channel blocker IBTX induced similar vasoconstriction in the control MCAs (13.3 ± 2.3%; n = 6) but changed the vascular diameter only by 3.1 ± 0.9% (n = 8) in IR arteries (difference, $P < 0.01$). Inhibition of the KATP channels with glibenclamide did not alter the vascular tone in either of the two groups. Vascular diameter decreased by 1.7 ± 0.5% (n = 8) and 1.2 ± 0.5% (n = 7) in the control and IR arteries, respectively (Fig. 1).

BK induced concentration-dependent relaxation in both the control and IR MCAs; however, responses were significantly reduced in the IR group (Fig. 2). Maximum relaxation to BK was 42 ± 4% in control MCAs (10$^{-6}$ M, n = 9) and only 19 ± 2% in the IR arteries (10$^{-6}$ M, n = 10; $P < 0.01$). As we have shown
IBTX, respectively. The combination of L-NAME and n/NAME (solutions (Fig. 3B). Maximum relaxations were 20% on the already diminished BK-induced vascular responses in the IR arteries 

In control arteries, blockade of KCa channels with either the nonselective KCa channel blocker TEA or the selective BKCa channel blocker IBTX reduced the dilation to BK (Fig. 3A). Maximum relaxations to BK were 19 ± 2% (n = 6) and 22 ± 1% (n = 6) in the presence of TEA and IBTX, respectively. In contrast, in IR arteries, neither TEA nor IBTX pretreatment had any effect on the already diminished BK-induced vascular responses (Fig. 3B). Maximum relaxations were 20 ± 3% (n = 7) and 23 ± 3% (n = 8) in the presence of TEA and IBTX, respectively. The combination of L-NAME and TEA completely blocked the BK-induced dilation in both the control and IR MCAs: the maximum changes in diameter were 3 ± 2% (n = 7) and −1 ± 4% (n = 4), respectively.

To examine whether the changes in BK-induced relaxations after KCa channel blockade were not simply the consequence of the increased vascular tone, we constricted the arteries to a similar level by applying the appropriate amount of ET-1 without blocking the KCa channels. Thus the vascular tone of MCAs from control and IR rats was augmented by 12.5 ± 2.6% (n = 4) and 12.0 ± 2.2% (n = 4), respectively, but responses to BK did not differ significantly from those of the untreated arteries (Fig. 3). Maximum relaxations to BK were 35 ± 5% (n = 4) and 23 ± 3% (n = 4) in the control and IR arteries, respectively.

Blockade of the KATP channels with glibenclamide also reduced the dilation to BK in the control MCAs [maximum relaxation was 21 ± 3% (n = 8); Fig. 4A]. In contrast, glibenclamide had no effect on BK-induced responses in the IR arteries [maximum relaxation to BK after glibenclamide administration was 19 ± 4% (n = 7); Fig. 4B]. The combination of L-NAME and glibenclamide completely abolished BK-induced dilation in both the control and IR arteries. Maximum changes in diameter were 3 ± 2% (n = 6) and 1 ± 2% (n = 4), respectively. Co-administration of TEA and glibenclamide had no additional effect in either the control or IR MCAs. Relaxation to BK was similar to the responses after TEA or glibenclamide administration alone. Maximum relaxations were 18 ± 3% (n = 6) and 23 ± 1% (n = 4) in the control and IR arteries, respectively (Fig. 4).

Finally, the function of the KATP channels was examined with the specific channel opener cromakalim. Cromakalim induced dose-dependent dilation in both the control and IR MCAs; however, the relaxation was markedly reduced in the IR arteries (Fig. 5). Maximum dilations to cromakalim were 48 ± 3% (10−5 M, n = 6) and 19 ± 2% (10−5 M, n = 6) in the control and IR arteries, respectively.

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Fig. 2. Cumulative dose-response curves to bradykinin (BK) in MCAs of control and IR rats. BK-induced dilation is significantly reduced in IR MCAs. *P < 0.01 compared with control group.

Fig. 3. Cumulative dose-response curves to BK in MCAs of control (A) and IR (B) rats. Application of TEA or IBTX reduced the BK-induced relaxation significantly in control MCAs but had no effect in IR arteries. Combination of N2-nitro-L-arginine methyl ester (L-NAME) and TEA completely abolished the BK-induced dilation in both control and IR MCAs. Increased vascular tone induced by endothelin-1 (ET-1) had no effect on responses to BK. *P < 0.05, treated vs. untreated groups; **P < 0.05 compared with arteries treated with TEA alone.

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DISCUSSION

The major finding of this study is that the functions of both \( K_{Ca} \) and \( K_{ATP} \) channels are altered in the cerebral arteries of IR rats. \( K_{Ca} \) channels are involved in the control of spontaneous vascular tone, and the blockade of these ion channels leads to significant vasoconstriction. Although the spontaneous vascular tone and the vasoconstriction induced by nonspecific blockade of the \( K_{Ca} \) channels are similar in the control and IR groups, it seems that the role of \( BK_{Ca} \) channels in the regulation of resting vascular tone is diminished in IR arteries compared with controls. Moreover, in control arteries, \( K_{ATP} \) and \( K_{Ca} \) channels contribute to the endothelium-dependent relaxation elicited by BK; however, in IR arteries, the relaxation mediated by these ion channels is missing. Dilation to the \( K_{ATP} \) channel opener cromakalim is also reduced in IR MCAs compared with control arteries. Thus insulin resistance alters the function of \( K_{ATP} \) and \( K_{Ca} \) channels in the regulation of both basal and stimulated vascular control mechanisms.

\( K_{Ca} \) channels play a key role in buffering pressure-induced constriction of small cerebral arteries (4, 9, 18). Thus in control arteries, both TEA (a nonspecific \( K_{Ca} \) channel blocker) and IBTX (a specific \( BK_{Ca} \) channel blocker) induced significant vasoconstriction of similar magnitude. These data are in accordance with previous studies (4, 9, 18) that suggest that under normal conditions, the \( BK_{Ca} \) channel is the major \( K_{Ca} \) channel subtype that regulates vascular tone. In contrast, specific blockade of the \( BK_{Ca} \) channels in IR arteries does not lead to significant vasoconstriction in addition to the pressure-induced tone. These data indicate that the role of \( BK_{Ca} \) channels in buffering the pressure-induced constriction is reduced in insulin resistance. The decreased \( K^+ \) efflux through the \( BK_{Ca} \) channels would be expected to result in increased vascular tone. However, there was no significant difference in the resting tone of arteries from the two experimental groups. This could be due to a compensatory increase in the contribution of other \( K_{Ca} \) channels, because TEA, which also blocks the intermediate- and small-conductance \( K_{Ca} \) channels in addition to the \( BK_{Ca} \) channels, induced a similar vasoconstriction in the IR arteries compared with the control arteries. Thus it is possible that these subtypes of the \( K_{Ca} \) channel family, which are probably not affected by insulin resistance, compensate for the loss of \( BK_{Ca} \) channel function. It should be kept in mind that the dysfunction of \( BK_{Ca} \) channels might be compensated in this way in isolated arteries, but in vivo, the control of vascular tone is more complex and particularly in certain pathological conditions such as hypertension, the role of \( BK_{Ca} \) channels is more important (18). In these cases, alteration in \( BK_{Ca} \) channel function may have a more significant impact.

\( K_{ATP} \) and \( K_{Ca} \) channels play an important role in the mediation of numerous dilator stimuli in the cerebral
arteries including endothelium-dependent relaxation (for review, see Refs. 6 and 7). BK, for example, has been shown to activate these ion channels in several circulatory beds including cerebral circulation (10, 16, 24, 28). In a previous study, we showed that in isolated MCAs of normal rats, BK-induced relaxation is mediated by NO and COX derivates (5). Products of COX [i.e., prostacyclin or reactive oxygen species (ROS)] are well-known openers of K<sub>ATP</sub> and K<sub>Ca</sub> channels (8, 11, 25, 29), and the data of the present study show that the NO-independent, COX-mediated responses to BK require the opening of these K<sup>+</sup> channels. In control arteries, glibenclamide reduced the magnitude of relaxation to BK and in combination with L-NAME led to complete blockade of BK-induced responses. Similarly, both TEA and IBTX diminished dilation to BK, and the combined blockade of NO synthesis and K<sub>Ca</sub> channels completely abolished the BK-induced dilation. Furthermore, the fact that IBTX and TEA had similar effects suggests that the dominant K<sub>Ca</sub> channel subtype in this mechanism is the BK<sub>Ca</sub> channel.

Although BK has been shown to activate either K<sub>Ca</sub> or K<sub>ATP</sub> channels (10, 16, 24, 28) depending on the species and the circulatory area examined, it was a surprising finding of the present study that in isolated rat MCAs, these two types of K<sup>+</sup> channels are both involved in the mediation of BK-induced dilation and contribute to the same regulatory pathway. Combined blockade of K<sub>ATP</sub> and K<sub>Ca</sub> channels with TEA and glibenclamide did not further inhibit the BK-induced relaxations. Previously, Schubert et al. (25) reported that relaxation to the prostacyclin analog iloprost involves the activation of both types of K<sup>+</sup> channels, and the combined blockade of the K<sub>ATP</sub> and K<sub>Ca</sub> channels provides no additional effects. However, the mechanisms that connect the activation of these two ion channels remain to be clarified.

Despite this uncertainty regarding the mechanisms that induce the simultaneous activation of K<sub>ATP</sub> and K<sub>Ca</sub> channels in control arteries, it is evident that in IR arteries, neither type of K<sup>+</sup> channel plays a role in the BK-induced relaxation. The already reduced dilations in IR MCAs were not affected by the application of glibenclamide, TEA, or IBTX. The combined blockade of K<sub>ATP</sub> and K<sub>Ca</sub> channels was also ineffective. Thus, the present data and our previous findings (5) demonstrate that BK-induced dilation is reduced in insulin resistance, and this appears to be due to impaired K<sup>+</sup> channel activation by COX metabolites leaving BK-induced relaxation entirely dependent on NO.

It is not clear at this time what kinds of mechanisms are responsible for the insulin resistance-induced impairment of this pathway. Theoretically, both the reduced endothelial release of COX products and the dysfunction of vascular smooth muscle K<sup>+</sup> channels activated by these mediators can explain our findings. However, several facts suggest that the function of K<sub>ATP</sub> and BK<sub>Ca</sub> channels is altered in insulin resistance. The data of the present study demonstrate that relaxation to cromakalim is significantly reduced in IR arteries compared with control MCAs, which indicates that K<sub>ATP</sub> channel mediated dilation was impaired even when the ion channels were stimulated by a specific channel opener. Although a similar specific agonist for the K<sub>Ca</sub> channels is not available, the fact that BK<sub>Ca</sub> channel function in the regulation of resting tone is diminished in IR MCAs indicates that these ion channels are also affected by insulin resistance. Moreover, insulin resistance impairs the function of K<sub>ATP</sub> and BK<sub>Ca</sub> channels, probably via the same mechanisms, in other circulatory beds such as small coronary and mesenteric arteries, which also leads to reduced endothelium-mediated dilation (13, 15, 22, 23).

Further investigation is needed to reveal how the changes in insulin signaling affect the function of these vascular smooth muscle cell K<sup>+</sup> channels. One possibility is that insulin resistance increases the production of ROS in the vascular tissues, which influences the structure and activity of these ion channels. The following evidence supports this hypothesis. First, superoxide production has been shown to be elevated in aortas of fructose-fed rats due to the activation of endothelial NADH/NADPH oxidase (12) and the dysfunction of endothelial NO synthesis caused by abnormal biopterin metabolism (26). Second, K<sup>+</sup> channel mediated relaxations are impaired in pathophysiological conditions when excessive ROS production occurs, for example, during ischemia-reperfusion (3, 20), brain injury (1, 2), or hyperglycemia (17, 19). Thus the impairment of K<sub>ATP</sub> and K<sub>Ca</sub> channel mediated vascular responses in IR cerebral arteries could also be the consequence of elevated ROS production.

In conclusion, results of the present study indicate that high-fructose-diet-induced insulin resistance impairs the function of BK<sub>Ca</sub> and K<sub>ATP</sub> channels in rat MCAs, and the dysfunction of these K<sup>+</sup> channels influences the control of resting vascular tone as well as the mediation of endothelium-dependent relaxation. The altered cerebrovascular regulation may be responsible for the increased risk of cerebrovascular events in insulin resistance.

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