Addendum

Selective Downregulation of the BKβ1 Subunit in Diabetic Arteriolar Myocytes

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ABSTRACT

Diabetic retinopathy is an important cause of visual loss. Functional abnormalities including vasoconstriction precede structural changes. Using the streptozotocin-model of diabetes in rats, we have identified downregulation of the β1 subunit of the BK channel in arteriole myocytes as a possible molecular mechanism underlying these early changes. BKβ1 mRNA levels were reduced as early as one month after induction of diabetes, and BK Ca2+-sensitivity and caffeine-evoked BK currents were reduced at three months. This effect appears to be selective for the regulatory subunit, as BKα subunit expression was not altered at the mRNA level, and voltage-activated BK currents were unaltered. No changes were seen in voltage activated Ca2+-current, Ca2+-activated Cl-current, or A-type voltage activated K+-currents. Reduced Ca2+-activated BK activity may promote depolarization, Ca2+-channel activation and increased contraction under resting conditions or in response to Ca2+-mobilizing agonists.

Diabetes mellitus is a major metabolic disorder which adversely affects most systems in the body. Many of the complications of this condition are microvascular in nature, leading to impaired tissue repair, nephropathy and retinopathy. Although the devastating complications of diabetes have long been recognised and are well documented, the underlying molecular mechanisms responsible remain unclear. Our recent paper on the effects of streptozotocin-induced diabetes on rat retinal arterioles suggests that one important cellular target may be large conductance, Ca2+-activated K+-channel (BK-channel) activity. This was dramatically reduced in arteriole smooth muscle three months after induction of diabetes. This did not appear to result from downregulation of the pore-forming α-subunit, since neither the relevant mRNA levels nor the voltage-activated BK-current was altered in arterioles from diabetic animals. Similarly, there was no change in caffeine evoked Ca2+-transients, while spontaneous Ca2+-sparks were actually increased in amplitude. This suggests that the reductions seen in both caffeine-evoked and spontaneous BK-current (spontaneous transient outward currents or STOCs) reflected uncoupling of Ca2+-signalling from BK channel activation. One possible explanation of such uncoupling could be reduced Ca2+-sensitivity of the BK channels themselves. Voltage-clamp experiments using off-cell, inside-out patches demonstrated that the [Ca2+] dependence of channel open probability was indeed shifted towards higher concentrations in myocytes from diabetic animals. This functional change was paralleled by a reduction in the gene and protein expression of the Bkβ1 subunit, which is known to influence the Ca2+-sensitivity of BK channels. It appears, therefore, that this downregulation decreases BK channel activity in these cells, reducing negative feedback by intracellular [Ca2+] and promoting contraction. This may play a role in the vasoconstriction and reduced retinal blood flow, which are often seen as the earliest functional changes in diabetic patients.

New, previously unpublished data suggests that this effect may occur very early in the diabetic state. Quantitative PCR, carried out as described in our previous paper (see ref. 1), showed that mRNA levels for the BKβ1-subunit (normalized to β-actin mRNA levels) were reduced by 30 ± 5% (mean ± SEM) after only one month of diabetes when compared to age-matched controls (p < 0.05, three replicates in each of three experiments). The effect was time-dependent, however, and the reduction in transcript levels was increased to 60% at three months. As well as being early in onset, it also appears that this effect is highly selective. No changes were seen when a range of other conductances were compared in three-month diabetic and age-matched control tissues. We have already...
Ca²⁺-channels was also largely unaffected three months after streptozotocin treatment (Fig. 1A). This was studied under divalent-free conditions to increase the amplitude of the currents, which are small in these cells (see Methods in ref. 5). The peak inward current was observed at -20 mV in both tissue groups, with a mean current density of 5.7±1.7 pA/pF (n = 4) in control arterioles and 4.5±0.4 pA/pF in vessels from diabetic animals (n = 5; NS). Caffeine-activated BK current was reduced by almost 100% over the physiologically relevant range of potentials (Fig. 2 in ref. 1). Taken together, these findings allow for a model in which reduced BK currents could result in increased vascular tone in the arterioles of diabetic animals through membrane depolarization and increased Ca²⁺-channel activity.⁶

Diabetic modulation of K⁺-currents in arteriolar myocytes also appears to be selective. We have previously identified a ‘transient outward’, or A-type K⁺-current in retinal arteriolar smooth muscle.⁷ Our evidence suggests that this current results from voltage-dependent activation of channels containing Kᵥ1.5 subunits, probably coassembled with Kᵥβ₁ subunits.⁸ When this current was compared for arteriolar myocytes from control and three-month diabetic rats, there was no change in the kinetics of the current, or in the peak-current density and voltage dependence of activation and inactivation (Fig. 1B and C). In particular, inactivation rates were unaltered, with a mean time constant of 0.097±0.009 s (n = 15) in myocytes from diabetic animals and 0.083±0.007 s in controls (n = 18; NS). Evidence from expression systems suggests that it is the Kᵥβ₁ subunit which results in the rapid inactivation of the Kᵥ1.5 current in retinal arteriole, since Kᵥ1.5 α-subunits generate slowly inactivating, delayed rectifier currents when expressed alone.⁹,¹⁰

One might speculate, therefore, that neither expression nor assembly of the α- and β-subunits responsible for the A-current is affected by diabetes in the smooth muscle of the retinal arterioles. This contrasts somewhat with the acute effects of glucose elevation, which reduces Kᵥ currents in isolated vascular myocytes.¹¹ This inhibition was seen over the physiological range of glucose, however, and may be unrelated to the complex, chronic effects of diabetes mellitus.

These findings suggest a number of important research questions related both to the cellular mechanisms responsible for the downregulation of BKβ₁ expression and the pathophysiological consequences resulting from it. It will be important to determine which signalling pathways and transcription factors downregulate BKβ₁ expression in vascular smooth muscle in diabetes. Further study will also be needed to establish what role if any this plays in the development of diabetic retinopathy. However, it may help explain the vascular constriction presumably resulting in relative retinal ischemia, which has been documented in human diabetes before retinopathy is established.⁴ It also seems likely that

Figure 1. Diabetes mellitus and ion channel function in retinal arterioles. All experiments compared control vessels with those from three-month diabetic animals, diabetes having been induced with streptozotocin (see Methods in ref. 1). (Ai). Voltage dependent activation of Ca²⁺-channels was recorded at 37°C in divalent free solution using depolarizing steps from a holding potential of -80 mV. K⁺-currents were inhibited using Penitrem A (100 nM) and 4-aminopyridine (10 mM) in the bath, and CsCl pipette solution, while Cl⁻ currents were blocked using 9-anthracene carboxylic acid (9AC, 1 mM) in the bath. A(ii) Average peak inward-current density ±SEM plotted against depolarizing voltage for arterioles from diabetic (n = 5) and non-diabetic (n = 4) animals. (B) Fast-inactivating or A-type K⁺-currents were recorded at 37°C in the presence of Penitrem A (100 nM) to block BK currents, and 9-AC (1 mM) to block Cl⁻ currents. Current traces are shown for arterioles isolated from both a nondiabetic (Bi) and a diabetic (Bii) animal. (C) Summary data for experiments similar to those in (Bi). (Ci). Average peak current density plotted against membrane potential for arterioles from diabetic (n = 15) and non-diabetic (n = 18) animals. (Cii) Data has been replotted in terms of normalized conductance to generate the activation curves (ascending plots). Separate, twp-step inactivation protocols were also carried out using an inactivating step to varying potentials and lasting 3 seconds followed by a test step to +60 mV (see Protocols and Equations in ref. 7). Data was normalized to the peak current during the test step and summarized for arterioles from diabetic (n = 12) and non-diabetic (n = 15) animals.
this effect will not prove to be restricted to the retinal vasculature. If a more generalised increase in arteriole tone resulting from decreased BK activity is confirmed in diabetic animals, then that may have a number of important clinical consequences. Hypertension is increased in incidence in diabetic patients and this compounds the risk of complications over that of an individual suffering from diabetes alone. Some, but not all, of this increased incidence results from diabetic nephropathy. It may be, however, that an increase in peripheral vascular resistance secondary to a reduction in resting or agonist-evoked BK current also contributes to the elevation of blood pressure, particularly in the early stages of the disease. Since angiotensin-induced hypertension itself can result in downregulation of the BKβ1 subunit, there is also potential for pathological positive feedback. Given the heavy personal, social, clinical and financial costs resulting from the vascular consequences of diabetes, these questions deserve urgent attention.

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