The Effect of Papaverine on the Calcium-dependent K+ Current in Rat Basilar Smooth Muscle Cells

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Objective : Papaverine has been used in treating vasospasm following subarachnoid hemorrhage(SAH). However, its action mechanism for cerebral vascular relaxation is not clear. Potassium channels are closely related to the contraction and relaxation of cerebral smooth muscle. Therefore, to identify the role of potassium and calcium channels in papaverine-induced vascular relaxation, we examine the effect of papaverine on potassium channels in freshly isolated smooth muscle cells from rat basilar artery.

Methods : The isolation of rat basilar smooth muscle cells was performed by special techniques. The whole cell currents were recorded by whole cell patch clamp technique in freshly isolated smooth muscle cells from rat basilar artery. Papaverine was added to the bath solution.

Results : Papaverine of $100\mu M$ into bath solution increased the amplitude of the outward K+ current which was completely blocked by BKCa(large conductance calcium dependent potassium channels)blocker, IBX(iberiotoxin), and calcium chealator, BAPTA(1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid), in whole cell mode.

Conclusion : These results strongly suggest that potassium channels may play roles in papaverine-induced vascular relaxation in rat basilar artery.

KEY WORDS : Papaverine · Vasospasm · Potassium channel · Patch-Clamp techniques.

Introduction

Papaverine normally relaxes smooth muscles and reduces the contractile response to excitatory agents[2]. However, there is no consensus of opinion regarding the mechanism of papaverine-induced relaxation. There is evidence that papaverine inhibits phosphodiesterase activity in various visceral smooth muscles[28,31], which implies an increase in cellular cyclic AMP concentration which, in turn, is thought to be the intracellular mediator of the relaxation[15,35]. However, a number of inconsistencies in the relationship between inhibition of phosphodiesterase and the relaxing effect of papaverine have been reported[1,32]. Huddart & Saad[16] showed that at least in ileal smooth muscle the relaxing effect of papaverine is associated with the blockade of Ca^{2+} influx. Also they obtained results that argue strongly against any

causal relationship between cyclic AMP and relaxation in the ileum and vas deferens[16].

Vasospasm is the leading cause of disability and death after intracranial aneurysm rupture, but the pathogenesis of the arterial narrowing is not completely understood, and the best form of treatment is not yet clear. Intracranial angioplasty with nondetachable silicone balloons and intracranial intraarterial papaverine infusion have been used in a number of institutions; however, the reported experience remains modest[5,8,13,14,18,19,23,25,27,29,45].

This study was undertaken to clarify the role of potassium channel in papaverine-induced vascular relaxation, and to investigate the effect of papaverine ($100\mu M$) on outward potassium currents using patch clamp technique in cerebral smooth muscle cells from rat basilar artery and found that papaverine activated the BKCa channel .

Materials and Methods

Cell isolation

The methods for isolation of rat basilar smooth muscle cells have been described[23]. Briefly, Spargue-Dawley female rats were anesthetized with Motofane and decapitated. The basilar arteries were removed to a medium consisting of (in mM) : NaCl 130, KCl 15, CaCl₂ 0.8, MgCl₂ 1.3, glucose 5, N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid] (Herpes) 10, penicillin (100units/ml) and streptomycin (0.1g/l). Arteries were then cleaned of connective tissue and small side branches. The arteries were cut into 2.0-mm rings and incubated for 1hour at room temperature in a medium containing 0.2mM CaCl₂ and collagenase (type II, 0.5g/l), elastase (0.5g/l), hyaluronidase (type IV-S, 0.5g/l) and deoxyribonuclease I (0.1g/l). The rings were washed in fresh solution containing CaCl₂ (0.2mM), trypsin inhibitor (0.5g/l) and deoxyribonuclease I (0.1g/l) and then triturated gently. Cells were plated on glass coverslips and stored at 4°C(used in 12hours) in the above-me-ntioned buffer containing CaCl₂ (0.8mM) and bovine serum albumin (2g/l) free of essential fatty acids. Isolated cells stained positive for α-actin and retained the ability to contract in response to KCl, caffeine, serotonin and hemolysate.

Whole-cell patch clamp technique

Cells were voltage-clamped using the whole-cell patch clamp technique[23]. Electrodes were prepared from glass capillary tubing (KIMAX-51, Kimble products, USA) by using a patch electrode puller (PP-83, Narishige, Tokyo, Japan), and pipette resistance was 2-10MΩ. These were positioned using a three-dimensional vernier-type hydraulic micromanipulator (MX-630R, SOMA SCIENTIFIC). Seals(5-10GΩ) were formed by applying gentle negative pressure. Voltage steps were applied with pulse protocols driven by a IBM 586 computer equipped with A-D and D-A converters (DigiData 1200, Axon Instruments Inc., Foster City, CA, USA). Data of membrane currents were collected and amplified using a patch clamp Axon-patch 1D and pCLAMP 5.7.1 programs (Axon Instruments). None of the record shown were leakage-corrected, and series compensation was not used. Data were filtered with a low-pass Bessel filter (-3dB at 1 kHz) and digitized on-line at a sampling frequency of 5~10 kHz for subsequent computer analysis. Data analysis was performed using pCLAMP 5/7/1. All experiments were carried out at room temperature (20~26°C).

Solutions and drugs

Calcium dependent K⁺ channels (BKca)

The normal bath solution for the whole-cell recordings was (mM) : NaCl 130, KCl 5, MgCl₂ 1.2, CaCl₂ 1.8, HEPES 10, glucose 5.2 and the pH was adjusted to 7.4 with NaOH. Pipettes were filled with (mM) : KCl 140, MgCl₂ 0.5, CaCl₂ 0.1, ethylenebis(oxonitrilo) tetraacetic acid (EGTA) 0.09, HEPES 10, glucose 10 and the pH was adjusted to 7.4 with KOH.

The free calcium concentration in the pipette solution was estimated to be 10nM. The pipette solution was filtered before use (pore size 0.2μm).

Data analysis

Data are expressed as mean ± SE. Differences among multiple groups were calculated by Student's t-test. A value of p < 0.05 was considered statistically significant.
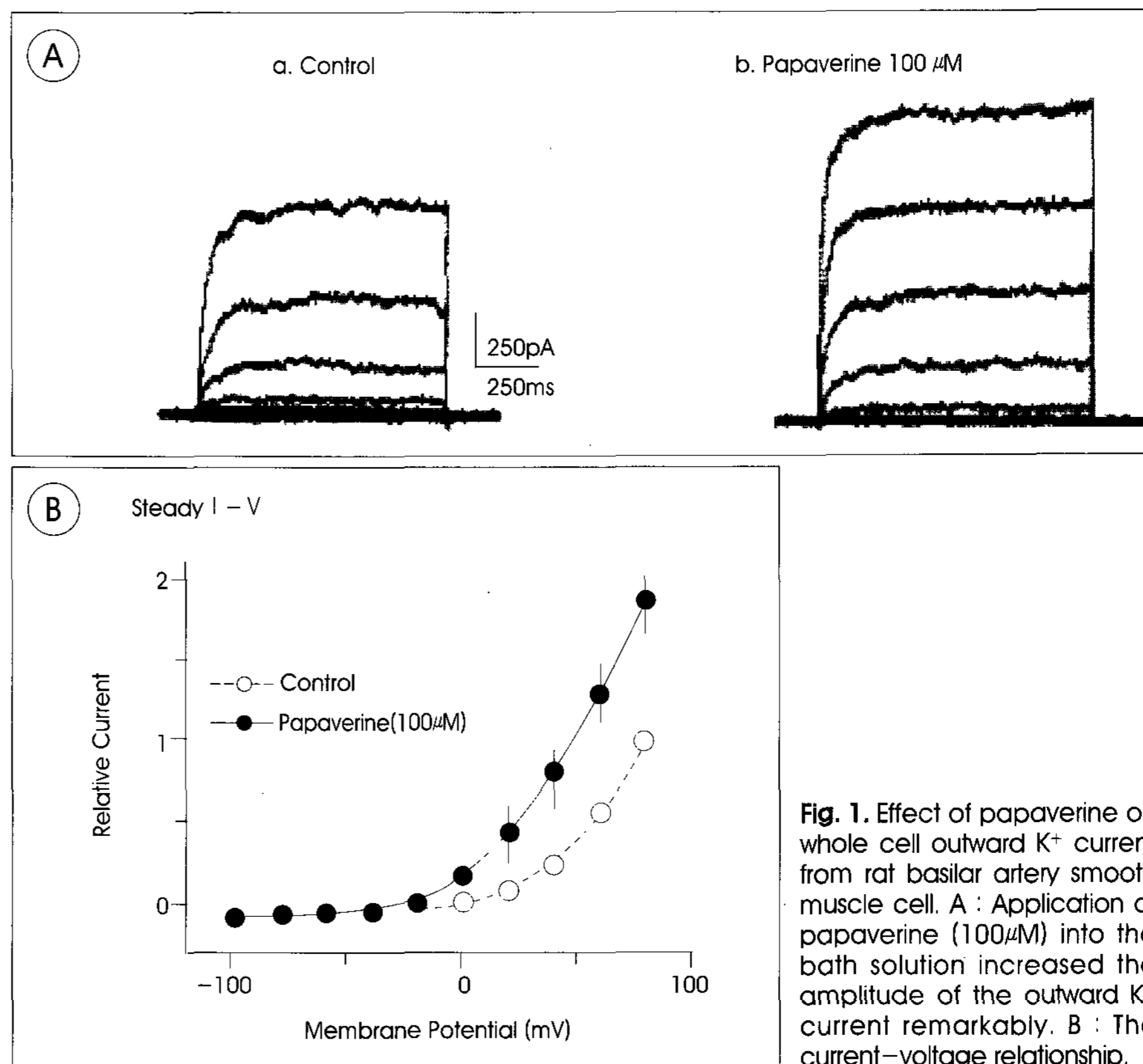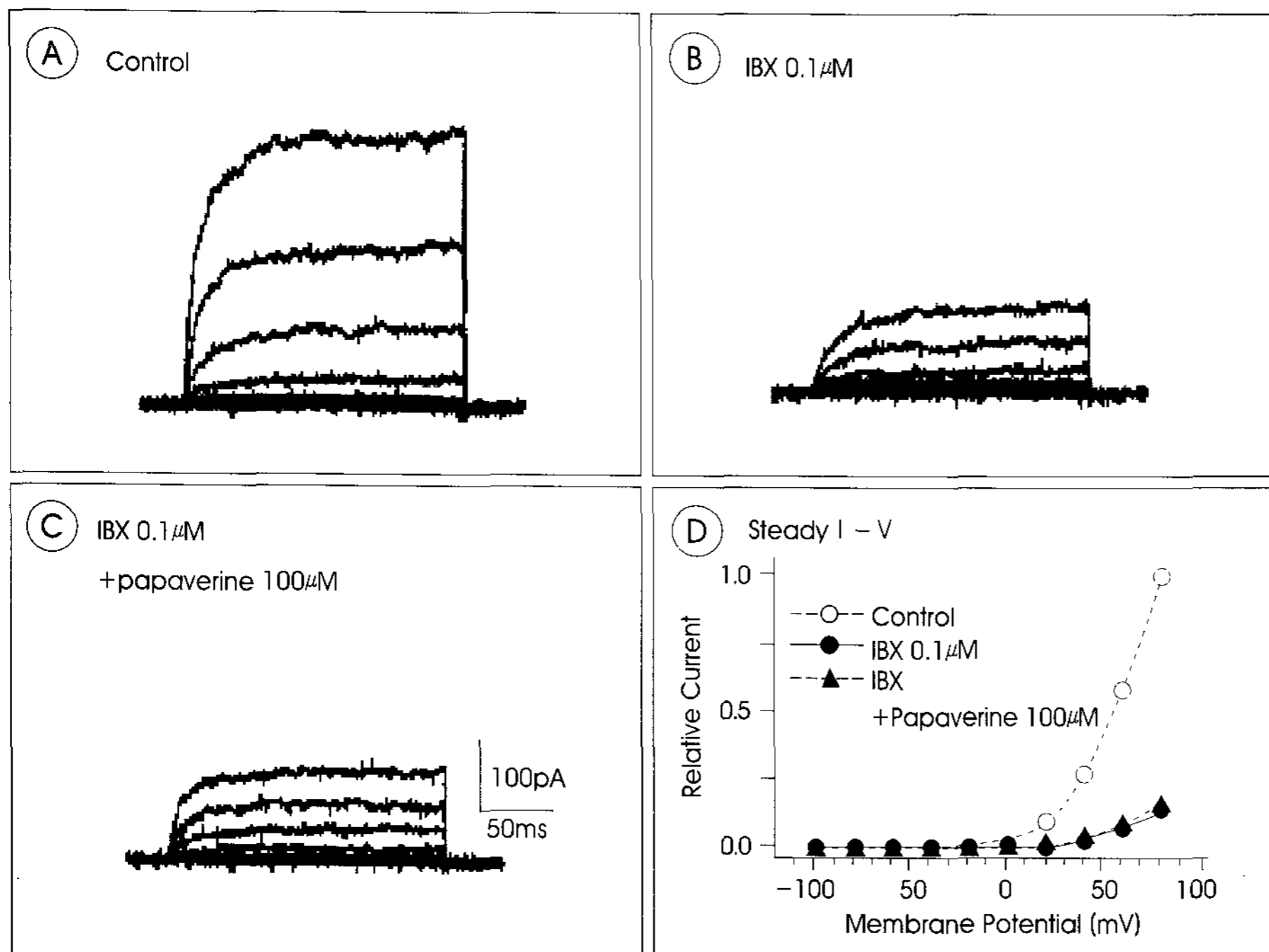

Fig. 1. Effect of papaverine on whole cell outward K⁺ current from rat basilar artery smooth muscle cell. A : Application of papaverine (100μM) into the bath solution increased the amplitude of the outward K⁺ current remarkably. B : The current-voltage relationship.
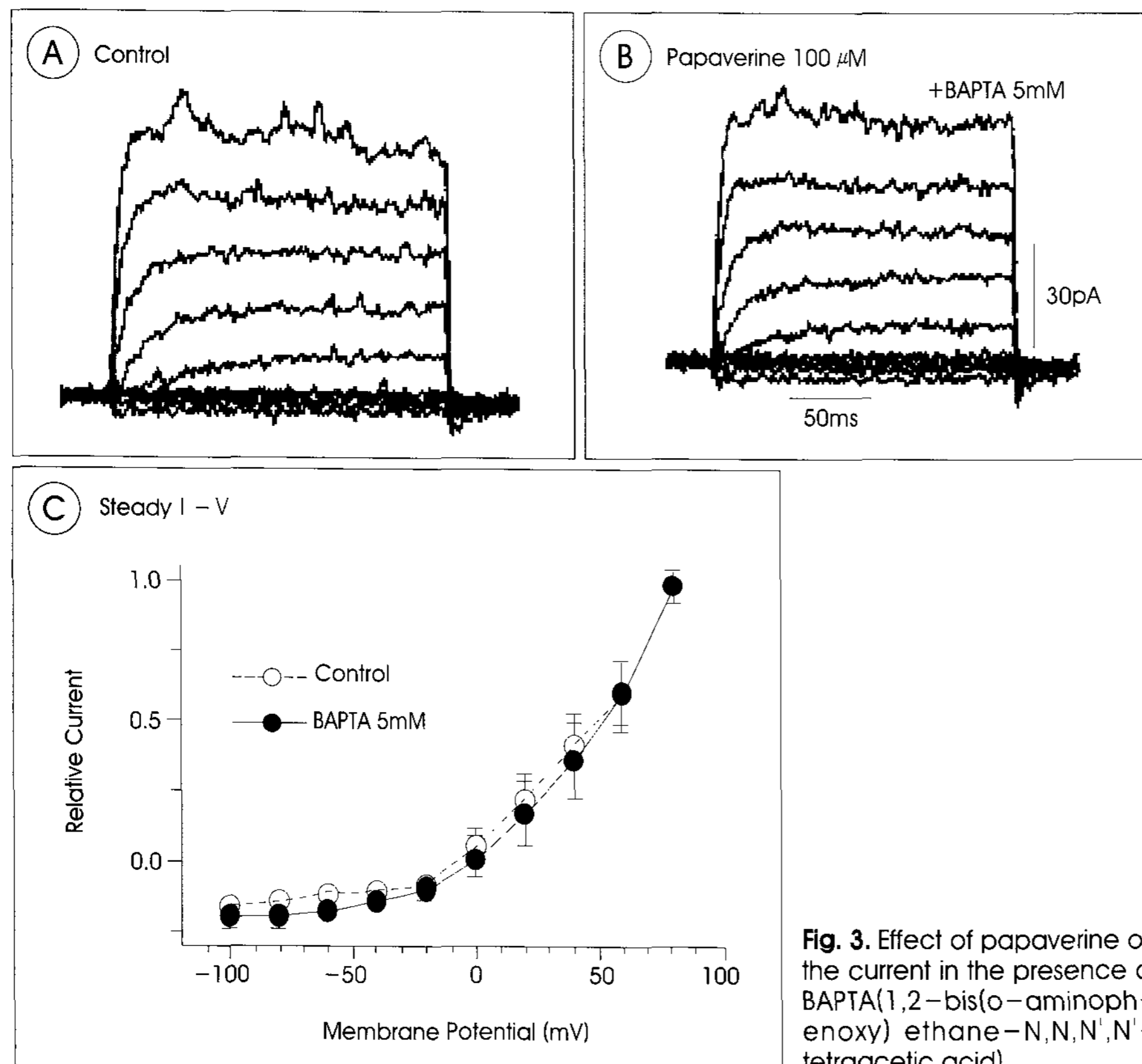
Results

Effect of papaverine on K+ channels

Outward K+ currents from rat basilar artery smooth muscle

Fig. 2. IBX(Iberiotoxin) blocked the papaverine-induced current.

Fig. 3. Effect of papaverine on the current in the presence of BAPTA(1,2-bis(o-aminoph-enoxy) ethane-N,N,N',N'-tetraacetic acid).

cells were always elicited by potentials beyond -40mV by voltage pulses given every 3 s in 20-mV increments over the range of -100 to +80mV from the holding potential of -60mV. No marked inward current could be seen in the same voltage ranges. Replacement of potassium in the pipette with cesium eliminated the outward current(n=5). Application of caffeine (3-6mM; n=4) and calcium ionophore A23187 (10μM; n=2) into normal bath solution significantly p<0.05) enhanced the outward current and the current was reversibly blocked by addition of TEA (10-30mM; n = 8; p<0.01) to the bath solution, but was not blocked by glibenclamide (3-6μM;n=4; p>0.05), a selective ATP-sensitive potassium channel (K$_{ATP}$) blocking agent. Thus, the outward current had the properties of a calcium-activated potassium current, or KCa[44].

After establishment of whole-cell recordings and collecting control recordings for approximately 5minutes until the current elicited by depolarization stabilized, papaverine was applied into the bath solution. In control studies, the whole-cell current remained stable over 10 to 20 minutes in the absence of interventions. Fig. 1 showed whole cell potassium current from rat basilar artery smooth muscle cells. Application of papaverine (100μM) into the bath solution increased the amplitude of the outward K+ current remar-kably (n=10, P<0.05). The current voltage relationship of the study was shown in Fig. 1B.

Application of large conductance calcium-activated potassium channel (BKca) blocker, iberiotoxin (0.1 μM, IBX), into bath solution blocked the increased amplitude of the papaverine-induced outward current remarkably (Fig. 2. n=10, P<0.05). The current voltage relationship of the study was shown in Fig. 2D. Fig. 3 illustrated the effect

of BAPTA, a calcium chealator on the papaverine induced current. Papaverine (n=3) did not increase the amplitude of the outward K⁺ current in the presence of BAPTA(5mM). The current voltage relationship of the study was shown in Fig. 3C.

Discussion

The membrane potential of cerebral arterial smooth muscle is depolarized during vasospasm[36,46] which follows about 30% of the cases of subarachnoid hemorrhage[21]. This depolarization may play a role in prolonged constriction and vascular hyperreactivity which underlies cerebral vasospasm[30,36,39]. The potassium channel agonist, nicorandil, hyperpolarizes the smooth muscle membrane by increasing potassium conductance and, at least partly, reverses the vasospasm in a canine model of subarachnoid hemorrhage[12]. The mechanisms of opening potassium channels which initiate the relaxation of vascular smooth muscle have been discussed by many authors[6,7,9,11,17,42]. When a potassium channel opens, potassium diffuses down its electrochemical gradient, transferring positive charge out of the cell, thereby marking the interior of the cell more negative and driving the membrane potential in an hyperpolarizing direction[39].

KCa, especially the large-conductance KCa(BKCa) has been identified in different cerebral arterial smooth muscles such as rat[34,38], guinea-pig[41], cat[10] and rabbit[4]. These BKCa are voltage-dependent, activated by increased internal calcium, and inhibited by TEA and charybdotoxin. The increase of intracellular calcium can be the result of either influx of extracellular calcium or release of calcium from intracellular stores. A voltage-, calcium- and charybdotoxin-sensitive, large conductance (220pS) potassium channel has been identified in rat basilar artery[34,43]. This channel is sensitive to the potassium channel openers, cromakalim and pinacidil. Both whole-cell current and single-channel opening probability were increased by bath application of those agents, suggesting that this channel may be involved in the regulation of cerebral smooth muscle tone and the potassium channel openers may be useful in the management of cerebral diseases, including vasospasm. Most known types of potassium channel have been discovered in at least one or two vascular preparations, but it seems likely that in cerebrovascular smooth muscle, most of the potassium current is carried by calcium-activated potassium channels[33,34,41,43], with delayed rectifiers[3,43] and inward rectifiers also present.

Intracellular Ca²⁺, [Ca²⁺]ᵢ, which plays an important role in the regulation of smooth muscle tone, has been shown to increase in major cerebral arteries after subarachnoid hemorrhage(SAH) and has been suggested to mediate the prolonged

contraction that is known as vasospasm of those vessels[20,26,30,40]. A massive Ca²⁺ accumulation with formation of intracytoplasmic vacuoles occurred in the smooth muscle cells of canine basilar arteries in vivo 15minutes after experimental SAH[22]. Erythrocyte hemolysate, oxyhemoglobin or hemin, have been shown to increase [Ca²⁺]ᵢ in cerebral smooth muscle cells[26,37,44].

BKCa channels are abundant in vascular smooth muscle and are target proteins for cyclic nucleotide-dependent protein kinases such as cAMP-dependent protein kinase A-kinase. A-kinase has been shown to activate BKCa channels in vascular smooth muscles through phosphorylation of the channel or closely associated proteins .

Thus, cAMP elevation via inhibition of phosphodiesterase by papaverine[15,35], may mediate the activation of BKCa channels, leading to the relaxant response of cerebral artery to papaverine. This idea can be supported by our present finding. In the present study, the increase of outward K⁺ current by papaverine was blocked by a selective BKCa blocker, iberiotoxin, as well as a Ca²⁺chelator, BAPTA. These results strongly indicate that papaverine opens the BKCa channel. Opening of the BKCa channel leads to hyperpolarization of the vascular smooth muscle, which inhibits the calcium entry through voltage-sensitive Ca²⁺ channel, thereby being able to relax the vascular smooth muscle. Considering all of these, our study could explain the underlying mechanism for papaverine-induced vascular relaxation on a new aspect. This is the first report clarifying that papaverine could induce the vascular relaxation through activation of the BKCa channel.

Conclusion

Our results suggest that potassium channels may play a role in papaverine-induced vascular relaxation in rat basilar artery. 1. Papaverine increased the outward K⁺ current which was completely blocked by IBX and BAPTA in whole cell mode. 2. Papaverine may induce the BKCa channel in a cytosol-dependent manner in intact cerebral basilar smooth muscle cells, thereby contributing to the relaxation of cerebral artery.

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