Enhanced BK ($I_{K(ca)}$) channel activity contributes to impaired myogenic response in the cerebral circulation of Fawn Hooded Hypertensive (FHH) Rats

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Abstract

The present study examined the role of alterations in Iberiotoxin (IBTX) sensitive IK(ca) channel activity in impaired myogenic response in the cerebral circulation of Fawn Hooded Hypertensive (FHH) rats. Isolated perfused middle cerebral arterioles from FHH rats exhibited no myogenic response when pressure was increased from 40 to 140 mmHg. In contrast, vascular diameter decreased by about 50% in an FHH.1BN AR+ congenic strain in which a 2.6 Mb region of BN chromosome 1 was introgressed into the FHH genetic background. Whole-cell patch-clamp (1μM free calcium; +40mV) of cerebral vascular smooth muscle cells revealed amplified IK(ca) current densities (pA/pF) in FHH rats compared to AR+ and SD rats (23.2+6;6.9+1.9 and 7.1+1.3 respectively). Additionally, in inside-out patch clamping, IK(ca) exhibited similar unitary conductance (235 and 245 pS) in both strains. However, open probability of IK(ca) were significantly higher in FHH rats compared to AR+ rats (0.82+0.04 and 0.3+0.09 respectively). These results suggest that enhanced activity of the IK(ca) channel contributes to loss of myogenic response in the cerebral circulation of FHH rats. NIH HL36279, HL59996, DK79306. Roman.

Introduction

The Fawn Hooded Hypertensive (FHH) rat is a genetic model of renal disease that develops systolic hypertension and end stage renal disease (ESRD). Previous studies from our laboratory suggest that FHH rats exhibit impaired autoregulation of renal blood flow (RBF) and transfer of a 12.8 Mb or 4.3 Mbp region on chromosome 1 from the BN rat onto the FHH genetic background (FHH. 1^{BN}) restored auto regulation of RBF. Further studies suggest that impaired RBF autoregulation in FHH rats is due to impaired myogenic response of renal afferent arteriole that leads to transfer of high systemic blood pressure to glomerular capillaries. Along these lines, we hypothesized that FHH rats will also exhibit impaired autoregulation of cerebral blood flow (CBF) due to impaired myogenic response in cerebral vasculature.

Hypothesis

Our Hypothesis is that "the impaired myogenic response in FHH rats is due to a mutation in one of the genes in the 2.6Mb region that contains 11 genes and that transfer of this region restores the myogenic response in FHH rats and the impaired myogenic response in FHH rats is associated with enhanced activity of large conductance potassium channel (BK)".

Materials and methods

Animals: Experiments were performed on 9-12 week old male FHH, FHH.1^{BN} congenic rats and SD rats that were obtained from inbred colonies maintained at the University of Mississippi Medical center (UMMC).

Protocol 1: *Vascular reactivity studies* in the presence and absence of calcium

Middle cerebral arteries (MCA) (inner diameter ,100 μm, 8-10mm length) were micro dissected under a stereomicroscope (X60, Leica, Bannockburn, IL). MCAs were cannulated with glass micropipettes (<100μm; FHC, Brunswick, ME) and pressurized with PSS to 40 mmHg. Vascular diameter was measured using a videomicrometer (VIA-100, Boeckeler Instruments, Tucson, AZ). After a 60-min equilibration period, a pre-IBTX myogenic response curve was constructed by measuring internal diameter of the vessels as transmural pressure was varied from 40 to 140 mmHg in steps of 20 mmHg was recorded.

Protocol 2: *Vascular reactivity studies* in the presence and absence of IBTX

The pressure-diameter curves were redetermined in the presence of 300nm IBTX.

Protocol 3. Isolation of cerebral vascular smooth muscle cells (VSMC).

For studies of BK channel vascular smooth muscle cells (VSMC) were isolated from middle cerebral arteries (inner diameter ,100 μm) from the brain of Sprague-Dawley (SD), FHH and FHH.1^{BN} rats (9-12 weeks).

Protocol 4. whole-cell Patch-clamp studies. BK currents were recorded from VSMC using the whole cell patch-clamp mode at room temperature. An Axopatch 200B amplifier (Axon Instruments, Foster City, CA) will be used to clamp pipette potential and record whole cell and single-channel currents. Membrane capacitance (in pF) will be obtained by integrating the average pulse (30mV). Peak current is expressed as current density (pA/pF) to normalize for differences of cell sizes.

Protocol 5. Single channel analysis:

An Axopatch 200B amplifier (Axon Instruments, Foster City, CA) was used to clamp pipette potential and record single-channel currents. Open-state probability (*NPo*) for single-channel currents, expressed as a percentage of the total recording time in which a channel was open, was calculated using the following equation $NPo = (\sum Tj \; X \; j)/T$; where *Tj* is the sum of the open time at a given conductance level, *j* represents multiples of a given conductance, and *T* is the total recording time.

Fig. 1

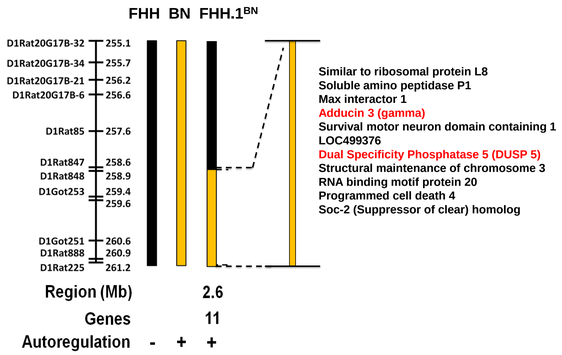

Fig 1. Genetic map illustrating the introgressed regions in control FHH AR- and FHH.1^{BN} AR+ congenic strains. *Left:* indicates the location of genetic markers used to genotype the animals on chromosome 1. The closed and open filled bars refer to Fawn hooded-hypertensive (FHH) and Brown Norway (BN) genomes, respectively. *Right:* indicates some of the known candidate genes in the 2.6 Mb region of interest. + Autoregulation present; - Autoregulation absent.

Fig. 2

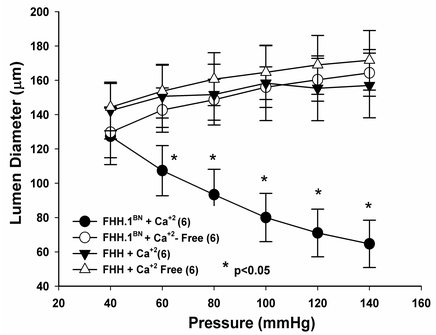

Fig 2. Middle cerebral arteries (MCA) of FHH rats exhibit impaired myogenic response that is restored in FHH.1^{BN} rats. Lumen diameter of middle cerebral arteries (MCA) dissected from FHH rats do not exhibit myogenic response in response to graded increase in perfusion pressure (mmHg) both in the presence (n = 6 vessels) and absence (n = 6 vessels) of Ca^{+2} in physiological salt solution (PSS). In contrary, the myogenic response is restored in FHH.1^{BN} rats. Values are means ± SE. *p <0.05 compared to FHH.

Fig. 3

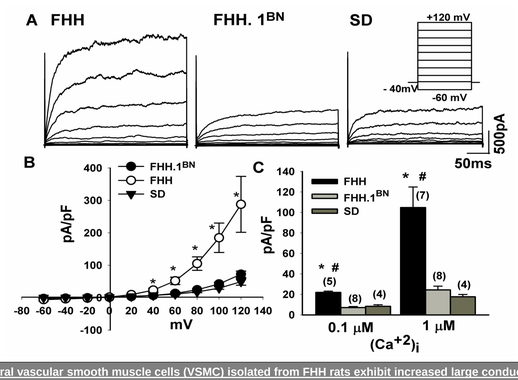

Fig 3. Cerebral vascular smooth muscle cells (VSMC) isolated from FHH rats exhibit increased large conductance calcium-activated potassium channel (BK) currents. Whole cell BK currents were recorded from freshly dissociated cerebral VSMC from FHH, FHH.1^{BN} and SD rats during 300-ms voltage steps from -60 to +120 mV in 20-mV increments from V_h of -40 mV at 1μM free cytosolic calcium (Ca^{+2})$_i$ (protocol) A. Representative BK currents from FHH, FHH.1^{BN} and SD rats. B. I-V curves show greater peak current density (pA/pF) in FHH rats (n = 7) than FHH.1^{BN} (n = 8) and SD rats (n = 3). *p <0.05 compared to FHH.1^{BN} and control rats C. Summary graph of BK current densities at +80mV in FHH, FHH.1^{BN} and SD rats at 0.1 and at 1μM free cytosolic calcium (Ca^{+2})$_i$. *p<0.05 compared to FHH.1^{BN} and # p<0.05 compared to control rats.

Fig. 4

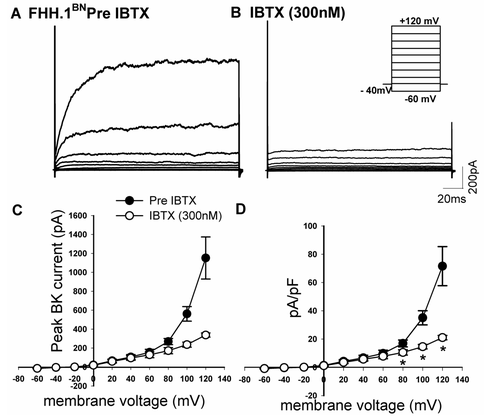

Fig 4. Cerebral vascular smooth muscle cells (VSMC) isolated from FHH.1^{BN} minimal congenic strain exhibit Iberiotoxin (IBTX) sensitive large conductance calcium activated potassium channel (BK) currents. Whole cell BK currents were recorded from freshly dissociated cerebral VSMC from FHH.1^{BN} minimal congenic strain at 300-ms voltage steps from -60 to +120 mV in 20-mV increments from V_h of -40 mV at 1μM free cytosolic calcium (Ca^{+2})$_i$ (protocol). Representative BK currents from FHH.1^{BN} rats before **(A)** and **(B)** after IBTX. I-V curves show peak currents **(C)** and current densities (pA/pF) (D) before and after IBTX in FHH.1^{BN} minimal congenic strain .*p <0.05 compared to Pre IBTX.

Fig. 5

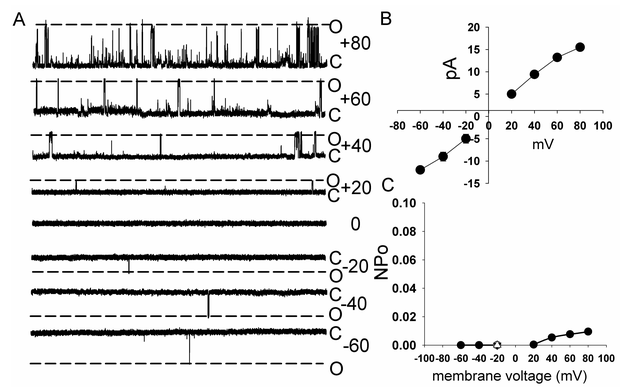

Fig 5 . BK single channel activity in VSMC isolated from FHH.1^{BN} rats. Inside-out patch clamp technique was used in cerebral vascular smooth muscle cells (VSMC) isolated from FHH. 1^{BN} rats. At 100nM (Ca^{+2})$_i$ IV curve of BK channel exhibit more activity at +Ve potentials compared to –Ve potentials (A and C). Note BK channel exhibit unitary conductance at both +Ve and –Ve membrane potentials (B).

Fig. 6

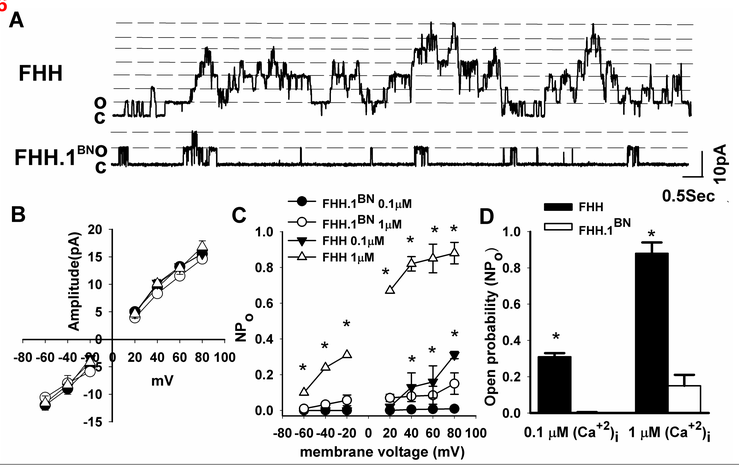

Fig 6. FHH rats exhibit increased large conductance calcium activated potassium channel (BK) activity. BK single channel currents recorded from inside-out patch clamp of VSMC obtained from FHH and FHH. 1^{BN} A. Representative BK single channel currents at -40mV pipette potential (PP) and 1μM free calcium in bath solution showing increased NPo and number of channels in FHH rats compared to FHH. 1^{BN} congenic rats B. single-channel current amplitudes obtained at 0.1 and 1μM bath-Ca^{+2} are plotted as a function of membrane potential. C. NP_o is plotted as a function of membrane voltage (mV). NPo is higher in FHH rats compared to FHH.1^{BN} rats at 0.1 and 1μM bath solution -Ca^{+2} (FHH: n = 3-5 patches per data point;– at 0.1 and 1μM free calcium) (FHH. 1^{BN}: n = 3-5 patches per data point present ;– at 0.1 and 1μM free calcium) D. summary graph of NPo at -80mV PP in FHH and FHH. 1^{BN} rats at 0.1 and 1μM bath -Ca^{+2}.

Fig. 7

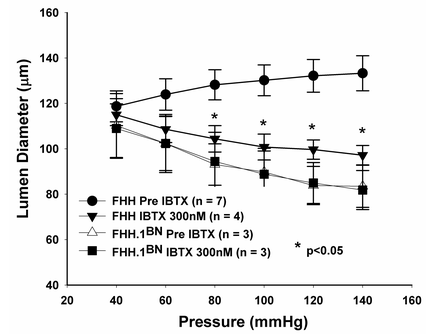

Fig 6. Impaired myogenic response in middle cerebral arteries (MCA) of FHH rats can be restored by the BK channel blocker iberiotoxin (IBTX). MCA isolated from FHH rats exhibit impaired myogenic response (n=7 vessels) that is restored after IBTX application (300nm; n=4 vessels). In contrast MCA isolated from FHH.1^{BN} exhibit myogenic constriction in response to increases in perfusion pressure both in the presence (n=3 vessels) and absence (n=3 vessels) of 300nm IBTX. Values are means ± SE. *p <0.05 compared to FHH pre-IBTX data.

Summary and Conclusions

- **FHH rats exhibit impaired myogenic response which is restored in FHH.1^{BN} minimal congenic strain**
- **FHH rats exhibit increased IBTX sensitive BK channel activity compared to FHH.1^{BN} and SD rats**
- **IBTX partially restores impaired myogenic response in FHH rats**
- **The impaired myogenic response in FHH rats is due to a mutation in one of the genes in the 2.6Mb region that contains 11 genes and that transfer of this region restores myogenic response.**

Acknowledgements

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