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Effect of chronic insulin on cromakalim-induced relaxation in established streptozotocin–diabetic rat basilar artery

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Abstract

Our goals were to determine whether the response of the rat isolated basilar artery to activation of ATP-sensitive potassium (K_{ATP}) channels is altered in diabetes mellitus, and to determine the effect of chronic insulin treatment on this response in established diabetic rats. The relaxation induced by cromakalim, an activator of K_{ATP} channels, was significantly weaker in insulin-untreated streptozotocin-induced diabetic rats than in the controls. This impairment was significantly improved following chronic administration of insulin. The relaxations induced by two Ca^{2+}-activated K^+-channel activators [1-ethyl-2-benzimidazolinone (1-EBIO) or 1, 3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one (NS1619)] were not significantly different between control and insulin-untreated diabetic rats. The sodium nitroprusside-induced relaxation was similar among the three groups (control, diabetic, and insulin-treated diabetic). These results suggest that: (a) the impaired cromakalim-induced relaxation seen in diabetic rats is not due to a nonspecific effect of diabetes mellitus on vasorelaxation, but at least partly to an effect on K_{ATP} channels, and (b) that this impaired relaxation can be restored by chronic insulin treatment.

Keywords: ATP-sensitive potassium channel; Basilar artery; Cromakalim; Diabetes; Insulin; Relaxation

1. Introduction

Vascular resistance in large arteries appears to be greater in the cerebral circulation than in other vascular beds. Large arteries such as the basilar artery make an important contribution to total cerebral vascular resistance and are also major determinants of local microvascular pressure (Faraci and Heistad, 1990). While the basic principles of blood-flow regulation apply to all vascular beds, there are some important differences between cerebral blood vessels and vessels in other organs in their response to humoral, neural, and metabolic stimuli, in their response to hypercapnia/hypoxia, and in their autoregulation (Faraci and Heistad, 1998). Changes in the activity of potassium channels represent a major mechanism for the regulation of vascular tone. Activation of potassium channels in blood vessels produces hyperpolarization of the cell membrane, closure of voltage-dependent calcium channels, a decrease in the intracellular calcium level, and vascular relaxation (Faraci and Heistad, 1998). ATP-sensitive potassium (K_{ATP}) channels are present both in large cerebral arteries and in cerebral arterioles, and opening of these channels is a major mechanism for both cerebral vasodilation (Faraci and Heistad, 1993, 1998) and increases in cerebral blood flow (Takaba et al., 1996). In addition, Ca^{2+}-activated potassium (K_{Ca}) channels have been described in smooth muscle in cerebral vessels, amongst others (Nelson, 1993; Faraci and Heistad, 1998). K_{Ca} channels are characterized by their activation by increases in the concentration of intracellular calcium. In cerebral arteries, inhibitors of K_{Ca} channels produce contraction both in vitro and in vivo (Faraci and Heistad, 1998), suggesting that these channels

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are active under basal conditions and that they are important in the regulation of vascular tone.

Diabetes mellitus is a risk factor in the pathogenesis of many cerebrovascular events, including cerebral ischemia, vertebrobasilar transient ischemic attacks, and stroke (Tuomilehto et al., 1996; Ho et al., 2003). Diabetes is associated with endothelial dysfunction in extracranial blood vessels in experimental animals (Kamata et al., 1989a; Cohen, 1995; Pieper, 1998; De Vriese et al., 2000) and in humans (Poston and Taylor, 1995). Similarly, an impaired relaxation of cerebral arterioles (Mayhan, 1989; Mayhan et al., 1991) and of the basilar artery (Abiru et al., 1991; Mayhan, 1992; Kamata and Kondoh, 1996) has been observed during diabetes. We reported some years ago that the relaxations of the thoracic aorta induced by cromakalim and nicorandil, both of which are K_{ATP}-channel openers, are significantly attenuated in streptozotocin-induced diabetic rats (Kamata et al., 1989b). Since then, a number of investigators have confirmed that the relaxation responses to K_{ATP}-channel openers are attenuated in diabetic states (Mayhan and Faraci, 1993; Mayhan, 1994; Zimmermann et al., 1997).

In animal models of streptozotocin-induced diabetes, chronic insulin treatment starting from the onset of glycosuria has been shown to prevent the impairment of acetylcholine-induced endothelium-dependent relaxation seen in mesenteric resistance arteries and aortic rings (Taylor et al., 1994; Heygate et al., 1996; Pieper, 1997). Moreover, Mayhan et al. (2001) noted that insulin treatment (via a sustained-release insulin implant inserted 3 days after streptozotocin injection) reverses the impaired acetylcholine-induced relaxation of the rat basilar artery seen during diabetes mellitus in vivo. On the other hand, there is little evidence that chronic administration of insulin to an established diabetic model can prevent diabetic vasculopathy. We recently reported that in rats with established streptozotocin-induced diabetes, chronic insulin treatment reverses the development of an impaired endothelium-dependent relaxation in the thoracic aorta (Kobayashi and Kamata, 1999a, 2001, 2002). However, no study has been performed to assess whether chronic administration of insulin in established diabetes can reverse the diabetes-related abnormalities of the relaxation response to K^+-channel activation in the isolated basilar artery. The first goal of the present study was to determine whether the relaxing effects induced by activation of K_{ATP} and K_{Ca} channels in the isolated basilar artery might be altered in the diabetic state. For this, we used isometric tension recording. We also examined the effects of K_{Ca}-channel inhibitors on vascular tone in basilar arteries isolated from streptozotocin-induced diabetic and age-matched control rats. Our second goal was to investigate the influence of chronic insulin treatment on the alteration in the relaxation response induced by activation of K_{ATP} channels that is seen in basilar arteries from rats with established streptozotocin-induced diabetes.

2. Materials and methods

2.1. Reagents

Streptozotocin, 1, 3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619), glibenclamide, tetraethylammonium chloride (TEA), iberiotoxin, sodium nitroprusside, and 9, 11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_2\alpha$ (U46619) were all purchased from Sigma (St. Louis, MO, U.S.A.). Cromakalim was from Toronto Research Chemicals, (North York, ON, Canada), and 1-ethyl-2-benzimidazolinone (1-EBIO) from Tocris (Natick, MA, U.S.A.). The superoxide dismutase mimetic 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl (Tempol) was from Calbiochem (La Jolla, CA, U.S.A.). All drugs were dissolved in saline, except 1-EBIO, NS1619, glibenclamide, and cromakalim, which were dissolved in dimethyl sulfoxide (DMSO). Control experiments confirmed the absence of significant effects on vascular tone at the final vehicle concentration used. All concentrations are expressed as the final molar concentration of the base in the organ bath.

2.2. Animals and experimental design

Male Wistar rats (8 weeks old and 180–230 g body weight) received a single injection via the tail vein of streptozotocin 65 mg/kg dissolved in a citrate buffer. Age-matched control rats were injected with the buffer alone. Food and water were given ad libitum. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Science, Sports, and Culture, Japan).

2.3. Insulin treatment

About 10 weeks after the streptozotocin injection, streptozotocin-induced diabetic rats were treated with a gradually increasing dose of insulin (human insulin 5–30 U/kg per day) for 2 weeks. Twelve weeks after the streptozotocin injection, the rats were killed by decapitation under ether anesthesia. Control rats were killed in the same way 12 weeks after receiving their buffer injection. Thus, we studied three groups: controls, insulin-untreated diabetic rats, and insulin-treated diabetic rats.

2.4. Measurement of plasma glucose, cholesterol, and insulin

Twelve weeks after the administration of streptozotocin (insulin-untreated or -treated diabetic groups) or buffer (control group), plasma glucose was determined using a commercially available enzyme kit (Wako, Osaka, Japan), which made use of the *O*-toluidine method (Dubowski,

1962). Plasma total cholesterol and triglyceride levels were determined using a commercially available enzyme kit (Wako, Osaka, Japan), the plasma triglyceride level being assayed by the method described by Spayd et al. (1978). High-density lipoprotein (HDL) cholesterol was measured following phosphotungstic-$MgCl_2$ precipitation of apolipoprotein B containing very low density lipoprotein (VLDL; Wako, Osaka, Japan). Plasma insulin was measured by enzyme-immunoassay (Shibayagi, Gunma, Japan).

2.5. Measurement of isometric force

Rats from the three groups mentioned in Section 2.3 were anesthetized with diethyl ether and euthanized by decapitation 12 weeks after treatment with streptozotocin or buffer. A section of the basilar artery was then removed and placed in ice-cold, oxygenated, modified Krebs–Henseleit solution (KHS). This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 $NaHCO_3$, 1.8 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, and 11.0 dextrose. Each basilar artery was separated from the surrounding connective tissue and cut into rings (2 mm long). The ring segments were suspended by a pair of stainless-steel pins in a well-oxygenated (95% O_2–5% CO_2) bath of 10-ml KHS at 37 °C. The rings were stretched until an optimal resting tension of 0.3 g was loaded, and then allowed to equilibrate for at least 60 min. Force generation was monitored by means of an isometric transducer (model TB-611T; Nihon Kohden, Tokyo, Japan). Tension was readjusted when necessary and the bath fluid was changed every 15 min. After this period of equilibration, the reactivity of the rings was checked by depolarization with 64 mM KCl. There were no significant differences in the response to KCl among the three groups [257±21, 258±25, and 266±18 mg in control (n=12), insulin-untreated diabetic (n=12), and insulin-treated diabetic (n=12) rats, respectively].

Once the contraction induced by U46619, a thromboxane analogue (3 μM), was established, a concentration–response curve was constructed for the relaxation induced by cromakalim, 1-EBIO, NS1619, or sodium nitroprusside. After the addition of sufficient aliquots of the agonist to produce the chosen concentration, a plateau response was allowed to develop before the addition of the next dose of the same agonist.

In separate experiments, we examined the contractions induced by application of TEA (2.5 mM), a nonselective K_{Ca}-channel blocker, or iberiotoxin (100 nM), a large-conductance K_{Ca}-channel (BK_{Ca}) blocker, in diabetic and age-matched control rats. These contractile responses were each expressed as a percentage of that previously induced by 64 mM KCl in the same ring.

In addition, to investigate the influence of oxidative stress on the cromakalim-induced relaxation in the diabetic basilar artery, a superoxide dismutase mimetic (Tempol, 1 mM) was added to the organ bath 30 min before precontraction of diabetic arteries with U46619. This was followed by the application of 10 μM cromakalim.

2.6. Statistical analysis

Data are expressed as the mean±S.E. When appropriate, statistical differences were assessed by Dunnett's test for multiple comparisons after a one-way analysis of variance (ANOVA), a probability level of $P<0.05$ being regarded as significant. Statistical comparisons between concentration-response curves were made using a two-way ANOVA, with Bonferroni's correction for multiple comparisons being performed post hoc ($P<0.05$ again being considered significant).

3. Results

3.1. Blood glucose, insulin levels, and animal body weights

As reported previously (Matsumoto et al., 2003), at the time of the experiment, all streptozotocin-treated rats exhibited hyperglycemia, their blood glucose concentrations being significantly higher than those of the age-matched nondiabetic control rats (Table 1). Treatment with insulin (5–30 U/kg per day for 2 weeks) in our established diabetic rats produced a plasma glucose concentration that was significantly decreased and a body weight that was significantly increased compared with those of the insulin-

Table 1
Values of various parameters in control rats and insulin-treated and -untreated streptozotocin-induced diabetic rats

Parameters	Control	Diabetic	Insulin-treated diabetic
Body weight (g)	583.3±27.9 (8)	232.7±12.2 (8)[a]	368.5±17.3 (6)[b]
Plasma glucose (mg/dl)	176.0±2.7 (8)	709.8±37.4 (8)[a]	218.3±94.8 (6)[b]
Plasma insulin (ρg/ml)	1050.0±70.0 (8)	192.5±30.5 (8)[a]	6206.7±459.1 (6)[c]
Plasma cholesterol (mg/dl)	94.5±4.5 (8)	154.7±11.8 (6)[a]	120.0±8.9 (6)[c]
Plasma HDL (mg/dl)	57.5±1.2 (8)	61.6±5.3 (8)	62.2±5.6 (6)
Plasma triglyceride (mg/dl)	168.3±15.2 (8)	623.4±72.5 (5)[a]	92.5±10.0 (6)[b]

Number of determinations is shown within parentheses.
[a] $P<0.001$ vs. controls.
[b] $P<0.001$ vs. diabetic.
[c] $P<0.05$ vs. diabetic.

untreated diabetic rats (Table 1). Plasma insulin levels were significantly lower in streptozotocin-induced diabetic rats than in the controls. The plasma insulin concentration was higher in the insulin-treated group than in the insulin-untreated one (Table 1).

3.2. Plasma cholesterol and triglyceride levels

As shown in Table 1, the plasma total cholesterol and triglyceride levels were significantly higher in streptozotocin-induced diabetic rats than in the age-matched controls, while insulin treatment significantly reduced these raised levels. The plasma HDL level did not differ significantly among the three groups.

3.3. Relaxation response to cromakalim

To investigate K_{ATP} channel-induced relaxation, we added cromakalim (10^{-9}–10^{-5} M) cumulatively to rings precontracted by U46619 (3 μM; Fig. 1A, B). The tension developed in response to 3 μM U46619 did not differ significantly among the three groups [224±15, 234±32, and 209±30 mg in control (n=6), insulin-untreated diabetic (n=6), and insulin-treated diabetic (n=6) rats, respectively]. In rings from age-matched control rats, cromakalim (10^{-9}–10^{-5} M) induced a concentration-dependent relaxation, with the maximum response at 10^{-5} M. This relaxation was significantly weaker in rings from streptozotocin-induced diabetic rats (P<0.01 vs. control group). This impaired relaxation response recovered significantly following chronic insulin treatment (P<0.001 vs. diabetic group). Furthermore, rings from streptozotocin-induced diabetic rats chronically treated with insulin relaxed more strongly than those from control rats. The EC_{50} values for the cromakalim-induced relaxations exhibited no significant difference among the three groups, but tended to be increased in the insulin-untreated diabetic group [1.52±0.33, 2.74±0.68, and 1.22±0.39 μM in control (n=6), insulin-untreated diabetic (n=6), and insulin-treated diabetic (n=6) groups, respectively]. The relaxation induced by cromakalim was inhibited by 1 μM glibenclamide, a K_{ATP}-channel blocker, in all three groups (data not shown).

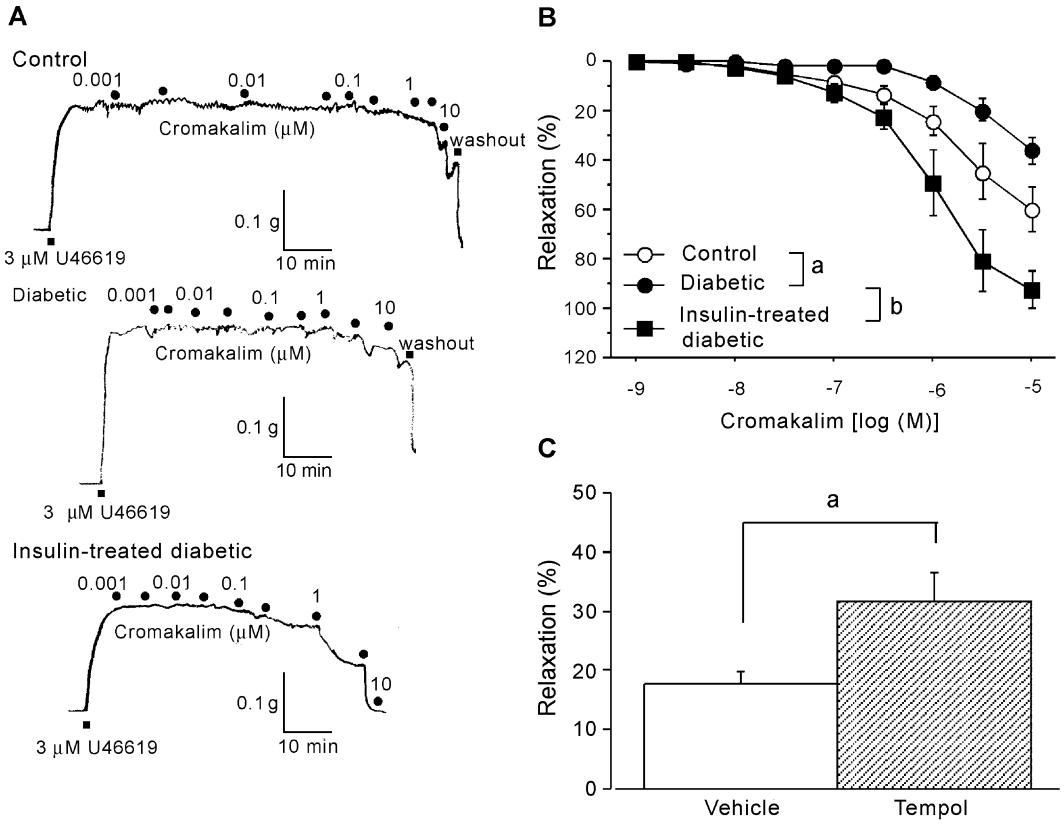

Fig. 1. Relaxation response to an ATP-sensitive potassium-channel opener in the isolated basilar artery. (A) Representative traces showing cromakalim-induced relaxation in basilar arteries taken from age-matched control, diabetic, and insulin-treated diabetic rats. (B) Concentration–response curves for the relaxations induced by cromakalim in rings cut from basilar arteries isolated from age-matched control (○), diabetic (●), and insulin-treated (■) diabetic rats. The y-axis shows relaxation as a percentage of the contraction induced by U-46619 (3 μM). Each data-point represents the mean±S.E. from six experiments, the S.E. mean being included only when it exceeds the dimension of the symbol used. [a]P<0.01, diabetic vs. control. [b]P<0.001, diabetic vs. insulin-treated diabetic. (C) Effect of a superoxide dismutase mimetic on the 10 μM cromakalim-induced relaxation in U46619-precontracted basilar arteries from diabetic rats. Tempol (1

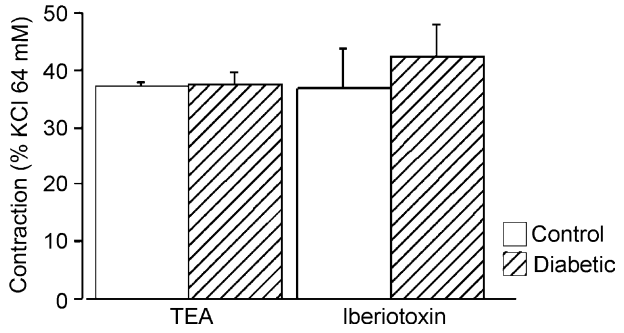

Fig. 2. Effects of tetraethylammonium chloride (TEA, 2.5 mM) and iberiotoxin (100 nM) on vascular tone in basilar artery rings isolated from age-matched controls and diabetic rats. The ordinate shows contraction expressed as a percentage of that previously induced by 64 mM KCl. Each column represents the mean±S.E. of four experiments.

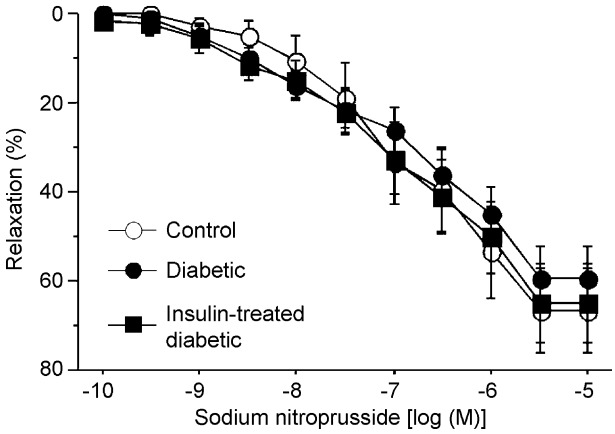

Fig. 4. Concentration–response curves for the sodium nitroprusside-induced relaxation of basilar artery ring isolated from age-matched controls (○), diabetic (●), and insulin-treated diabetic (■) rats. The *y*-axis shows relaxation as a percentage of the contraction induced by U-46619 (3 μM). Each data-point represents the mean±S.E. of eight experiments.

To investigate whether this impaired K_{ATP} channel-induced relaxation in the diabetic basilar artery was associated with oxidative stress, we examined the effect of Tempol, a superoxide dismutase mimetic, on the cromakalim-induced relaxation (Fig. 1C). When the streptozotocin-induced diabetic basilar artery was pretreated with 1 mM Tempol, a reagent that did not alter the U46619-induced contraction, the cromakalim (10 μM)-induced relaxation was significantly enhanced (Fig. 1C).

3.4. Effect of Ca^{2+}-activated potassium channel inhibitors on vascular tone

K_{Ca} channels play important roles in dilation and in the modulation of contractile responses in blood vessels (see Introduction). To relate K_{Ca}-channel activity to vascular tone in the basilar artery, we applied one of two representative K_{Ca}-channel inhibitors to basilar artery rings. When we applied TEA (2.5 mM), a nonselective K_{Ca}-channel blocker, or iberiotoxin (10^{-7} M), a large-conductance K_{Ca}-channel (BK_{Ca}) blocker, a contractile response was observed, and neither response was significantly different between diabetic and age-matched control rats (Fig. 2). On the other hand, the K_{ATP}-channel blocker glibenclamide did not induce a contractile response in isolated basilar artery rings from age-matched control or diabetic rats (data not shown).

3.5. Relaxation responses to 1-EBIO and NS1619

To investigate K_{Ca} channel-induced relaxation, we added one of two commercially available K_{Ca}-channel activators, 1-EBIO (10^{-6}–3×10^{-4} M) or NS1619 (10^{-6}–10^{-4} M), in a cumulative fashion to rings precontracted by U46619 (3 μM; Fig. 3). The relaxation induced by 1-EBIO, an intermediate-conductance K_{Ca}-channel activator, was not significantly different between the age-matched control and diabetic rats (Fig. 3A). Likewise, the relaxation induced by NS1619, a BK_{Ca}-channel activator, did not differ between these two groups (Fig. 3B).

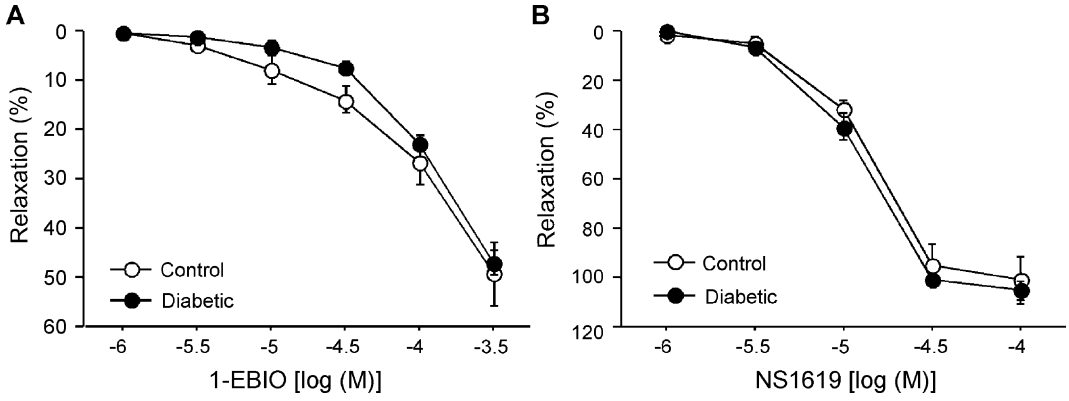

Fig. 3. Concentration–response curves for the relaxations induced by activation of Ca^{2+}-activated K^+ channels in basilar artery rings isolated from age-matched control (○) and diabetic (●) rats. (A) Relaxation induced by an intermediate-conductance Ca^{2+}-activated K^+-channel activator (1-EBIO). (B) Relaxation induced by a large-conductance Ca^{2+}-activated K^+-channel activator (NS1619). The *y*-axis shows relaxation as a percentage of the contraction induced by U-46619 (3 μM). Each data-point represents the mean±S.E. from five (NS1619) or seven (1-EBIO) experiments, the S.E. mean being included only when it exceeds the dimension of the symbol used.

3.6. Relaxation response to sodium nitroprusside

To determine whether the impairment of the relaxation response to cromakalim seen in streptozotocin-induced diabetic rats might be due to some nonspecific effect of diabetes on vasodilation, we examined the responses of basilar artery rings to sodium nitroprusside. This agent induced a dose-dependent relaxation that was similar among age-matched control, insulin-untreated diabetic, and insulin-treated diabetic rats (Fig. 4).

4. Discussion

The main conclusions to be drawn from the present study are that, in rats with established streptozotocin-induced diabetes: (i) activation of K_{ATP} channels, but not of K_{Ca} channels, is impaired in the isolated basilar artery and (ii) chronic insulin treatment reverses the impairment of the relaxation response to cromakalim seen in the diabetic basilar artery.

K_{ATP} channels play important roles in the regulation of the membrane potential and tone in vascular smooth muscle cells, as well as in the regulation of cerebral blood flow. K_{Ca} channels have also been described in vascular smooth muscle, including that in cerebral vessels, and are important targets for mediators released from the endothelium (Faraci and Heistad, 1998). Although activation of K_{ATP} channels appears to be an important mechanism for the cerebral vasodilations evoked by several stimuli, these channels do not appear to influence resting tone in the cerebral circulation (Faraci and Heistad, 1998). On the other hand, K_{Ca} channels are active under basal conditions, and their inhibitory influence on basal tone may be more important in large cerebral arteries than in cerebral arterioles (Faraci and Heistad, 1998). In the present study, glibenclamide did not alter vascular tone in basilar artery rings isolated from diabetic and age-matched control rats (data not shown). On the other hand, TEA and iberiotoxin each induced contractile responses of similar amplitude between diabetic and control rats (Fig. 2). Thus, the present data are congruent with the current consensus and suggest that the inhibition of vascular tone induced by K_{Ca}-channel activation is not impaired in the diabetic state (at least in streptozotocin-induced diabetic rats).

The dilation of the isolated basilar artery induced by sodium nitroprusside was not altered in our diabetic rats (Fig. 4). Thus, the impaired relaxation observed in response to cromakalim cannot be explained by a nonspecific impairment of relaxation in such rats. These findings suggest that K_{ATP}-channel activity is specifically impaired in the diabetic basilar artery. This conclusion is supported by the results of two previous studies on cerebral arteries from diabetic rats (Mayhan and Faraci, 1993; Mayhan, 1994). Mayhan and Faraci (1993) suggested that the dilation of pial arterioles induced by aprikalim (RP52891, an activator of K_{ATP} channels) was not related to the synthesis/release of nitric oxide (NO) or of an NO-containing compound. Thus, it would appear that the impairment of the K_{ATP} channel-induced dilation of cerebral blood vessels seen in diabetes is not related to an effect on the NO pathway. In addition, Mayhan and Faraci (1993) showed that the relaxation of pial arterioles induced by aprikalim was not altered by inhibitors of large-and small-conductance K_{Ca} channels. Thus, such channels would appear not to play a role in the relaxation of cerebral blood vessels induced by activation of K_{ATP} channels nor to play a role in the impaired responses of cerebral blood vessels seen in the diabetic state.

Investigations to assess the vascular responses induced by activation of K_{Ca} channels by chemical stimuli need to be interpreted with caution. For example, NS1619 produces a relaxation of cerebral arteries that appears to involve opening of K_{Ca} channels in vascular smooth muscle (Holland et al., 1996). However, this compound has other vasoactive effects, including inhibition of calcium channels (Holland et al., 1996; Lawson, 1996). 1-EBIO, a putative intermediate-conductance K_{Ca}-channel (IK_{Ca}) activator, has been employed to investigate the role played by IK_{Ca} channels in the arterial EDHF (endothelium-dependent hyperpolarizing factor) pathway (Edwards et al., 1999). Walker et al. (2001) demonstrated that, at concentrations greater than 100 μM, 1-EBIO selectively activates an outward current in endothelial cells, which presumably underlies the smooth muscle hyperpolarization and is a component of the observed relaxation. However, this compound can also relax smooth muscle by an undefined mechanism, independent of any change in membrane potential (Walker et al., 2001). In the present study, these compounds (NS1619 and 1-EBIO) induced relaxations that were not altered in streptozotocin-induced diabetic rats (as compared with the age-matched controls; Fig. 3A, B). Taking all this together, we speculate that K_{Ca} channels do not play a role in the impairment of basilar artery responses seen in streptozotocin-induced diabetic rats. It remains to be determined in future studies whether the relaxations induced by specific K_{Ca} activators (if such compounds are developed) are directly impaired in streptozotocin-induced diabetic rats.

It is not yet clear what kinds of mechanisms might be responsible for the decrease in the relaxation induced by K_{ATP}-channel activators in streptozotocin-induced diabetic rats. One possibility is that diabetes mellitus increases the production of reactive oxygen species in the vascular tissues and that this influences the structure and activity of these ion channels. The following evidence supports this notion. First, in streptozotocin-induced diabetic rats superoxide production is elevated in the aorta due to activation of NAD(P)H oxidase (Hink et al., 2001; Kanie and Kamata, 2002). Moreover, the additional superoxide anions may not be metabolized to H_2O_2 because superoxide dismutase activity is decreased in streptozotocin-induced diabetic rats (Kamata and Kobayashi, 1996; Kobayashi and Kamata, 1999b).

Moreover, the manganese superoxide dismutase mRNA level is significantly lower in basilar arteries from streptozotocin-induced diabetic rats than in those from the controls, whereas the mRNA for gp91phox, an NAD(P)H oxidase subunit, is increased (Matsumoto et al., submitted for publication). In consequence, the local accumulation of superoxide will be enhanced in the diabetic state. Second, K^+ channel-mediated relaxations are impaired in pathophysiological conditions in which excessive production of reactive oxygen species occurs, such as ischemia–reperfusion (Bari et al., 1996), brain injury (Armstead, 2001), and hyperglycemia (Liu and Gutterman, 2002a). The above is supported by evidence suggesting that excess production of superoxide is implicated in the impaired dilator responses to K_{ATP}-channel openers seen in the aorta and in the mesenteric and cerebral arteries of streptozotocin-induced diabetic rats (Liu and Gutterman, 2002b). Furthermore, it has recently been reported that insulin-resistant Zucker obese rats are characterized by the presence of a diminished cromakalim-induced relaxation in the basilar artery (Erdos et al., 2004b). These data suggest that Type 2 diabetic rats have a defective cromakalim-induced relaxation, as also observed in Type 1 diabetic rats in the present study (Fig. 1). In addition, the sodium nitroprusside-induced relaxation is not impaired in Type 2 diabetes (Erdos et al., 2004b) or in Type 1 diabetes (our Fig. 4). Moreover, Erdos et al. (2004a,b) reported (a) that whereas both protein kinase C (PKC) activity and superoxide anion production were increased in the cerebral arteries of Type 2 diabetic rats, pretreatment with superoxide dismutase, but not with PKC inhibitors, restored the impaired cromakalim-induced relaxation response, and (b) that pretreatment of middle cerebral arteries with superoxide dismutase plus catalase restored the diminished dilator response to the K_{ATP} opener pinacidil seen in fructose-fed insulin-resistant rats. In view of the above evidence, it seems likely that continuous production of reactive oxygen species exerts a constant inhibitory effect on K_{ATP} channels in the cerebral arteries of these diabetic models. However, not all K^+ channels are adversely affected by reactive oxygen species. For example, BK_{Ca} channels may compensate for the loss of other vasodilator mechanisms in disease states such as atherosclerosis in which the generation of reactive oxygen species is increased (Liu and Gutterman, 2002a,b). As mentioned above, the present data suggest that K_{Ca} channels do not play a role in the impairment of the basilar artery relaxation response seen in streptozotocin-induced diabetic rats. Thus, the impairment of K_{ATP} channel-mediated vascular responses in the basilar artery seen in our streptozotocin-induced diabetic rats could also be a consequence of an elevated production of reactive oxygen species. Indeed, the present results show that the cromakalim-induced relaxation is enhanced in diabetic basilar arteries pretreated with a superoxide dismutase mimetic (Fig. 1C). It remains to be fully determined, however, whether the presence of different susceptibilities to oxidative stress leads to a discrepancy between K_{ATP} and K_{Ca} channels in the streptozotocin-induced diabetic basilar artery.

The next issue to be discussed is the possible site at which insulin acts to restore cromakalim-induced relaxation responses following their impairment in the diabetic state. The presence of high cholesterol and triglyceride levels in the plasma is thought to be an important factor in cardiovascular diseases. In our previous studies, fructose-fed animals (a model of triglyceride-rich insulin-resistant diabetes) were found to exhibit a markedly increased plasma triglyceride level as well as an impaired endothelium-dependent relaxation, suggesting that an increased plasma triglyceride level may be a risk factor for vascular disease (Kamata and Yamashita, 1999; Kamata et al., 2001). Furthermore, it has been reported that an elevated triglyceride level is associated with an increased risk of mortality in cerebrovascular disease (Shahar et al., 2003). In fact, the insulin resistance induced by a high-fructose diet impairs the function of both K_{Ca} and K_{ATP} channels in rat middle cerebral arteries (Erdos et al., 2002). In the present study, chronic insulin treatment was found greatly to improve the abnormality of lipid metabolism and greatly to decrease the glucose level in streptozotocin-induced diabetic rats (Table 1). Thus, we demonstrated that the improvement of the relaxation response to cromakalim seen in insulin-treated diabetic rats was associated, perhaps causally, with an alleviation of oxidative stress via an improvement in lipid metabolism and a glucose-lowering effect. Moreover, we recently found that the mRNA for p22phox, an NAD(P)H oxidase subunit, is significantly decreased in the insulin-treated streptozotocin-induced diabetic basilar artery (vs. insulin-untreated diabetic; Matsumoto et al., submitted for publication). Because NAD(P)H oxidase is the major source of superoxide in the vascular wall, the resulting decreased NAD(P)H oxidase activity may lead to a reduced oxidative stress in this insulin-treated diabetic basilar artery. Taking the above evidence together suggests to us that chronic treatment with insulin restores the impaired K_{ATP} activity seen in the basilar artery in the streptozotocin-induced diabetic rats by reducing the production of reactive oxygen species through an improvement in metabolism and/or by reducing NAD(P)H activity.

Another possibility as to the site of action of insulin is indicated by the accumulating body of evidence indicating that insulin, independently of its effects on intermediary metabolism, may have a direct vasorelaxant action (Standley et al., 1991; Kobayashi and Kamata, 2002) and also attenuate the contractile responses of vascular smooth muscle to various agonists (Zemel et al., 1992; Han et al., 1995). Insulin has been shown to inhibit an inward Ca^{2+} current (Standley et al., 1991) and to stimulate the Na^+–K^+ pump, which leads to hyperpolarization of the cell membrane, thereby decreasing Ca^{2+}-ATPase activity in the plasma membrane and increasing Ca^{2+} extrusion from the cell (Zemel et al., 1992). On the other hand, Sugai et al. (1999) suggested that in the resting condition, levcromakalim

reduced $[Ca^{2+}]_i$ and vascular tone in basilar arteries more profoundly in preparations from subarachnoid hemorrhage-model dogs, through the opening of K_{ATP} channels, without any contribution by BK_{Ca} channels and independent by of their state. Furthermore, levcromakalim reduced the serotonin-induced increase in $[Ca^{2+}]_i$ and contractile force in subarachnoid hemorrhage-model dogs as well as in control dogs (Sugai et al., 1999). Recent evidence also suggests a potential link between insulin signaling and K_{ATP}-channel activation. Thus insulin has been shown to regulate K_{ATP}-channel activity (Spanswick et al., 2000) by increasing the open-state probability of the channel and by decreasing the channel's sensitivity to ATP (Tricarico et al., 1997). However, we did not try to observe a vascular response to direct insulin application in the isolated basilar artery, and it remains to be determined in future studies on the basilar artery whether insulin can directly alter vascular tone or modify K_{ATP}-channel activity in streptozotocin-induced diabetes.

In conclusion, the results of the present study indicate that, in streptozotocin-induced diabetes mellitus in rats, the function of K_{ATP} channels in the basilar artery may be impaired and that chronic insulin treatment in this model of established diabetes may be beneficial for the restoration of K_{ATP}-channel function. The altered cerebrovascular regulation resulting from the impairment of the functions of these channels may be responsible, at least in part, for the increased risk of cerebrovascular events seen in diabetes mellitus.

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