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Temporal Profile of Potassium Channel Dysfunction in Cerebrovascular Smooth Muscle after Experimental Subarachnoid Haemorrhage

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Abstract

The pathogenesis of cerebral vasospasm after subarachnoid hemorrhage (SAH) involves sustained contraction of arterial smooth muscle cells that is maximal 6 to 8 days after SAH. We reported that function of voltage-gated K⁺ (K_V) channels was significantly decreased during vasospasm 7 days after SAH in dogs. Since arterial constriction is regulated by membrane potential that in turn is determined predominately by K⁺ conductance, the compromised K⁺ channel dysfunction may cause vasospasm. Additional support for this hypothesis would be demonstration that K⁺ channel dysfunction is temporally coincident with vasospasm. To test this hypothesis, SAH was created using the double hemorrhage model in dogs and smooth muscle cells from the basilar artery, which develops vasospasm, were isolated 4 (early vasospasm), 7 (during vasospasm) and 21 (after vasospasm) days after SAH and studied using patch-clamp electrophysiology. We investigated the 2 main K⁺ channels (K_V and large-conductance voltage/Ca²⁺ activated (K_{Ca}) channels). Electrophysiologic function of K_{Ca} channels was preserved at all times after SAH. In contrast, function of K_V channels was significantly decreased at all times after SAH. The decrease in cell size and degree of K_V channel dysfunction was maximal 7 days after SAH. The results suggests that K_V channel dysfunction either only partially contributes to vasospasm after SAH or that compensatory mechanisms develop that lead to resolution of vasospasm before K_V channels recover their function.

Keywords

Cerebral vasospasm; K⁺ channels; subarachnoid haemorrhage

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INTRODUCTION

Cerebral vasospasm is transient narrowing of intradural subarachnoid arteries seen angiographically 4 to 12 days after subarachnoid hemorrhage (SAH). It causes cerebral ischemia, infarction and sometimes death in these patients. A mechanism of vasospasm may be sustained contraction of smooth muscle cells in the large conducting arteries of the circle of Willis [11].

The double hemorrhage model of SAH in dogs reproduces vasospasm with a similar temporal profile and severity to that seen in patients [14]. We reported that the function of voltage-gated K^+ (K_V) channels was significantly decreased during vasospasm 7 days after SAH in dogs [6]. Arterial diameter is regulated by smooth muscle membrane potential that controls influx of external Ca^{2+} through voltage-gated Ca^{2+} channels [19]. Membrane potential in turn is largely, but not solely, determined by K^+ conductance. This suggests that decreased K^+ channel function contributes to vasospasm. Additional evidence to support this hypothesis would be that pharmacologic or molecular manipulation of K_V channels prevented vasospasm and that the time course of alteration in K_V channels correlated with vasospasm. Pharmacologic blockade of K^+ channels has prevented experimental vasospasm but these drugs are nonspecific [9;10;23]. Molecular manipulation of proteins in dog basilar artery is not possible at present. Therefore, this study investigated function of the 2 main K^+ channels in dog basilar artery (K_V and large-conductance voltage/ Ca^{2+} -activated K^+ [K_{Ca}] channels) during the time course of vasospasm after SAH in dogs.

MATERIALS AND METHODS

Dog Model of SAH and Isolation of Smooth Muscle Cells

Mongrel dogs weighing 15-20 kg were randomly allocated to be controls or subjected to SAH [13;21]. Dogs were placed under general anesthesia and underwent baseline cerebral angiography on day 0. After angiography, they were turned prone and the cisterna magna was punctured. 0.3 ml kg^{-1} cerebrospinal fluid was removed and 0.5 ml kg^{-1} of autologous, arterial, nonheparinized blood was injected into the cisterna magna at 5 ml min^{-1} . The cisternal blood injection was repeated on day 2. Control animals were sacrificed after angiography on day 0, while all other animals underwent repeat angiography 4, 7 or 21 days after SAH and were then sacrificed. Animals sacrificed on day 21 had angiography on day 7. Procedures on animals were approved by the Institutional Animal Care Committee.

Smooth muscle cells were isolated from basilar arteries by enzymatic dissociation [6;15;21]. Animals were euthanized under general anesthesia by exsanguination and perfusion with ice-cold phosphate-buffered saline (PBS, pH 7.4). The basilar artery was removed and placed in dissection solution (in mmol l^{-1} : NaCl 130, KCl 5, $MgCl_2$ 1.3, 4-[2-hydroxyethyl]piperazine-1-ethanesulfonic acid [HEPES] 10, glucose 5 with 100 units ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin). Arteries were cut into small pieces and smooth muscle cells isolated by enzymatic digestion in the dissection solution containing 500 units ml^{-1} collagenase type IV, 50 units ml^{-1} elastase, 100 units ml^{-1} DNase I and 1 mg ml^{-1} trypsin inhibitor.

Electrophysiology

Cells were plated in a chamber (180 μl , Warner Instruments, Hamden, CT) mounted on an inverted microscope (Nikon Diaphot 200, Nikon, Tokyo, Japan)[6;15;21]. Macroscopic currents were recorded using the whole-cell configuration of the patch-clamp method. Currents were amplified using an Axopatch-1D amplifier (Axon Instruments, Union City, CA) and digitized at 12-bits using a DigiData 1200 interface controlled by pClamp 6 (Axon Instruments, Union City, CA). Series resistance in whole-cell recordings was usually $< 5 M\Omega$ and was

compensated by $\geq 80\%$. Recordings were at room temperature (22°C). Current analyses were performed using custom-written software in Igor (Wavemetrics, Lake Oswego, OR). K_V currents were isolated from K_{Ca} currents by intracellular Ca^{2+} buffering with 1,2-bis(2-aminophenoxy)ethane- N,N,N',N' -tetraacetic acid (BAPTA, tetrapotassium salt) and block of L-type Ca^{2+} channels by external Cd^{2+} . The intracellular solution for K_V currents contained (in mmol l^{-1}): KCl 88.6, KOH 6.4, K_2ATP 5, NaGTP 0.1, BAPTA 10, MgCl_2 2.5, HEPES 10, pH 7.2 with KOH. The resulting currents were insensitive to paxilline ($1 \mu\text{mol l}^{-1}$) and glyburide ($10 \mu\text{mol l}^{-1}$), showing no contamination with K_{Ca} current and no contribution of ATP-sensitive K^+ (K_{ATP}) channels. For lengthy protocols such as inactivation and recovery from inactivation, brief hyperpolarizing pulses were included at the end of each episode to facilitate calculation and subtraction of linear leak.

K_{Ca} currents recorded with standard $140 \text{ mmol l}^{-1} \text{ K}^+$ internal solutions usually exceeded 15–20 nA in response to the large depolarizing steps ($> 120 \text{ mV}$), which was necessary to determine K_{Ca} voltage-dependency and kinetics. Such currents imposed significant voltage-control errors despite series resistance compensation and usually resulted in oscillations in the compensation circuitry and seal loss. To minimize this, K^+ was lowered and replaced with choline, as previously reported by others [2]. The intracellular K_{Ca} solution thus consisted of (in mmol l^{-1}): KCl 20, choline-Cl 120, MgCl_2 1.5, CaCl_2 2.65, ethylene glycol-bis(2-aminoethylether)- N,N,N',N' -tetraacetic acid (EGTA) 5, HEPES 5, glucose 5, pH 7.2 with KOH. Under these conditions, outward K^+ currents were dominated by K_{Ca} current, as demonstrated by near complete block of such currents by $1 \mu\text{mol l}^{-1}$ paxilline, a fungal mycotoxin that specifically blocks K_{Ca} channels with high affinity [8;18].

All chemicals were from Sigma-Aldrich (St. Louis, MO).

Data Analysis

Vasospasm was assessed by comparing diameters of basilar arteries on angiograms taken at constant exposure and magnification and measured at 5 predetermined points by 2 blinded observers using an optical micrometer. Macroscopic current analyses were performed using custom-written software in Igor. Data were compared using t-tests or analysis of variance (ANOVA, SigmaStat, SPSS, Chicago, IL). $P < 0.05$ was considered significant. Linear regression was performed using the least-squares method and fits to curves were performed using the Levenburg-Marquardt algorithm in Sigmaplot (Sigmaplot, SPSS) or Igor. All data are means $\pm$ standard errors of the means.

RESULTS

Time Course of Vasospasm

Angiography showed significant reduction in basilar artery diameter at all 3 times after SAH (4, 7 and 21 days, $P < 0.001$ as compared to control, Fig. 1). The reduction of basilar artery diameter was 55% 4 (0.71 ± 0.07 , $n = 8$), 54% 7 (0.72 ± 0.34 , $n = 17$) and 33% 21 days (1.04 ± 0.28 , $n = 7$) after SAH compared to a control diameter of $1.55 \pm 0.15 \text{ mm}$ ($n = 39$). The basilar diameter was significantly larger 21 days after SAH compared to 4 or 7 days post SAH ($P < 0.01$) whereas there was no difference between the diameter 4 and 7 days after SAH. There were no significant differences in physiological parameters (blood pressure, endtidal PCO_2 , PAO_2 , PACO_2 , body temperature and heart rate) between groups at day 0 or in SAH dogs between day 0 and the day of sacrifice (data not shown).

Temporal Changes in Size of Basilar Artery Myocytes After SAH

Cell capacitance, which is a measure of cell size, was measured during whole-cell patch clamp experiments (Fig. 1B). There was a significant decrease in cell capacitance at all 3 times after

SAH as compared to controls (28.3 ± 0.6 , $n = 76$ cells, $P < 0.01$). There was a 9% decrease in cell capacitance 4 (25.7 ± 0.8 , $n = 71$), a 27% decrease 7 (20.6 ± 0.5 , $n = 48$) and a 19% decrease 21 days after SAH (22.8 ± 0.6 , $n = 43$, each group significantly different from any other, ANOVA, $P < 0.01 - 0.001$). There were no significant differences between groups in membrane and series resistance of the patch-clamped smooth muscle cells (Fig. 1C and 1D, $P > 0.05$).

Temporal Profile of K_V Channels: Voltage-Dependence and Kinetics

We previously characterized K_V current (IK_V) from dog basilar artery and validated protocols to characterize these currents [6]. The same protocols were used in the present study. IK_V amplitude was significantly smaller at all 3 times after SAH at voltages more positive than 0 mV (Fig. 2A to 2D, Fig. 3A, $P < 0.05$). Although at a holding potential of 10 to 40 mV, the absolute IK_V amplitude 7 days after SAH was smaller than that 4 days after SAH (Fig. 3A), the current density was not significantly different at any holding potential between any of the 3 times after SAH (Fig. 3B, $P > 0.05$).

The voltage-dependence of activation and inactivation was similar between control and SAH myocytes (Fig. 3I and 3J) except that the conductance of myocytes from the 3 SAH groups was significantly less than control cells at holding potentials of -20 and -10 mV (Fig. 3J, $P < 0.05$). The mid-potentials for activation in SAH myocytes were shifted to more negative voltages at all times after SAH (-3.40 ± 0.71 mV 4 days, -3.65 ± 0.62 mV 7 days and -4.43 ± 0.61 mV 21 days after SAH, Fig. 3C, $P < 0.05 - 0.001$ compared to control cells [-1.33 ± 0.56 mV]). However, there were no statistically significant differences between the SAH groups (Fig. 3C, $P > 0.05$). There also was an identical shift of the mid-potential for inactivation at all 3 times after SAH compared to controls but no difference among the times after SAH (Fig. 3F, $P < 0.01 - 0.001$ as compared to controls).

In contrast, the slope factors, K , for both activation and inactivation for myocytes 4 and 21 days after SAH were not different from control (Fig. 3D and 3G, $P > 0.05$). However, the slope factor for activation and inactivation from cells 7 days after SAH was significantly higher than controls and cells 4 and 21 days after SAH (Fig. 3D and 3G, $P < 0.05 - 0.001$). Similarly, there was no difference in activation time constant (τ) for cells 4 and 21 days and controls at depolarized potentials ≥ 0 mV (0 mV to +40 mV). At these times, IK_V activation was well fitted by single exponentials for both control and SAH myocytes (Fig. 3E). However, τ for myocytes 7 days after SAH at +30 and +40 mV was significantly longer than all other groups (Fig. 3E, $P < 0.05 - 0.001$).

Inactivation was incomplete for all groups. However, 7 days after SAH, the remainder of the inactivation-resistant fraction was significantly larger than controls as well as cells 4 and 21 days after SAH. The remainder of inactivation was $16 \pm 2\%$ for controls and $18 \pm 1\%$ 4, $22 \pm 1\%$ 7 and $17 \pm 1\%$ 21 days after SAH (Fig. 3H, $P < 0.01$).

Temporal Profile of K_{Ca} Channels in Vasospastic Cerebrovascular Myocytes

Whole-cell patch clamp studies of the voltage-dependence and kinetics the large-conductance K_{Ca} channel were conducted in control and SAH myocytes. Both control and vasospastic myocytes generated large outward currents upon depolarization, which could be completely blocked by the K_{Ca} -specific toxin, paxilline (data not shown)(Fig. 2E to 2H)[8]. Normalized tail current amplitudes were fit to the Boltzmann function $G/G_{max} = 1 / [1 + e^{-(V-V_{1/2})/k}]$. There were no significant changes in half-activation potentials ($V_{1/2}$, Fig. 4C), slope factors (k , Fig. 4D), activation τ (Fig. 4E), deactivation τ (Fig. 4F) and normalized tail currents (Fig. 4G). The absolute amplitudes of K_{Ca} currents at all 3 times after SAH (at 100 - 160 mV holding potential) were significantly smaller than in control cells (Fig. 4A, $P < 0.05 - 0.01$). However, currents adjusted for cell capacitance (current density) demonstrated only a significant difference

between cells 4 and 7 days after SAH and only at more depolarized potentials (from 100 to 160 mV, Fig. 4B, $P < 0.01$).

DISCUSSION

Vasospasm is a major cause of mortality and morbidity after aneurysmal SAH. It occurs in 70% of these patients and causes morbidity and mortality in up to 30% [11]. Treatment has been directed at preventing secondary injury to the brain, augmenting cerebral blood flow by hemodynamic manipulations and administering the L-type Ca^{2+} channel antagonist, nimodipine [11]. These measures are complicated to use and not very effective.

Vasospasm is at least initially a problem of smooth muscle contraction [4]. Contraction involves alterations in membrane potential, receptor-operated agonists, altered Ca^{2+} sensitivity or failure of relaxation mechanisms. Regarding the first possibility, we reported that smooth muscle cells from dogs with SAH had significantly decreased $\text{K}_V2.2$ messenger ribonucleic acid (mRNA) and protein and mRNA but not protein of the β_1 subunit of the large-conductance K_{Ca} channel [1]. There was no significant change in mRNA for L-type Ca^{2+} channels and the K_{Ca} α subunit. Our prior electrophysiological and functional studies of K_V and K_{Ca} channels found that K_V2 current density was halved in myocytes 7 days after SAH in dogs. $\text{K}_V2.1$ and $\text{K}_V2.2$ mRNA and protein were decreased whereas there were no changes in the K_{Ca} channel [6;7]. Vasospastic myocytes were significantly depolarized and had a smaller contribution of K^+ conductance toward maintenance of their membrane potential. This depolarization was reproduced by pharmacological block of K_V current in normal myocytes and the degree of membrane depolarization was compatible with the degree of vasoconstriction observed after SAH. These observations suggested K_V2 dysfunction contributes to vasospasm.

The electrophysiology findings of our previous work are extended here. Vasospasm was maximal 4 and 7 days after SAH and was resolving by 21 days. This correlated with cell capacitance and probably reflects contraction of the cells during vasospasm and relaxation as vasospasm resolves. On the other hand, K_V current amplitude and mid-potentials for activation and inactivation decreased with vasospasm but did not recover as vasospasm resolved. The shift in activation and inactivation mid-potentials to more negative voltage indicates these channels are more sensitive to K^+ , which is consistent with findings in rabbits with SAH [22]. An increased slope factor for both activation and inactivation was detected only 7 days after SAH, suggesting that K_V dysfunction peaks at this time in this model. The lack of direct correlation between K_V channel alteration and vasospasm raises questions about the causal effect of K_V channel dysfunction in vasospasm.

One possibility is that resolution of vasospasm involves compensatory changes in other regulators of membrane potential or other pathways. For example, inwardly rectifying K^+ channels were increased during vasospasm after SAH, suggesting one possible mechanism for such reversal [20]. Another is that the decrease in K_V channel function does not cause vasospasm, although function studies suggested that this is not the case [6]. Also, studies reported that vasospasm in rabbits was decreased with K^+ channel agonists [9;10;23]. These studies used cromakalim which is not a specific K_V channel activator and they studied relatively acute, mild vasospasm. In support of a role for decreased K_V channel function in vasospasm was the finding that the more specific K_V channel antagonist, 4-aminopyridine, produced less depolarization and basilar artery constriction 2 days after SAH in rats compared to control arteries [16]. Furthermore, Ishiguro, et al., reported that oxyhemoglobin, a component of subarachnoid blood that may cause vasospasm [3;12], decreases 4-aminopyridine sensitive K_V currents in myocytes isolated from rabbit cerebral arteries but does not directly alter activity of voltage-dependent Ca^{2+} or K_{Ca} channels [5]. Finally, although there was less vasospasm 21 days after SAH in this model, vasospasm did not reverse so examination of K_V channel function

at a longer time after SAH might show recovery. Prior studies show a 15% reduction in dog basilar artery diameter 60 days after SAH so this might be a reasonable time to examine in future studies [17].

In contrast to the decrease in K_V function, we previously reported that K_{Ca} channels were not altered 7 days after SAH [7]. It is thus unlikely that a primary alteration in K_{Ca} channel function contributes to vasospasm. The present study did find significantly smaller absolute K_{Ca} current amplitude at all times after SAH although after correction for cell size, there was only a significant decrease 4 days after SAH. Whether this is important in vasospasm needs further study, although it seems unlikely given the small and transient change.

In conclusion, these data support a role for dysfunction in K_V channels in vasospasm after SAH. However, recovery of vasospasm seems to occur without concomitant improvement in K_V channel function, suggesting other factors contribute to resolution of vasospasm.

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References

1. Aihara Y, Jahromi BS, Yassari R, Nikitina E, Agbaje-Williams M, Macdonald RL. Molecular profile of vascular ion channels after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2004;24:75–83. [PubMed: 14688619]
2. Catacuzzeno L, Pisconti DA, Harper AA, Petris A, Franciolini F. Characterization of the large-conductance Ca-activated K channel in myocytes of rat saphenous artery. *Pflugers Arch* 2000;441:208–218. [PubMed: 11211105]
3. Clark JF, Sharp FR. Bilirubin oxidation products (BOXes) and their role in cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2006
4. Humphrey JD, Baek S, Niklason LE. Biochemomechanics of cerebral vasospasm and its resolution: I. A new hypothesis and theoretical framework. *Ann Biomed Eng* 2007;35:1485–1497. [PubMed: 17487586]
5. Ishiguro M, Morielli AD, Zvarova K, Tranmer BI, Penar PL, Wellman GC. Oxyhemoglobin-induced suppression of voltage-dependent K^+ channels in cerebral arteries by enhanced tyrosine kinase activity. *Circ Res* 2006;99:1252–1260. [PubMed: 17068294]
6. Jahromi BS, Aihara Y, Ai J, Zhang ZD, Nikitina E, Macdonald RL. Voltage-gated $K(+)$ channel dysfunction in myocytes from a dog model of subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2007
7. Jahromi BS, Aihara Y, Ai J, Zhang ZD, Weyer GW, Nikitina E, Yassari R, Houamed KM, Macdonald RL. Preserved BK channel function in vasospastic myocytes from a dog model of subarachnoid hemorrhage. *J Vasc Res*. 2008in press
8. Knaus HG, McManus OB, Lee SH, Schmalhofer WA, Garcia-Calvo M, Helms LM, Sanchez M, Giangiacomo K, Reuben JP, Smith AB III. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. *Biochemistry* 1994;33:5819–5828. [PubMed: 7514038]
9. Kwan AL, Lin CL, Wu CS, Chen EF, Howng SL, Kassell NF, Lee KS. Delayed administration of the K^+ channel activator cromakalim attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. *Acta Neurochir* 2000;142:193–197.
10. Kwan AL, Lin CL, Yanamoto H, Howng SL, Kassell NF, Lee KS. Systemic administration of the potassium channel activator cromakalim attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. *Neurosurgery* 1998;42:347–350. [PubMed: 9482186]
11. Macdonald, RL.; Weir, B. Cerebral Vasospasm. Academic; San Diego: 2001.

12. Macdonald RL, Weir BK, Runzer TD, Grace MG, Findlay JM, Saito K, Cook DA, Mielke BW, Kanamaru K. Etiology of cerebral vasospasm in primates. *J Neurosurg* 1991;75:415–424. [PubMed: 1869943]
13. Macdonald RL, Zhang J, Sima B, Johns L. Papaverine-sensitive vasospasm and arterial contractility and compliance after subarachnoid hemorrhage in dogs. *Neurosurgery* 1995;37:962–967. [PubMed: 8559346]
14. Megyesi JF, Vollrath B, Cook DA, Findlay JM. In vivo animal models of cerebral vasospasm: a review. *Neurosurgery* 2000;46:448–460. [PubMed: 10690735]
15. Nikitina E, Zhang ZD, Kawashima A, Jahromi BS, Bouryi VA, Takahashi M, Xie A, Macdonald RL. Voltage-dependent calcium channels of dog basilar artery. *J Physiol* 2007;580:523–541. [PubMed: 17185332]
16. Quan L, Sobey CG. Selective effects of subarachnoid hemorrhage on cerebral vascular responses to 4-aminopyridine in rats. *Stroke* 2000;31:2460–2465. [PubMed: 11022080]
17. Sakaki S, Ohue S, Kohno K, Takeda S. Impairment of vascular reactivity and changes in intracellular calcium and calmodulin levels of smooth muscle cells in canine basilar arteries after subarachnoid hemorrhage. *Neurosurgery* 1989;25:753–761. [PubMed: 2586728]
18. Sanchez M, McManus OB. Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel. *Neuropharmacology* 1996;35:963–968. [PubMed: 8938726]
19. Wellman GC. Ion channels and calcium signaling in cerebral arteries following subarachnoid hemorrhage. *Neurol Res* 2006;28:690–702. [PubMed: 17164032]
20. Weyer GW, Jahromi BS, Aihara Y, Agbaje-Williams M, Nikitina E, Zhang ZD, Macdonald RL. Expression and function of inwardly rectifying potassium channels after experimental subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2006;26:382–391. [PubMed: 16079788]
21. Xie A, Aihara Y, Bouryi VA, Nikitina E, Jahromi BS, Zhang ZD, Takahashi M, Macdonald RL. Novel mechanism of endothelin-1-induced vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2007;27:1692–1701. [PubMed: 17392694]
22. Young HA, Kolbeck RC, Schmidek H, Evans JN. Reactivity of rabbit basilar artery to alterations in extracellular potassium and calcium after subarachnoid hemorrhage. *Neurosurgery* 1986;19:346–349. [PubMed: 3762882]
23. Zuccarello M, Bonasso CL, Lewis AI, Sperelakis N, Rapoport RM. Relaxation of subarachnoid hemorrhage-induced spasm of rabbit basilar artery by the K⁺ channel activator cromakalim. *Stroke* 1996;27:311–316. [PubMed: 8571429]

Figure 1. Quantification of arterial diameter and myocytes properties

(A) Arterial diameter from SAH and control animals (* $P < 0.001$ compared to control, § $P < 0.01$ compared to 21 days after SAH, Ctl; control [n = 39], D4 [n = 8], D7 [n = 17] and D21 [n = 7] refer to 4, 7 and 21 days after SAH). (B) Membrane capacitance of myocytes from all cells patched (* $P < 0.01$ as compared to control cells, n = 76 control cells, 71 D4, 48 D7 and 43 D21 cells for all graphs). (C) Membrane resistance and (D) series resistance of all patched myocytes from control or SAH animals.

Figure 2. Current traces of K_V and K_{Ca} currents from dog basilar artery smooth muscle cells (A-D) K_V currents from control myocytes (A) and myocytes 4 (B), 7 (C) and 21 days (D) after SAH. Reduction in K_V current can be seen in cells at all times after SAH. In contrast, K_{Ca} currents for control cells (E) and those obtained 4 (F), 7 (G) and 21 days (H) after SAH show no significant differences.

Figure 3. Changes of Kv channel functions in SAH myocytes

Diminished K_V current amplitude (A) and current density (B) in SAH cells (* $P < 0.05$ as compared to all 3 times after SAH). Both half activation (C) and inactivation (F) potentials were decreased in at all 3 times after SAH (* $P < 0.05$ - 0.001 as compared to controls). An increased slope factor for activation (D) and inactivation (G) and remainder of inactivation (H) found in cells 7 days after SAH (* $P < 0.05$ - 0.001 as compared to control, day 4 or day 21 SAH cells). (E) Activation τ (* $P < 0.05$ - 0.001 for 7 days after SAH compared to all other groups). (I) Activation of normalized tail current. (J) Inactivation of normalized tail current (* $P < 0.05$ - 0.001 control versus the 3 SAH groups).

Figure 4. Changes of BK channel functions in SAH myocytes

Compared to control cells, there was decreased K_{Ca} current amplitude (A, * $P < 0.05$ as compared to all 3 times after SAH), but not current density (B, * $P < 0.05$ day 7 versus day 4 SAH cells). No difference was found between control cells and cells from any time after SAH in half activation potential (C), slope factor (D) activation τ (E), as well as deactivation τ (F) or normalized tail current conductance (G).