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3-Morpholinosydnonimine (SIN-1) and K^+ channels in smooth muscle cells of the rabbit and guinea pig carotid arteries

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Abstract

Experiments were designed to determine the subtype of K^+ channels activated by the nitrovasodilator 3-morpholinosydnonimine (SIN-1) in smooth muscle cells of the rabbit and guinea pig carotid arteries. Membrane potential was recorded in isolated segments with intracellular microelectrode and K^+ currents in freshly dissociated smooth muscle cells, with the patch–clamp technique. In the guinea pig carotid artery, SIN-1 caused a glibenclamide-sensitive hyperpolarization. The nitrovasodilator did not affect the whole-cell K^+ current, but activated a glibenclamide-sensitive K^+ current. In the rabbit carotid artery, SIN-1 induced only an iberiotoxin-sensitive repolarization in phenylephrine-depolarized tissue and in isolated cells, enhanced the activity of an iberiotoxin-sensitive K^+ current. These findings demonstrate that the population of K^+ channels activated by nitric oxide (NO) is species-dependent and support the conclusion that, in the guinea pig carotid artery, in contrast to the rabbit carotid artery, the release of NO cannot account for the responses attributed to endothelium-derived hyperpolarizing factor (EDHF).

Keywords: 3-morpholinosydnonimine (SIN-1); Endothelium-derived hyperpolarizing factor (EDHF); K^+ channel, Ca^{2+}-activated; K^+ channel, ATP-sensitive; Membrane potential

1. Introduction

Endogenous nitric oxide (NO) released by endothelial cells or NO released from nitrovasodilators produces relaxation of the vascular smooth muscle cells. The principal physiological action of NO is the activation of cytosolic soluble guanylate cyclase and the consequent formation of cyclic-GMP (Moncada et al., 1991), but endothelial NO has many other targets in smooth muscle cells, including K^+ channels (Cohen and Vanhoutte, 1995). Indeed, in species, such as the guinea pig (Tare et al., 1990; Parkington et al. 1995; Corriu et al., 1996) and the rabbit (Cohen et al., 1997), NO causes hyperpolarization of the vascular smooth muscle. In the rabbit carotid artery, inhibitors of NO-synthase produce an incomplete blockade of the enzyme and the remaining production of NO may be sufficient to produce hyperpolarization of the smooth muscle cells and, thus, relaxation. In this artery, endothelial NO could account for the effects usually attributed to endothelium-derived hyperpolarizing factor (EDHF) (Cohen et al., 1997). However, in the guinea pig carotid artery, endothelium-dependent hyperpolarizations, elicited by EDHF and NO, are not sensitive to the same K^+ channel blockers, suggesting that NO and EDHF are not the same entity (Corriu et al., 1996).

The purpose of the present study was to determine the type of K^+ channels activated by NO donors in arterial smooth muscle cells of carotid arteries of both the guinea pig and the rabbit.

2. Materials and methods

2.1. Microelectrode studies

Male Hartley guinea pigs and male New Zealand rabbits were anesthetized. Their carotid arteries were dis-

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sected, cleaned of adherent connective tissues and pinned to the bottom of an organ chamber. The tissues were superfused with a thermostated modified Krebs–Ringer bicarbonate solution of the following composition (in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.026 and glucose 11.1. Transmembrane potential was recorded by using glass capillary microelectrodes (tip resistance of 30–90 MΩ) filled with KCl (3 M) and connected to the headstage of a recording amplifier (intra 767, WPI). Successful impalements were signaled by a sudden negative drop in potential from the baseline (zero potential reference), followed by a stable negative potential for at least 3 min. All the experiments were performed in the presence of N^{ω}nitro-L-arginine (100 μM) and indomethacin (5 μM) to inhibit NO synthase and cyclooxygenase, respectively (Corriu et al., 1996).

2.2. Patch–clamp studies

The media of guinea pig and rabbit carotid arteries were dissected from cleaned arteries. The smooth muscle cells were dissociated enzymatically (Quignard et al., 1999). Whole-cell K^+ currents were recorded at room temperature using the patch–clamp technique. The cells were superfused with a solution containing (in mM): NaCl 125, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1.2, HEPES 10 and glucose 11. In order to record K_v current, an intracellular Ca^{2+}-free solution was used with the following composition (in mM): KCl 130, $MgCl_2$ 2, ATP 3, GTP 0.5, HEPES 25, EGTA 10, glucose 11. In order to record K_{Ca} currents, the concentration of EGTA was reduced to 1 mM and $CaCl_2$ (0.5 mM) was added. In order to record K_{ATP} current, ATP was reduced to 0.1 mM, in the presence of oxaloacetic (5 mM) and pyruvic acid (2 mM). For the outside–out configuration, the extracellular solution was (in mM): KCl 130, $MgCl_2$ 2, HEPES 10, $CaCl_2$ 2, glucose 11, and the intra-pipette medium was: KCl 130, $MgCl_2$ 2, ATP 3, GTP 0.5, HEPES 25, $CaCl_2$ 0.01, glucose 11. For the cell-attached configuration, the pipette solution was identical to the intracellular solution used to record K_{Ca}, but with out ATP and GTP.

Data were recorded with pClamp6 software (Axon Instruments, USA) through an RK-400 amplifier (Biologic, France). Passive capacitive currents and leakage currents were subtracted using the P/4 protocol (for the whole-cell patch–clamp configuration). The cells that showed a leakage current larger than 20 pA, when clamped at a holding potential of −100 mV, were not studied.

2.3. Statistics

Data are shown as mean ± S.E.M.; n indicates the number of cells in which membrane potential was recorded. Statistical analysis was performed using Student's t-test for paired or unpaired observations. Differences were considered to be statistically significant when P was less than 0.05.

3. Results

3.1. Microelectrode studies

3.1.1. Guinea pig

In the guinea pig isolated carotid artery, the resting membrane potential was −51.5 ± 1.1 mV ($n = 20$). 3-morpholinosydnonimine (SIN-1: 10 μM) induced a hyperpolarization of the vascular smooth muscle cells (−12.8 ± 2.0 mV, $n = 6$; Fig. 1). The hyperpolarization produced by the nitrovasodilator was endothelium-independent (data not shown). Glibenclamide (1 μM) abolished this hyperpolarization (+4.5 ± 4.6 mV, $n = 4$; Fig. 1). Apamin (0.5 μM), charybdotoxin (0.1 μM) and iberiotoxin (0.1 μM) alone, or the combination of apamin plus charybdotoxin,

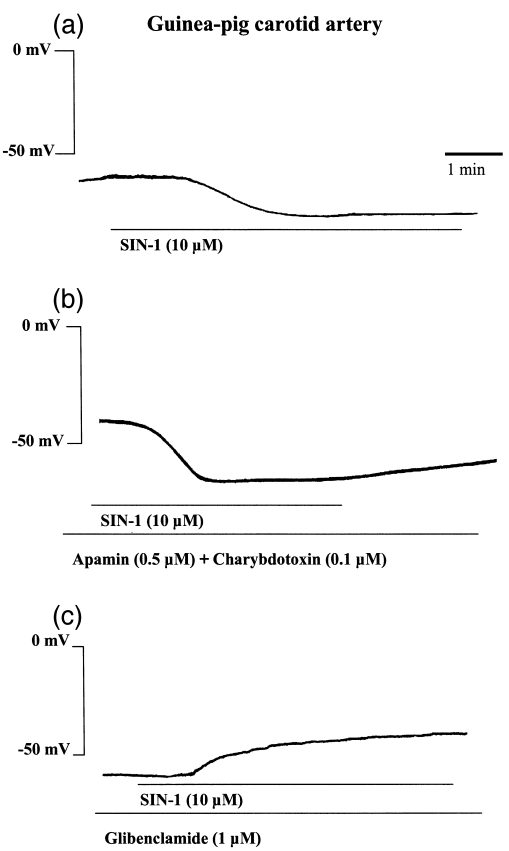

Fig. 1. Effects of different K^+ channel inhibitors on SIN-1-induced hyperpolarization in a guinea pig isolated carotid artery, in the presence of N^{ω}-nitro-L-arginine (100 μM) and indomethacin (5 μM). (a) Original trace showing the effect of SIN-1 (10 μM), on the cell membrane potential. (b) In the presence of the combination of apamin (0.5 μM) plus charybdotoxin (0.1 μM). (c) In the presence of glibenclamide (1 μM). Recordings in (a), (b) and (c) originate from different cells.

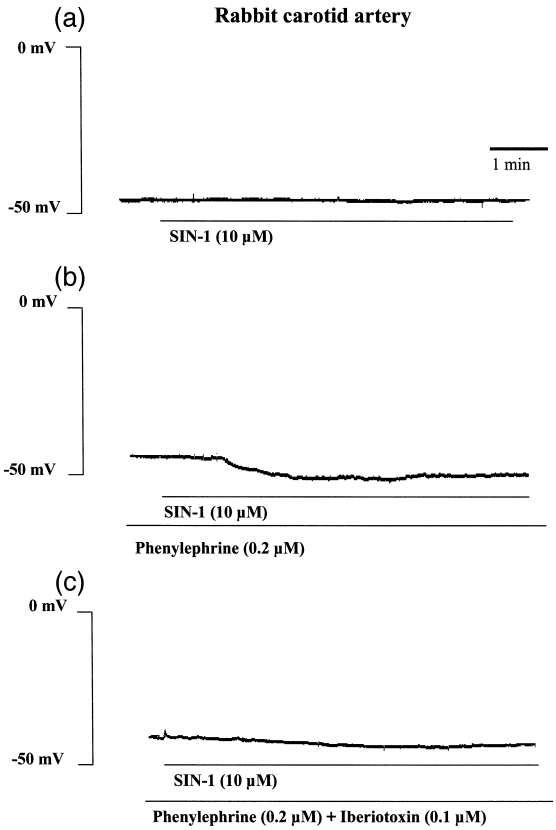

Fig. 2. Effects of different K^+ channel inhibitors on SIN-1-induced hyperpolarization in a rabbit isolated carotid artery, in the presence of N^{ω}-nitro-L-arginine (100 μM) and indomethacin (5 μM). Original traces, showing the effect of SIN-1 (10 μM), on the cell membrane potential of three different myocytes. (a) Control. (b) In the presence of phenylephrine (0.2 μM). (c) In the presence of phenylephrine (0.2 μM) plus iberiotoxin (0.1 μM).

did not alter the hyperpolarization induced by SIN-1 (Fig. 1 and data not shown).

3.1.2. Rabbit

In the rabbit isolated carotid artery, the resting membrane potential was -57.9 ± 1.6 mV ($n = 9$). SIN-1 (10 μM) did not induce any significant changes in the membrane potential of the vascular smooth muscle cells (-0.2 ± 0.1 mV, $n = 9$; Fig. 2). Similar results were obtained with another nitrovasodilator, sodium nitroprusside (10 μM, $n = 3$; data not shown). Cromakalim (10 μM) produced a sustained hyperpolarization (-21.3 ± 3.7 mV, $n = 3$). In the presence of phenylephrine (0.2 μM), the cell membrane was significantly depolarized (-50.6 ± 2.1 mV, $n = 9$). The addition of SIN-1 (10 μM) repolarized the rabbit carotid artery smooth muscle cells (by -5.1 ± 0.6 mV, $n = 3$; Fig. 2). The repolarization was inhibited in the presence of iberiotoxin (0.1 μM; -1 ± 0.9 mV, $n = 2$; Fig. 2).

3.2. Patch–clamp studies

3.2.1. Guinea pig

Freshly dissociated myocytes from the guinea pig carotid artery were studied with the whole-cell configuration of the patch–clamp technique at a holding potential of 0 mV, to inactivate voltage-dependent K^+ channels (Kv) and in the presence of a high intracellular concentration of free Ca^{2+} (0.5 μM), in order to more accurately record Ca^{2+}-activated K^+ channels (Quignard et al., 2000). SIN-1 (up to 10 μM) did not affect the current density (Fig. 3 and Table 1) or the current–voltage relationship ($n = 8$, data not shown) of the K^+ current. Similarly, SIN-1 did not affect the amplitude or the apparent activation threshold of

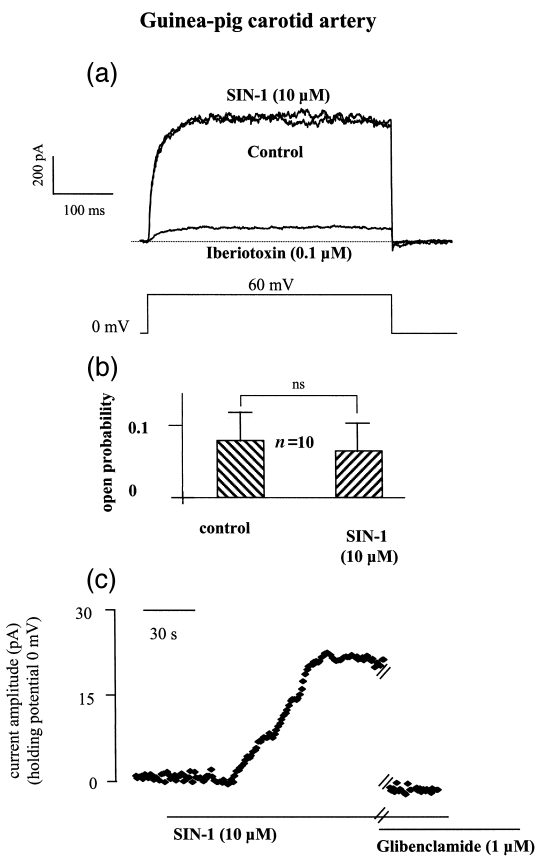

Fig. 3. Effects of SIN-1 on outward K^+ currents in freshly isolated smooth muscle cells from the carotid artery of the guinea pig. (a) In the presence of a high free-Ca^{2+} concentration (0.5 μM) in the intracellular solution; recording of K^+ currents before and after exposure to SIN-1 (10 μM). The current was inhibited by iberiotoxin (0.1 μM). Currents were generated by a depolarizing step from a holding potential of 0 mV to the test potential of 60 mV. (b) Effects of SIN-1 (10 μM) on the mean open probability of large conductance outward K^+ channel (cell-attached configuration, patch potential −20 mV). (c) SIN-1 (10 μM) induced a glibenclamide (1 μM) sensitive outward K^+ current (holding potential: 0 mV, intracellular solution with a low concentration of ATP). ns Indicates no statistical difference between the two groups; *n* represents the number of cells studied.

Table 1
Current density of different types of K^+ channels in freshly dissociated vascular smooth muscle cells of guinea pig and rabbit carotid arteries

K^+ channels		Guinea pig (pA/pF)	Rabbit (pA/pF)
BK_{Ca}	Control	14.6 ± 1.8	20.5 ± 2.5
	SIN-1 (10 μM)	14.1 ± 2.4	34.0 ± 4.0 *
	Db-cGMP (10 μM)	14.3 ± 2.5	38.0 ± 4.2 *
	Iberiotoxin (0.1 μM)	4.0 ± 2.2 *	4.0 ± 2.0 *
K_v	Control	4.5 ± 1.5	6.1 ± 1.8
	SIN-1 (10 μM)	4.3 ± 0.8	6.1 ± 1.4
	Db-cGMP (10 μM)	4.0 ± 0.4	7.2
	4-AP (5 mM)	0.4 ± 0.1*	0.2 ± 0.1*
K_{ATP}	Control (glibenclamide sensitive current)	0.0 ± 0.0	0.0 ± 0.0
	Cromakalim (10 μM)	2.1 ± 0.2 *	2.1 ± 0.1*
	Cromakalim + glibenclamide (1 μM)	0.1 ± 0.1	0.2 ± 0.0
	SIN-1 (10 μM)	1.4 ± 0.3	−0.1 ± 0.0
	SIN-1 + glibenclamide	0.1 ± 0.1	–
	Db-cGMP (10 μM)		0.0 ± 0.0

Large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}): currents were generated by a depolarizing step from a holding potential of 0 mV to the test potential of 60 mV (intracellular solution with a high concentr ation of Ca^{2+}). Voltage-gated K^+ channels (K_v). Currents were generated by a depolarizing step from a holding potential of −100 mV to the test potential of 30 mV (intracellular solution with a low concentration of Ca^{2+}). ATP-sensitive K^+ channels (K_{ATP}): currents were recorded from a holding potential of 0 mV (intracellular solution with a low concentration of ATP). Db-cGMP: dibutyril-cyclic GMP, 4-AP: 4-aminopyri dine.

* Statistically significant difference vs. control values ($P < 0.05$).

the outward current elicited by a ramp depolarization from 0 to 80 mV. This current was inhibited by iberiotoxin (0.1 μM), but was unaffected by 4-aminopyridine (5 mM). With a holding potential of −100 mV and a low intracellular Ca^{2+} concentration (in order to more accurately record voltage-gated K^+ channels, Quignard et al., 2000), SIN-1 (10 μM) did not significantly affect the current density elicited by a step or a ramp depolarization or the current–voltage relationship ($n = 10$) of the outward K^+ current (Table 1). This current was inhibited by 4-aminopyridine (5 mM), but was unaffected by iberiotoxin (0.1 μM) or charybdotoxin (0.1 μM). Dibutyril-cyclic GMP (10 μM) did not alter the amplitude of the K^+ currents, recorded either at a low or a high concentration of intracellular Ca^{2+} ($n = 5$; Table 1).

Unitary currents with a unitary conductance of 280 ± 20 pS ($n = 5$) were recorded in cell-attached and outside–out membrane patches (Fig. 3). With the outside–out configuration, at a stable holding potential of +30 mV, the open probability of the unitary current was reduced by iberiotoxin (0.1 μM) or by charybdotoxin (0.1 μM) ($n = 3$, data not shown). SIN-1 (10 μM) did not modify significantly the open probability or the mean open time of this current (Fig. 3). Dibutyril-cyclic GMP (10 μM, $n = 10$) did not significantly affect these parameters. However, in 2 out of these 10 cells, dibutyril-cyclic GMP increase the open probability transiently (+30 ± 10%) over 3 min before returning to control values.

In the whole-cell configuration and in the presence of a low concentration of ATP, SIN-1 induced, in 4 out of 10 cells, an outward current that was inhibited by glibenclamide (1 μM) at 0 mV (Fig. 3 and Table 1). This current reversed near the equilibrium potential for K^+ ions (−74 ± 5 mV, $n = 3$) and its density at 0 mV was 1.2 ± 0.3 pA/pF ($n = 4$). Under the same conditions, cromakalim also induced a glibenclamide-sensitive K^+ current ($n = 6$; Table 1).

3.2.2. Rabbit

3.2.2.1. Currents in the presence of intracellular calcium (0.5 mM). The capacity of the carotid arterial myocytes was 27.4 ± 2.9 pF ($n = 60$). With a holding potential of −100 mV, depolarization of the myocytes induced a large whole-cell outward current, which was partially inhibited by iberiotoxin (0.1 μM) or charybdotoxin (0.1 μM), and by 4-aminopyridine (5 mM), indicating that it was the resultant of currents carried by different K^+ channel types. After inhibition of the current by iberiotoxin, addition of apamin (0.5 μM) did not further reduced the current induced by a step-depolarization to +60 mV. Subsequent additional exposure to charybdotoxin induced a further inhibition of the whole-cell current. The current density of this specific current for a step depolarization of +30 mV was 3.1 ± 0.4 pA/pF ($n = 6$). The inactivation kinetic of this current was fast and best fitted with a double exponential ($\tau_1 = 130 \pm 30$ ms, $n = 5$ and $\tau_2 = 25 \pm 5$ ms, $n = 5$), whereas the inactivation kinetic of the global outward current was best fitted with a single exponential ($\tau_1 = 561 \pm 109$, $n = 5$). 4-Aminopyridine (5 mM) inhibited the residual current ($n = 4$). The iberiotoxin-insensitive but charybdotoxin-sensitive current could not be recorded in the presence of 4-aminopyridine (5 mM).

With a holding potential of 0 mV (to inactivate voltage-dependent K^+ channels, K_V), and with a high concentration of intracellular Ca^{2+} (0.5 μM), depolariza-

tion of the myocytes induced a noisy outward current. The current became activated at potentials more positive than +10 mV and its density at +60 mV was 20.5 ± 2.5 pA/pF ($n = 12$). This current was inhibited by charybdotoxin (0.1 μM, inhibition: 93%) or by iberiotoxin (0.1 μM; inhibition: 92%; Table 1). A K^+ current sensitive to apamin (0.5 μm) could not be observed in the various cells studied. The iberiotoxin-insensitive but charybdotoxin-sensitive current could not be recorded in those conditions (holding potential 0 mV).

3.2.2.2. Currents in the presence of a low intracellular Ca^{2+} concentration. At a holding potential of −100 mV and with a low intracellular Ca^{2+} concentration, step-depolarizations induced a slowly inactivating current. Its activation threshold was −30 mV and the current density was 6.1 ± 1.8 pA/pF ($n = 6$) for a step-depolarization to +20 mV. 4-Aminopyridine (0.1–25 mM) inhibited this current in a concentration-dependent manner (5 mM, inhibition: 96%; Table 1). The 4-aminopyridine-sensitive K^+ current was voltage-dependent. The voltage for half-inactivation calculated from the steady state curve of the current inhibited by 4-aminopyridine (5 mM) averaged −32 mV. An iberiotoxin-sensitive current could be recorded only for depolarizations to potentials more positive than +20 mV (data not shown).

3.2.2.3. Currents in inside–out and outside–out patches. Unitary currents through large-conductance, Ca^{2+}-sensitive K^+ channels were recorded in inside–out and outside–out membrane patches. The amplitude of the current and the channel open probability were dependent on the holding potential. With the inside–out configuration, from the current–voltage relationship, only one single channel type with a unitary conductance of 221 ± 4 pS was observed. With the outside–out configuration, at a stable holding potential of +30 mV, the open probability of the unitary current was virtually abolished by the presence of iberiotoxin (data not shown).

3.2.2.4. Effects of SIN-1. In the whole-cell configuration and in the presence of a low concentration of ATP, cromakalim (10 μM) induced an outward current that was inhibited by glibenclamide (1 μM; Table 1). This current reversed near the equilibrium potential for K^+ ions (−76 mV) and its density at 0 mV in the presence of cromakalim was 2.1 ± 0.1 pA/pF (Table 1). Under the same conditions, SIN-1 (10 μM) did not elicited a glibenclamide-sensitive K^+ current (Table 1).

In the presence of a low intracellular Ca^{2+} concentration and with a holding potential of −100 mV, the application of SIN-1 (10 μM) did not alter the K^+ current generated by a step depolarization to +30 mV. In the presence of a high intracellular Ca^{2+} concentration and with a holding potential of 0 mV, the administration of SIN-1 (10 μM) increased the noisy outward K^+ current,

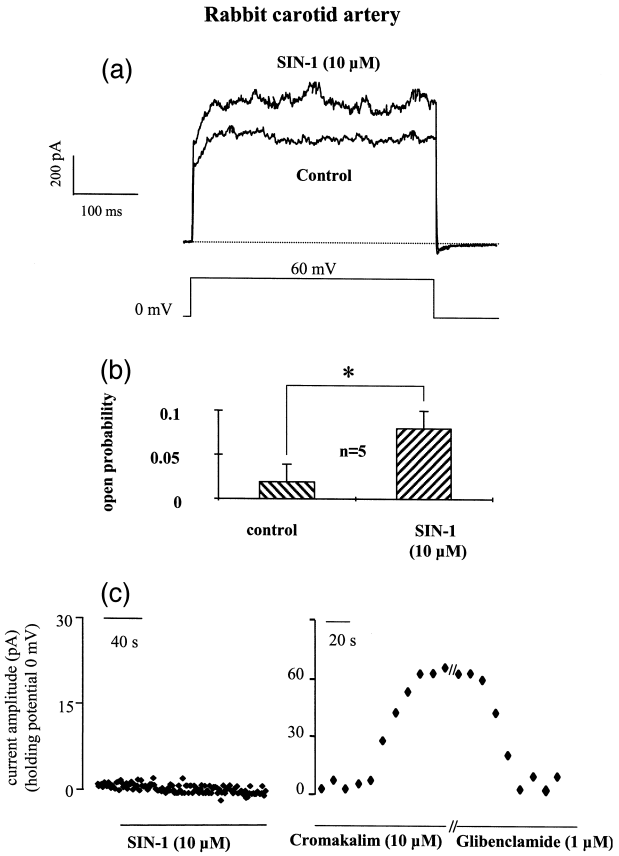

Fig. 4. Effects of SIN-1 on outward K^+ currents in freshly isolated smooth muscle cells from the carotid artery of the rabbit. (a) In the presence of an intracellular solution with a high concentration of Ca^{2+} (0.5 mM), recording of K^+ currents before and after exposure to SIN-1 (10 μM). Currents were generated by a depolarizing step from a potential of 0 mV to the test potential of 60 mV. (b) Effects of SIN-1 (10 μM) on the mean open probability of large conductance outward K^+ channel (cell-attached configuration, patch potential −20 mV). (c) Left: SIN-1 (10 μM) did not induced a glibenclamide (1 μM) sensitive outward K^+ channel (holding potential: 0 mV, intracellular solution with a low concentration of ATP). Right panel: cromakalim (10 μM) induced a glibenclamide-sensitive outward current under the same conditions. The asterisk indicates a statistically significant difference vs. control values ($P < 0.05$); n represents the number of cells studied.

generated by a step depolarization to +60 mV (+63 ± 7%, $n = 7$; Fig. 4 and Table 1). SIN-1 also increased the outward K^+ current, elicited by a ramp depolarization from 0 to 80 mV. The apparent activation threshold of this Ca^{2+}-activated K^+ current was shifted to the left by SIN-1 (by 5.5 ± 1.1 mV, $n = 6$). Similar results were observed with the current–voltage relationship (data not shown). The effects of SIN-1 were mimicked by dibutiryl-cyclic GMP (10 μM, +42 ± 4%, $n = 3$; Table 1). This current was inhibited by the addition of iberiotoxin (0.1 μM, inhibition: −88 ± 3%, $n = 5$).

Unitary currents of large conductance Ca^{2+}-activated K^+ channels were also recorded in the cell-attached configuration. Application of SIN-1 induced a rapid increase in the mean open probability of this channel (Fig. 4).

SIN-1 increased an iberiotoxin-sensitive outward current, only in the presence of a high intracellular concentration of Ca^{2+} ($+34 \pm 4\%$, $n = 7$). This effect could be mimicked by dibutiryl-cyclic GMP (10 μM, $+38 \pm 4\%$, $n = 3$).

Similar results were obtained in the smooth muscle cells of the aorta. SIN-1 (10 μM) increased an iberiotoxin-sensitive outward current only in the presence of a high intracellular concentration of Ca^{2+} ($+31 \pm 4\%$, $n = 3$). This effect was also mimicked by dibutiryl-cyclic-GMP (10 μM, $+25 \pm 3\%$, $n = 2$).

4. Discussion

The results of the present study suggest that the population of K^+ channels activated by NO is species-dependent and support the conclusion that, in the guinea pig carotid artery, the release of endothelial NO cannot account for the acetylcholine-induced hyperpolarization that is observed in the presence of inhibitors of NO-synthase and cyclooxygenase.

In the guinea pig carotid artery, NO is a hyperpolarizing factor. Indeed, SIN-1 produced a glibenclamide-sensitive hyperpolarization, and patch–clamp experiments demonstrated that SIN-1 activated a K^+ conductance. The characteristics of the current activated by SIN-1 are those of an ATP-sensitive K^+ current. This current, which was not observed under control conditions, was inhibited by the specific inhibitor of ATP-sensitive K^+ channels, glibenclamide. Furthermore, the reversal potential of the current was at the equilibrium potential for K^+ ions, indicating that K^+ ions are generating it. The activation of ATP-sensitive K^+ channels by NO has also been reported in isolated smooth muscle cells of the porcine coronary artery (Miyoshi et al., 1994) and in various isolated tissue (for review, see Félétou and Vanhoutte, 2000). In the guinea pig carotid artery, SIN-1 did not affect large conductance Ca^{2+}-activated and voltage-dependent K^+ currents. In the presence of a high concentration of EGTA to chelate intracellular Ca^{2+}, a 4-aminopyridine-sensitive current, which possessed the characteristics of a voltage-gated K^+ current, was recorded. In contrast to myocytes from rat pulmonary and bovine coronary arteries, (Yuan et al., 1996; Li et al., 1997), SIN-1 did not increase the activity of this voltage-gated K^+ current. In isolated smooth muscle cells of the guinea pig carotid artery, a whole-cell current, which was inhibited by iberiotoxin (a specific large conductance Ca^{2+}-activated K^+ channel blocker), was observed, while with the cell-attached configuration, a large conductance unitary current was also recorded. The characteristics of this unitary current (conductance 280 pS, inhibition by iberiotoxin, activation by voltage and Ca^{2+}; Quignard et al., 2000) are similar to the large conductance Ca^{2+}-activated unitary current (Kuriyama et al., 1995). SIN-1 and the permeant analogue of cyclic-GMP did not affect the activity of this Ca^{2+}-activated K^+ channel. This observation was further confirmed by intracellular recordings of the membrane potential, since SIN-1-induced hyperpolarization was unaffected by the presence of Ca^{2+}-activated K^+ channel inhibitors, such as iberiotoxin, apamin, charybdotoxin or the combination of charybdotoxin plus apamin (Corriu et al., 1996).

In the guinea pig isolated carotid artery, in the presence of N^{ω}-nitro-L-arginine and indomethacin, acetylcholine induces an endothelium-dependent hyperpolarization (Corriu et al., 1996). This hyperpolarization is not altered by glibenclamide, but is abolished by the combination of the two toxins, apamin plus charybdotoxin. Taken in conjunction, these findings and the present results clearly demonstrate that, in the guinea pig carotid artery, NO cannot account for the response that is attributed to EDHF.

In the rabbit carotid artery, two different NO-donors, SIN-1 and sodium nitroprusside, did not hyperpolarize the smooth muscle cells. However, in preparations depolarized by phenylephrine, SIN-1 repolarized the smooth muscle cells. Similar findings have previously been reported for carotid artery of the rabbit and for other tissues, such as rabbit femoral, guinea pig uterine and canine mesenteric arteries (Tare et al., 1990; Plane et al., 1995; Cohen et al., 1997). In the present study, the repolarization was inhibited by iberiotoxin. This apparently contradicts a previous report showing that SIN-1-induced relaxation of the rabbit carotid artery is unaffected by iberiotoxin (Plane et al., 1998). However, the relaxation produced by the nitrovasodilator involves more than one mechanism. Indeed, Plane et al. (1998) showed that the relaxation to SIN-1 is minimally affected by KCl, while the repolarization produced by the agent is nearly abolished.

In isolated myocytes of the rabbit carotid artery and aorta, the current, through large conductance Ca^{2+}-activated K^+ channels, was enhanced by SIN-1. Activation of the large conductance Ca^{2+}-activated K^+ channel by NO has been reported in aorta, cerebral and carotid arteries of the rabbit (Robertson et al., 1993; Bolotina et al., 1994), as well as in numerous species, including human (Peng et al., 1996; Bychkov et al., 1998). The activation of the channel may be dependent on the production of cyclic-GMP, but may occur independently, i.e. direct activation of the K^+ channel by NO (Bolotina et al., 1994; Shin et al., 1997). Whether or not SIN-1 produces a direct or indirect activation of the large conductance Ca^{2+}-activated K^+ channel was not determined in the present study. The observation that the permeant analogue of cyclic-GMP was able to mimic the effect of SIN-1, rather supports the latter interpretation. In contrast to the guinea pig carotid artery (Quignard et al., 2000), isolated myocytes of the rabbit carotid artery do not show an apamin-sensitive potassium current.

The smooth muscle cells of the rabbit carotid artery (and aorta) express two types of voltage-dependent K^+ channels, the delayed rectifier and the A type current (rapidly inactivating, charybdotoxin-sensitive and iberi-

otoxin-insensitive current, for review, see Kuriyama et al., 1995), the latter being not expressed in the carotid artery of the guinea pig. SIN-1 did not affect the global voltage-dependent K^+ current. Similarly, in the rabbit carotid artery, SIN-1 did not activate an ATP-sensitive K^+ channels, although the expression of this channel was evidenced by the cromakalim-dependent activation of a glibenclamide-sensitive current in isolated smooth muscle cells and by the cromakalim-induced hyperpolarization of the smooth muscle in isolated tissues.

The observation that SIN-1 induced repolarization, but was unable to hyperpolarize the smooth muscle cells of the carotid artery, is consistent with the activation by the nitrovasodilator of large conductance Ca^{2+}-activated K^+ channels, without affecting other K^+ conductances. Large conductance Ca^{2+}-activated K^+ currents are K^+-gated mainly by intracellular concentrations of free Ca^{2+} and by the membrane potential (Kuriyama et al., 1995). The activity of this current is generally low or absent in normal, non-stimulated arteries (Benham et al., 1986). However, phenylephrine, by increasing the intracellular Ca^{2+} concentration and producing depolarization, enhances the open probability of large conductance Ca^{2+}-activated K^+ channels.

Some hypotheses could be suggested in order to explain the species specificity of NO-induced activation of K-ATP or BK_{Ca}. The nature of some sub-unit of the BK_{Ca} (hSlo) or K-ATP channels (Kir6, SUR2B), expressed in the two species, may be different (Dworetzky et al., 1996; Alioua et al., 1998), or alternatively, the presence or absence of some regulatory proteins, such as phosphatase (Zhou et al., 1996, 1998).

In conclusion, the population of K^+ channels activated by NO is species-dependent. In the rabbit isolated carotid artery, acetylcholine like SIN-1 repolarizes phenylephrine-contracted strips (Cohen et al., 1997). In this tissue, even in the presence of an elevated concentration of NO-synthase inhibitor, the inhibition of the enzyme is incomplete and NO release can still be measured (Cohen et al., 1997). Thus, in the carotid artery of the rabbit, NO may act as an endothelium-derived hyperpolarizating factor. In contrast, in the guinea pig carotid artery, the release of endothelial NO cannot account for the acetylcholine-induced hyperpolarization that is observed in the presence of inhibitors of NO-synthase and cyclooxygenase.

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