The vasodilator, minoxidil (2,4-diamino-6-piperidenyl-pyrimidine 3-oxide), is an effective antihypertensive agent, similar in structure to hydralazine but minimally effective in the in vitro antagonism of norepinephrine-induced contraction of rat tail artery. It has been suggested that the efficacy of minoxidil is mediated by a metabolite. In vivo, minoxidil is sulfated to form minoxidil-0-sulfate (MNXS), which has a direct relaxing effect on rings of rabbit mesenteric artery. Although the efficacy of in vivo MNXS and in vitro MNXS have been well characterized, the mechanism of action on vascular smooth muscle has not been conclusively identified.

Electrophysiological Mechanisms of Minoxidil Sulfate–Induced Vasodilation of Rabbit Portal Vein

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The electrophysiological and mechanical properties of the vasodilator minoxidil sulfate (MNXS) were examined in isolated smooth muscle cells and strips from rabbit portal vein. At micromolar concentrations, MNXS inhibited norepinephrine (0.1–1.0 μM)–induced contractions in isolated muscle strips. In isolated cells, norepinephrine caused a dose-dependent depolarization of the resting membrane potential, which was significantly attenuated by MNXS (5 μM); MNXS alone caused a hyperpolarization of the membrane potential. This hyperpolarization was insensitive to Na⁺-K⁺ pump blockade by ouabain, but was inhibited by the K⁺ channel antagonist, tetraethylammonium (20 mM). In voltage-clamp experiments, a resting (background) conductance associated with the resting membrane potential was identified. This conductance, which previously has been shown to be reduced by Ba²⁺ as well as tetraethylammonium (2 μM). In additional experiments, whole-cell L-type Ca²⁺ currents were inhibited by micromolar concentrations of MNXS. These experiments show that concentrations of MNXS that inhibit norepinephrine-induced contractions promote K⁺ conductance and inhibit Ca²⁺ entry through voltage-dependent Ca²⁺ channels in vascular smooth muscle cells. These electrophysiological effects of MNXS may be responsible for the vasorelaxant effects of the drug observed in vitro and in vivo. (Circulation Research 1989;65:1102–1111)

Vascular smooth muscle cells (VSMCs) of rabbit portal vein provide a model for the investigation of drug effects on membrane ionic currents. Some of the membrane channels in these cells have recently been characterized by Inoue and coworkers. They identified at least three distinct K⁺ channels in cell-attached and excised membrane patches in VSMCs of rabbit portal vein. Corollary whole-cell voltage-clamp experiments in the same preparation have recently identified four distinct K⁺ currents and a Ca²⁺ current. Modulation of K⁺ or Ca²⁺ channels in VSMCs may be important mechanisms for the hypotensive actions of some vasoactive drugs. For example, BRL 34915 and 2-nicotinamido-ethyl nitrate (nicorandil) hyperpolarize rat portal vein by increasing the K⁺ conductance of the membrane. In addition, Ca²⁺ channel blocking agents like verapamil, nitrendipine, or nifedipine are well known for treatment of hypertension.

In a recent study it has been shown that MNXS-induced relaxation of isolated rabbit superior mesenteric artery is endothelium independent, blocked by tetraethylammonium (TEA) pretreatment, and is accompanied by a stimulation of K⁺ efflux, suggesting that part of the relaxing effects of MNXS might be due to activation of membrane K⁺ channels. We have, therefore, directly examined the
effects of MNXS on resting membrane potential and whole-cell ionic currents in VSMCs isolated from rabbit portal vein and have compared the electrophysiological potency of this compound with the concentrations required to cause significant reduction of the norepinephrine (NE)-induced contractions in syncytial strips of the same preparation. A preliminary report of these results has been presented in abstract form. 11

Materials and Methods

Cell Isolation

Single vascular smooth muscle cells were isolated from rabbit portal vein by a collagenase dispersion technique as described elsewhere. 7 Briefly, albino rabbits of either sex were euthanized and the portal vein rapidly removed and placed in cold oxygenated Tyrode’s solution having the following composition (mM): NaCl 130, NaHCO3 10, KCl 4.2, KH2PO4 1.2, MgCl2 0.5, CaCl2 1.8, dextrose 11, aspartic acid 1, glutamic acid 1, HEPES-NaOH 10 (pH 7.35). Fat and connective tissue are carefully removed, and the vessel is then cut into small pieces (4x4 mm) and incubated for 60 minutes at room temperature in the same Tyrode’s solution in which Ca2+ is omitted. SMCs were dispersed in this solution in which 150-200 units/ml of collagenase type I (Sigma Chemical, St. Louis, Missouri), 0.1% bovine albumin, and 1 mM ATP (disodium salt) were added. The dispersion was allowed to proceed for 50-70 minutes at 35° C without agitation. At this time, the tissue fragments were gently titrated with a pasteur pipette and transferred to the experimental chamber, which was mounted on the stage of an inverted microscope for electrophysiologic studies.

Electrophysiological Techniques

Resting membrane potentials of isolated cells were measured using conventional patch electrodes filled with (mM) K-gluconate 110, KCl 30, NaCl 10, ATP (dipotassium salt) 1.0, MgCl2 0.5, EGTA 0.1, creatine phosphate 5.0, and HEPES-KOH (pH 7.2) 5. For voltage-clamp experiments, small tip diameter pipettes (<1 μm), which minimize internal dialysis, 12,13 were also used in some experiments. In other experiments it was necessary to block whole-cell K+ currents to study Ca2+ currents, in which case the whole-cell configuration of the patch-clamp technique 14 using larger tip diameter pipettes was used to dialyze isolated cells with K+ channel blockers and impermeant ions. Small tip-diameter pipettes were filled with a solution containing 2 M K+ gluconate and 100 mM KCl. Conventional patch electrodes were filled with a solution that contained (mM) cesium aspartate 110, cesium chloride 20, TEA 20, MgCl2 0.5, ATP-Mg salt 5, creatine phosphate 5, EGTA 0.1, and HEPES-CsOH (pH 7.2) 5. Both types of pipettes when filled with their respective solutions had resistances that ranged from 2–6 MΩ.

Solutions and Drugs

The standard external solution contained (mM) NaCl 130, NaHCO3 10, KCl 4.2, KH2PO4 1.2, MgCl2 0.5, CaCl2 1.8, dextrose 5.5, and HEPES-NaOH (pH 7.35) 10. All experiments were carried out at room temperature (21°–23° C.). MNXS was dissolved in polyethylene glycol 400 to make a 5-mM stock solution each day and was diluted in standard external solution to give the desired concentrations.

NE was obtained from Sigma Chemical, St. Louis, Missouri. Unless otherwise noted, cells and tissue were exposed to MNXS or ouabain for at least 20 minutes before electrical or mechanical measurements were made. Exposure times for NE and TEA are indicated in the figures. Although lower MNXS concentrations are effective in vitro, for electrophysiological studies we examined concentrations of MNXS in the micromolar range to accelerate the time course of drug action, since lower concentrations are much slower in onset. 10

Mechanical Methods

A 10-15-mm segment of the rabbit portal vein was removed and cleaned of connective tissue, fat, and blood. The vessel was mounted in the longitudinal direction for contractile measurements by tying each end with suture to stainless steel wires in the tissue bath (30 ml). One wire was stationary and the other was attached to a Gould Metrigram isometric force transducer (Gould Instruments, Cleveland, Ohio). Force was monitored on a Western Graphtec WR3701 strip chart recorder (Irvine, California). A resting force of 0.5 g was applied, and a 1-hour equilibration time was allowed before agonist-induced contractions were initiated.

Results

MNXS Inhibition of NE Contractions in Intact Muscle Strips

As in the case of BRL 34915 8 and pinacidil, 15 MNXS has been reported to be more effective in inhibiting NE contractions compared with K+-depolarization contractions. 10 Since this latter study was carried out in rabbit superior mesenteric artery, we tested the potency of MNXS in inhibiting NE contractions in intact muscle strips from rabbit portal vein. As shown in Figure 1A, isolated muscle strips were spontaneously active, and the addition of NE caused dose-dependent (0.1 and 1.0 μM) contractions. The NE-induced contraction was completely reversed after washout of the drug and spontaneous contractions resumed. Subsequent exposure to 3 μM MNXS alone abolished spontaneous contractile activity in the preparation shown in Figure 1 and in the other three preparations significantly reduced the amplitude and frequency of spontaneous contractions. MNXS addition had no significant effect on the resting level of tone. The responses to both 0.1 and 1.0 μM NE were significantly attenuated in the presence of MNXS. Panel
FIGURE 1. Effects of minoxidil sulfate (MNXS) on the spontaneous and norepinephrine-induced contractions recorded in a portal vein muscle strip. In panel A, spontaneous contractions are shown on the left part of the recording; two doses of norepinephrine (NE) were sequentially applied to the bath. After washout of NE (W), the muscle was incubated with MNXS (3 μM; right part of the recording) for 20 minutes during which time spontaneous contractions were abolished. NE was then reapplied as described above in the continued presence of MNXS. Panel B summarizes the contractile data obtained in the presence of MNXS (3 μM) and NE in four muscle preparations. Each column was normalized against the contraction produced by 10 μM NE and is expressed as a mean±SEM. The differences between CONTROL and MNXS in the left and middle sets of columns are significant with p<0.001; the difference between the two columns of the right set is significant with p<0.05 (evaluated with paired Student's t test).

B summarizes the effects of MNXS on the contractile response to NE in four isolated muscle strips. MNXS (3 μM) produced a significant reduction in the amplitude of contraction to both 0.1 and 1.0 μM NE (62% and 55% inhibition, respectively) and significantly decreased the amplitude of spontaneous contractions. Thus, as in the rabbit mesenteric artery, moderate concentrations of MNXS are capable of significant inhibition of NE-induced contractions.

Effects of NE and MNXS on Resting Membrane Potential of Isolated VSMCs

In a previous study,7 we characterized the passive membrane properties of smooth muscle cells isolated from rabbit portal vein. In external solutions containing 5.4 mM K+, nondialyzed isolated cells had a mean resting membrane potential of -47.9±4.6 mV (n=9) and an input resistance of 378±91 MΩ (n=9).
FIGURE 2. Effects of minoxidil sulfate (MNXS) and norepinephrine (NE) on the resting potential recorded in single portal vein smooth muscle cells. The recordings shown in A and B are from different cells. Large suction micropipettes (dialysis) were used in current-clamp mode to record the membrane potential. A: Effects of NE and its subsequent washout on the resting membrane potential (RMP). B: Effects of MNXS (5 μM) and subsequent addition of NE on RMP. Panel C summarizes the effects of MNXS on the resting membrane potential of single smooth muscle cells in absence and in the presence of NE in a number of cells. Each column reflects a mean ± SEM. In the right set of columns, C (control) represents the value of membrane potential recorded in the presence of MNXS immediately before application of NE. Each data set was collected from four to seven cells. Highly significant differences (p<0.001) were detected for the left and middle set of columns; no detectable difference was observed between the two mean values of the right set of columns.
Figure 2 illustrates experiments in which we examined the effects of NE and MNXS on the resting membrane potential of isolated cells. In panel A, the cell exhibited a resting potential of \(-50\) mV under control conditions. Exposure of the cell to 10 \(\mu\)M NE caused a rapid depolarization of the membrane potential to \(-11\) mV. This depolarization was almost completely reversed on washout of NE. Panel B illustrates an experiment in which we examined the effects of MNXS on the resting membrane potential of a VSMC of rabbit portal vein. This cell exhibited a resting potential of \(-44\) mV before the application of MNXS. MNXS (5 \(\mu\)M) caused a hyperpolarization of the membrane potential to \(-46\) mV. Subsequent exposure of this cell to 10 \(\mu\)M NE in the continued presence of MNXS failed to depolarize the membrane potential.

Panel C summarizes the effects of NE and MNXS on the resting membrane potential of isolated VSMC of rabbit portal vein. Under control conditions, the mean resting membrane was \(-47\) mV and exposure to 5 \(\mu\)M MNXS produced a hyperpolarization to a mean value of \(-59\) mV (\(n=9\)). NE (10 \(\mu\)M) alone depolarized the resting membrane potential from a mean level of \(-57\) to \(-35\) mV (\(n=7\)). MNXS reduced the magnitude of depolarization to 10 \(\mu\)M NE from 22 to 8 mV (\(n=7\)). These results suggest that NE and MNXS have antagonistic actions on the membrane potential of VSMCs and that these antagonistic effects of the two compounds on resting membrane potential occur at concentrations similar to those in which the two compounds exert antagonistic effects on contractile activity (Figure 1).

**Effects of Ouabain and TEA on MNXS-Induced Membrane Hyperpolarization**

Additional experiments have been carried out to distinguish between two possible mechanisms responsible for the hyperpolarizing effects of MNXS on resting membrane potential: activation of an outward current due to stimulation of the electrogenic Na\(^+\)-K\(^+\) pump or activation of a K\(^+\) conductance that contributes to the resting membrane potential. In the experiment illustrated in Figure 3A, we examined the effects of a Na\(^+\)-K\(^+\) pump inhibitor, ouabain, on the resting membrane potential of VSMC of rabbit portal vein and whether acute blockade of the pump would prevent the hyperpolarizing effects of MNXS. Ouabain (50 \(\mu\)M) depolarized the resting membrane potential of this cell from \(-44\) to \(-35\) mV suggesting that a component of the resting membrane conductance of these cells is due to an electrogenic pump.\(^6\) In two additional cells, 50 \(\mu\)M ouabain produced depolarizations of 12 and 7 mV. In the continued presence of ouabain, 5 \(\mu\)M MNXS still hyperpolarized the membrane potential nearly 14 mV, a value similar to that previously observed with this dose of MNXS in the absence of ouabain (Figure 2). The ability of MNXS to hyperpolarize the resting membrane potential, therefore, appears to involve a mechanism other than pump stimulation.

In Figure 3B, we examined the effects of the K\(^+\) channel antagonist TEA on resting membrane potential and on the MNXS-induced membrane hyperpolarization. TEA (20 mM) produced a significant depolarization of the membrane potential in this cell from \(-30\) to \(-6\) mV. Exposure to MNXS (5 \(\mu\)M) in the continued presence of TEA failed to elicit any significant hyperpolarization of the membrane potential. On attempted washout of both drugs, the membrane potential hyperpolarized to a level considerably more negative than observed in the control (to approximately \(-54\) mV). This likely reflects the hyperpolarizing effect of MNXS, which becomes manifest when TEA is washed out, since MNXS is not easily reversible within this period (15-minute washout). The ability of TEA to prevent the MNXS-induced hyperpolarization of the resting membrane potential was observed in two other experiments and is consistent with the hypothesis that MNXS augments K\(^+\) conductance of the membrane of isolated VSMC.

**Effect of MNXS on Membrane Currents of VSMC of Rabbit Portal Vein**

In our recent analysis of macroscopic currents in VSMC isolated from rabbit portal vein,\(^7\) four distinct outward K\(^+\) currents were described based on differences in voltage dependence and sensitivity to Ca\(^{2+}\) concentration and K\(^+\) channel antagonists. When cells are voltage-clamped using small tip-diameter pipettes, which minimize internal dialysis (see "Materials and Methods"), two components of whole-cell outward current are consistently observed: 1) a quasi-instantaneous current that is blocked by Ba\(^{2+}\) ions and TEA, sensitive to external Ca\(^{2+}\) concentration, and may reflect the activity of K\(^+\) channels involved in maintenance of the resting membrane potential (background or resting conductance), and 2) a component of outward current that activates with time during depolarizations to positive membrane potentials, shows little if any inactivation, is insensitive to Ca\(^{2+}\), and is selective for K\(^+\) ions (delayed rectifier K\(^+\) current).

An example of these currents is illustrated in Figure 4A. This cell was clamped to a holding potential of \(-50\) mV and 500 msec duration voltage-clamp steps were applied in 10 mV increments to potentials ranging from \(-70\) to \(+60\) mV. In response to hyperpolarizing voltage-clamp steps, only quasi-instantaneous (time-independent) inward currents were activated. In response to depolarizing voltage-clamp steps, an initial time-independent outward current is observed followed by the time-dependent activation of an outward current that begins near \(-20\) mV and becomes larger as pulses are applied to more positive potentials. The current-voltage relations for these two currents are shown in Figure 5A. Currents measured immediately following discharge of the membrane capacitance (open circles) reflect the voltage dependence of the background...
con ductance; currents measured at the end of the voltage-clamp steps (open squares) reflect activation of time-dependent outward K⁺ current.

Figure 4B illustrates the effects of 2 μM MNXS on membrane currents in SMCs of rabbit portal vein. MNXS caused a shift in holding current in the outward direction required to clamp the cell to -50 mV. This is analogous to a hyperpolarization of the resting membrane potential. In addition, the amplitude of the background current activated during hyperpolarizing and depolarizing voltage-clamp steps was increased. There was little change in the amplitude or kinetics of the time-dependent K⁺ current in the presence of MNXS. These effects are more easily observed by comparing the current-voltage relations for these currents before and after exposure to MNXS, panels A and B of Figure 5, respectively. Similar effects of MNXS on membrane currents have been observed in nine separate experiments. In each case MNXS produced a shift in holding current in the outward direction and increased the magnitude of the background, time-independent conductance. These results and our earlier demonstration that this background conductance is blocked by external application of TEA or Ba²⁺ are consistent with the finding that MNXS produces a hyperpolarization of the resting membrane potential of these cells and that this hyperpolarization can be prevented by TEA. Both current and voltage-clamp data are therefore consistent with an effect of MNXS to enhance the activity of K⁺ channels which can modulate the cell resting membrane potential.

We have found empirically that if we use conventional patch pipettes to voltage clamp these cells, which allows internal dialysis, in addition to the two types of outward currents just described, two additional types of outward K⁺ currents can be observed in these VSMC: a smooth transient outward current and spontaneous transient outward currents, both of which are Ca²⁺-activated currents (blocked by...
FIGURE 4. Effects of minoxidil sulfate (MNXS) on the membrane currents recorded in a nondiaryzed portal vein cell. The small micropipette suction technique (see "Materials and Methods"; these are filled with 2 M K-Gluconate and 100 mM KCl) was used to voltage clamp the cell. From a holding potential of —50 mV, 500-msec duration step depolarizations to various potential levels were applied to the cell at a frequency of 0.2 Hz in control (left set of traces), and after incubating the cell with MNXS for 15 minutes (right set of traces).

A. B.

FIGURE 5. Current-voltage relations for the experiment shown in Figure 4. Membrane currents at each potential level imposed were estimated by measuring the absolute amount of current at the beginning (open circles) and at the end of the pulse (open squares). A: Control. B: In the presence of minoxidil sulfate (2 µM). Note that minoxidil sulfate shifts the zero current potential in the hyperpolarizing direction and enhances the background current measured at the beginning of the pulse but has very little influence on the time-dependent current as judged from the difference between the two curves.
FIGURE 6. Effects of two concentrations of minoxidil sulfate on the calcium current recorded in a single portal vein cell. In this experiment, a dialyzing micropipette filled with cesium and tetraethylammonium to block the outward K+ currents (see "Materials and Methods") was used to voltage clamp the cell. A: Transient inward calcium currents were elicited by voltage-clamp steps to 0 mV (peak of the current-voltage relation, see panel B) from a holding potential of -50 mV under control conditions (c) and in the presence of two doses of minoxidil sulfate (middle trace: 2 nM; upper trace: 5 nM). B: Current-voltage relations obtained from the same experiment shown in panel A. Consecutive steps from -40 mV to 60 mV were applied in 10-mV increments at a frequency of 0.2 Hz in control conditions, and the peak inward current was plotted as a function of the imposed voltage (empty circles). An identical protocol was repeated after application of 2 and 5 nM minoxidil sulfate, respectively, and the amplitude of the inward current measured under steady-state block (∆ and ○, respectively).

mV under control conditions. These inward currents can be identified as L-type Ca2+ currents due to their sensitivity to [Ca2+]o, dihydropyridines, and D600. These Ca2+ currents are activated near -40 mV and exhibit a peak amplitude near 0 mV (panel B). After recording control currents and ensuring their stability, sequential doses of 2 and 5 μM MNXS were superfused for periods of 10 minutes before Ca2+ current-voltage relations were measured. As shown in panel A, MNXS produced a dose-dependent block of calcium current, without causing any apparent shift in the shape of the current-voltage relation (panel B). In a total of five experiments, 5 μM MNXS produced a mean reduction in the peak amplitude of calcium current of 61 ± 10.5%.

Discussion

It has recently been proposed that compounds such as pinacidil, BRL 34915, and minoxidil represent a new class of antihypertensive agents that have as their primary mechanism of action the opening of K+ channels in the membrane of vascular smooth muscle cells. This hypothesis is based on the observations that 1) relaxation is endothelium independent, 2) each compound is more effective in relaxing agonist-induced contractions compared with K+ -induced contractions, 3) each compound increases 86Rb efflux from vascular muscle, and 4) relaxation is antagonized by K+ blockers such as TEA. In addition, in the case of BRL 34915 and pinacidil, microelectrode studies have demonstrated that these compounds produce a hyperpolarization of the resting membrane potential of vascular smooth muscle at doses that are similar to those that antagonize agonist-induced contractions. It is not known if MNXS exerts a similar effect on the resting membrane potential of VSMCs. The objective of our studies was to determine if MNXS produces changes in the resting membrane potential of vascular smooth muscle at doses that are effective in antagonizing NE-induced contractions and to determine if such changes in membrane potential are mediated by alterations in membrane K+ conductance.

Our initial contractile studies on intact muscle strips established that MNXS has a similar potency for antagonizing NE-induced contractions in rabbit portal vein as was found previously in rabbit superior mesenteric artery. It was also found that MNXS reduced both the frequency and amplitude of spontaneous mechanical events in this tissue. Measurements of resting membrane potentials in isolated VSMCs of rabbit portal vein showed that MNXS alone, which are effective in reducing NE-induced contractions, produce significant hyperpolarization of the resting membrane potential (10–15 mV) and effectively antagonize NE-induced depolarization of the resting membrane potential. Moreover, it was found that MNXS-induced membrane hyperpolarization was not prevented by acute blockade of the Na+-K+ pump by
ouabain but was prevented by prior treatment with the K⁺ channel antagonist TEA. These results, therefore, confirm earlier predictions based on contractile, K⁺ efflux, and Ca⁺ influx studies that MNXS might elicit a membrane hyperpolarization of VSMCs, mediated by augmentation of K⁺ conductance of the membrane.

Despite the evidence that BRL 34915, pinacidil, and MNXS act to promote K⁺ permeability in VSMCs, it is not yet clear which K⁺ channels in VSMCs are involved in the actions of these compounds. Recent studies on isolated smooth muscle cells have provided evidence for the existence of a variety of different types of K⁺ channels. Time-dependent whole-cell K⁺ currents have been described in cultured aortic cells, rabbit pulmonary artery cells, toad stomach cells, rabbit and guinea pig stomach cells, and rabbit jejunal cells. Some, but not all, of these K⁺ currents seem to be partially activated by the influx of Ca²⁺ through voltage-dependent channels. Another type of K⁺ current, described as spontaneous transient outward currents, has been identified in rabbit jejunum and car artery cells and intestinal smooth muscle balls. The oscillatory K⁺ currents seem to be different from those described above since they appear to be induced by quantal release of Ca²⁺ from internal stores.

In isolated VSMCs of rabbit portal vein, three types of unitary K⁺ channel activity have been described (Kₛ, Kₘ, and K₁) corresponding to 92, 180, and 273 pS channels (142 mM symmetrical K⁺) and classified according to their voltage dependence and sensitivity to K⁺ channel antagonists and Ca²⁺. Although the precise physiological role of these different K⁺ channels is unknown, it was speculated that the Kₛ-type channel might be responsible for the resting membrane potential since it exhibited little, if any, voltage dependence. Our own whole-cell current measurements in these isolated cells have demonstrated the existence of a time-independent background conductance, which underlies the resting membrane potential and exhibits sensitivity to external Ca²⁺ and TEA. Since the resting membrane potential in these and other VSMCs is believed to be due to a combined electrogenic potential as well as diffusion potentials for K⁺ and Cl⁻, it is likely that the background conductance measured under voltage-clamp conditions represents the sum of each of these individual conductances. Our present studies indicate that MNXS augments that part of the total background conductance that is due to a resting K⁺ permeability, since it is blocked by TEA. Whether this K⁺ permeability necessarily corresponds to K⁺ efflux through Kₛ-type channels remains to be determined. Our experiments failed to reveal any consistent effects of MNXS on the three other types of whole-cell K⁺ currents in these cells, which include the time-dependent, Ca²⁺-insensitive K⁺ current (delayed rectifier) and the Ca²⁺-activated currents, the transient outward current and spontaneous transient outward currents.

A certain degree of caution is always necessary in attempting to extrapolate results obtained from isolated cells to in vivo conditions. The rabbit portal vein is somewhat unusual in its properties as a model of vascular smooth muscle since spontaneous electrical and mechanical activity are present. The existence of a background conductance in these cells, which can at least in part be attributed to a K⁺ conductance however, may be rather common in smooth muscle, since we have also observed a similar background current in cells isolated from rabbit coronary arteries (authors' unpublished observations) and recent experiments in cells isolated from dog colon have also revealed a similar type of current.

A novel finding in our studies was the ability of MNXS to block L-type Ca²⁺ channels in this preparation. Such a property might be an important mechanism that contributes to the ability of this compound to inhibit NE-induced contractions. However, the importance of this mechanism is difficult to assess, since it is not clear what role, if any, Ca²⁺ entry through voltage-dependent channels plays in NE-induced contractions in VSMCs. It has recently been shown in VSMCs of rabbit portal vein that NE-induced depolarization of the membrane potential may be mediated by an increase in Cl⁻ conductance. NE has been reported to reduce or activate voltage-dependent Ca²⁺ currents in VSMCs. It is possible that the Ca²⁺ channel-blocking properties of MNXS may not be involved in the relaxation of agonist-induced contractions but may be involved in inhibition of spontaneous activity. Further studies are required to determine the importance of such a mechanism.

The issue of which particular K⁺ channels in VSMCs are being modulated by these compounds may be resolved by recent voltage-clamp studies of the effects of these compounds in isolated cardiac myocytes. BRL 34915 has been shown to activate ATP-sensitive K⁺ channels in these cells, which is antagonized by inhibitors of the ATP-sensitive K⁺ channel like glyburide. It has also been reported recently that the relaxation produced by BRL 34915 and MNXS in rat portal vein is antagonized by the ATP-sensitive K⁺ channel antagonist glyburide, suggesting the possibility that both compounds might be acting on ATP-sensitive K⁺ channels in VSMCs. However, the existence of ATP-sensitive K⁺ channels in VSMCs has not yet been demonstrated. It is clear that further single channel studies are required to establish which types of K⁺ channels in VSMCs are modulated by these compounds and the relative specificity of antagonists of ATP-sensitive K⁺ channels needs to be carefully assessed in VSMCs.

Note added in proof: ATP-sensitive K⁺ channels have recently been demonstrated in rabbit and rat mesenteric arteries (Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT: Hyperpolarizing vasodilators activate ATP-sensitive K⁺ channel...
nels in arterial smooth muscle. Science 1989;245:177–180), which may be modulated by MNXS.

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