BRIEF REPORTS / RAPPORTS BREFS

Nitric oxide induced membrane hyperpolarization in the rat aorta is not mediated by glibenclamide-sensitive potassium channels

Bert Vanheel and Johan Van de Voorde

Abstract: Using conventional intracellular microelectrode techniques, the membrane potential (E_m) of vascular smooth muscle cells in isolated segments of thoracic rat aorta was measured. The influence of exogenous and of endothelium-derived nitric oxide on the E_m was assessed, and the involvement of glibenclamide-sensitive channels in the observed membrane hyperpolarization was investigated. Exposures of the aorta strips to sodium nitroprusside (10^{-8}–10^{-5} M) caused a concentration-dependent and endothelium-independent hyperpolarization. Maximal hyperpolarization (5.7 ± 0.4 mV) was obtained with 10^{-5} M sodium nitroprusside. Acetylcholine (10^{-8}–10^{-5} M) produced endothelium-dependent hyperpolarizations. At low concentrations, a slow E_m change was elicited, which was sustained in the presence of the vasodilator. Higher concentrations of acetylcholine caused hyperpolarizations consisting of an initial transient peak followed by a more sustained component. Pre-exposure to N^G-nitro-L-arginine (L-NNA, 2×10^{-4} M), which depolarized E_m by 2.5 ± 0.7 mV, significantly attenuated the later component of hyperpolarization, indicating that it is NO dependent. Glibenclamide (10^{-5} M) did not significantly affect the hyperpolarization induced by 10^{-6}–10^{-5} M sodium nitroprusside nor the maintained component of hyperpolarization induced by 10^{-5} M acetylcholine. It is concluded that the hyperpolarization caused by exogenous or endogenous NO in the rat aorta is not mediated by activation of glibenclamide-sensitive potassium channels.

Key words: nitric oxide, endothelium, membrane potential, potassium channels, sodium nitroprusside.

Résumé : On a mesuré le potentiel de membrane (E_m) des cellules musculaires lisses vasculaires dans des segments d'aortes de rats, en utilisant des techniques à microélectrodes intracellulaires conventionnelles. On a évalué l'effet sur l'E_m du monoxyde d'azote dérivé de l'endothélium et exogène, et examiné le rôle des canaux sensibles au glibenclamide dans l'hyperpolarisation membranaire observée. Des expositions des bandes aortiques au nitroprussiate de sodium (10^{-8}–10^{-5} M) ont provoqué une hyperpolarisation indépendante de l'endothélium et dépendante de la concentration. Une hyperpolarisation maximale (5,7 ± 0,4 mV) a été obtenue avec une concentration de 10^{-5} M. L'acétylcholine (10^{-8}–10^{-5} M) a provoqué des hyperpolarisations dépendantes de l'endothélium. À faibles concentrations, une lente variation de l'E_m a été induite, variation qui a persisté en présence du vasodilatateur. Des concentrations plus élevées d'acétylcholine ont provoqué des hyperpolarisations constituées d'une crête transitoire initiale suivie d'une composante plus soutenue. Une pré-exposition à la N^G-nitro-L-arginine (L-NNA, 2×10^{-4} M), qui a dépolarisé l'E_m de 2,5 ± 0,7 mV, a significativement atténué la composante tardive d'hyperpolarisation, indiquant sa dépendance vis-à-vis le NO. Le glibenclamide (10^{-5} M) n'a pas significativement affecté l'hyperpolarisation induite par 10^{-6}–10^{-5} M de nitroprussiate de sodium ni la composante soutenue d'hyperpolarisation induite par 10^{-5} M d'acétylcholine. On conclut que l'hyperpolarisation induite par le NO endogène ou exogène dans l'aorte de rat n'est pas véhiculée par l'activation des canaux potassiques sensibles au glibenclamide.

Mots clés : monoxyde d'azote, endothélium, potentiel de membrane, canaux potassiques, nitroprussiate de sodium.
[Traduit par la Rédaction]

Received August 26, 1996.

B. Vanheel[1] and J. Van de Voorde. Department of Physiology and Physiopathology, University of Gent, De Pintelaan 185, B-9000 Gent, Belgium.

[1] Author to whom all correspondence should be addressed (e-mail: Bert.Vanheel@rug.ac.be).

Introduction

The membrane potential (E_m) of vascular smooth muscle cells plays an important role in determining the tone of vessels. Membrane potential largely determines the open probability of the voltage-operated calcium channels and, hence, the influx of calcium ions from the extracellular medium necessary for maintaining contractile activation. Moreover, E_m may change

agonist-induced inositol-1,4,5-triphosphate (IP_3) accumulation (Quast 1993) and interfere with IP_3-induced calcium release from intracellular stores (Ganitkevich and Isenberg 1993). Modest changes of E_m within the physiological range profoundly affect tension (Nelson et al. 1990).

Vascular smooth muscle membrane potential can be modulated by several substances released from the endothelium. Following stimulation of membrane receptors by various vasodilators such as acetylcholine, endothelial cells release the endothelium-derived hyperpolarizing factor (EDHF). The nature of this diffusable factor is as yet unknown. Although some reports suggested the involvement of a cytochrome P450 monooxygenase derived metabolite of arachidonic acid (Hecker et al. 1994; Bauersachs et al. 1994), inhibition of this metabolism does not influence endothelium-dependent hyperpolarization in several arteries (Corriu et al. 1996; Vanheel and Van de Voorde 1997). The hyperpolarization by EDHF is caused by an increase in K^+ permeability of the membrane of the smooth muscle cells (Chen and Suzuki 1989). While in cerebral arteries the endothelium-dependent hyperpolarization seems to be mediated by activation of ATP-regulated K^+ (K_{ATP}) channels (Brayden 1990; Plane and Garland 1993), in several vessels including the rat aorta (Van de Voorde et al. 1992) the hyperpolarization caused by EDHF is resistant to glibenclamide, pointing to another mechanism, perhaps activation of Ca-regulated K (K_{Ca}) channels (Chen et al. 1991; Eckman et al. 1992). In addition to EDHF, endothelium-derived nitric oxide (EDNO) and prostacyclin are liberated from the endothelium in response to acetylcholine and other vasoactive agonists. EDNO diffuses to the underlying vascular smooth muscle cells and activates soluble guanylyl cyclase, leading to increased smooth muscle cGMP levels and relaxation. In some vessels, such as the uterine (Tare et al. 1990) and coronary (Parkington et al. 1993) artery from guinea-pigs and the small mesenteric artery from rats (Garland and McPherson 1992), nitric oxide (NO) also causes a hyperpolarization of the vascular smooth muscle cells. However, differences seem to exist between the mechanism of the EDHF-induced and the NO-induced hyperpolarization. The membrane hyperpolarization in response to NO only is present in passively stretched preparations (Parkington et al. 1993), in depolarized and constricted preparations (Tare et al. 1990; Parkington et al. 1995), or only in the absence of active force (Garland and McPherson 1992), whereas the EDHF-induced hyperpolarization occurs, although with varying magnitude, independently of passive or active tone (Tare et al. 1990; Chen et al. 1991). In addition, both in the guinea-pig coronary artery and the rat small mesenteric artery the NO-induced but not the acetylcholine-evoked E_m change is completely blocked by glibenclamide (Garland and McPherson 1992; Parkington et al. 1995).

In previous work (Vanheel et al. 1994) we showed that the NO donors nitroglycerin (10^{-5} M) and SIN-1 (3×10^{-6} – 10^{-5} M), the active metabolite of molsidomine, produce a small sustained hyperpolarization of 1–2 mV in rat aortic smooth muscle at rest (in the absence of constriction). Moreover, by using N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA), inhibitors of endothelial NO synthesis, a component of hyperpolarization caused by endogenous NO could be identified as part of the membrane electrical response to acetylcholine (Vanheel et al. 1994). In the present study, we investigated the influence of sodium nitroprusside (SNP), which is another donor of NO, on the E_m of the rat aorta. In addition, we examined the influence of glibenclamide on the hyperpolarization in response to endogenous (endothelium derived) and exogenous (SNP generated) NO to determine whether ATP-regulated K^+ channels were involved.

Methods

Rats used in this study were treated in accordance with the principles and guidelines of the Canadian Council on Animal Care. Wistar rats were anesthetized with an intraperitoneal injection of a lethal dose of sodium pentobarbitone. The thoracic aorta was rapidly removed and placed in oxygenated cold Krebs–Ringer solution containing, in mmol/L, NaCl, 135; KCl, 5; $NaHCO_3$, 20; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; EDTA, 0.026; and glucose, 10. The preparation was dissected free of connective tissue and cut into segments about 4 mm in length. Ring segments were carefully slit along the longitudinal axis. The vessel strips were pinned down, luminal side upwards, to the bottom of an experimental chamber, and superfused with the Krebs–Ringer solution at 35°C. The solution was continuously gassed with a 95% O_2 – 5% CO_2 gas mixture, and pH was ~7.4.

Transmembrane potentials were measured by penetrating the muscle strip from the luminal side with conventional intracellular microelectrodes, pulled with a vertical pipette puller (model 750, David Kopf Instruments, Tujunga, Calif.). Filamented borosilicate glass tubing (1 mm o.d., Hilgenberg, Germany) was used. Microelectrodes were filled with 1 M KCl. Their electrical resistance ranged from 25 to 60 MΩ.

Data collection and analysis

Primary criteria for successful cell impalement were an extremely sharp voltage deflection on entering the cell and a fast return to the baseline value on withdrawal of the microelectrode. Values of the membrane potential were taken as the difference of the stabilized potential and the zero potential on withdrawal or dislodgement of the microelectrode from the cell.

The electrical signal was continuously followed on an oscilloscope and traced with a pen recorder set at 5 mm/min. In some experiments the membrane potential signal was also digitized (DAP 800/2, Microstar Laboratories Inc., Bellevue, Wash.) and stored on hard disk. In other experiments, the recorded pen traces were digitized off-line (2 data points/mm, corresponding to a sampling rate of 0.17 Hz) with a digitizing tablet connected to a PC.

Drugs

Acetylcholine chloride and N^G-nitro-L-arginine crystalline (L-NNA) were obtained from Sigma Chemical Co., St. Louis, Mo. Glibenclamide and sodium nitroprusside (SNP) were obtained from Merck, Darmstadt, Germany. Levcromakalim was a gift from Beecham Pharmaceuticals, Essex, U.K. These substances were added from the appropriate stock solutions a few minutes before use. The concentrations are expressed as final molar concentrations in the fluid superfusing the preparation.

Statistics

Results are expressed as means ± SEM; *n* indicates the number of aorta preparations, each obtained from a different rat. Glibenclamide was applied for at least 10 min before testing its influence on acetylcholine- or SNP-induced hyperpolarization. When the influence of glibenclamide on the acetylcholine-induced hyperpolarization was tested, the means ± SE of all digitized data points of the membrane potential values (*n* arrays of 200 integers) were calculated both in control conditions and after glibenclamide application. Only the data from experiments in which the microelectrode was kept in the same

cell throughout the whole experimental protocol (paired observations) were taken. In the SNP experiments, a decrease of the E_m response to repeated applications of the NO donor was observed. Therefore, values for the E_m change in the presence of glibenclamide in the test group were compared with values for the hyperpolarization obtained during a second exposure to SNP in control muscle strips, in the absence of the inhibitor. Statistical significance between mean values was evaluated using Student's *t* test for paired or unpaired observations, as appropriate. Values of $p < 0.05$ were considered to indicate a significant difference between means.

Results

Influence of glibenclamide on EDNO-induced hyperpolarization

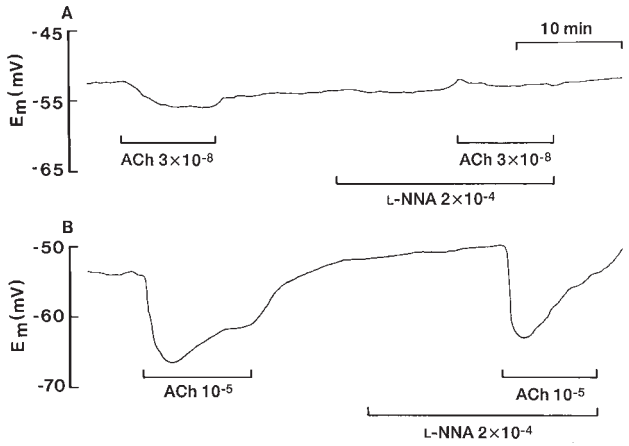
Influence of endogenous NO

In rat aortic smooth muscle superfused with the control Krebs–Ringer solution, a relatively stable and quiescent membrane potential of –52.4 ± 0.8 mV ($n = 24$) was recorded. The addition of acetylcholine caused a concentration-dependent hyperpolarization. The membrane potential response to low and to high concentrations of acetylcholine in the absence and the presence of L-NNA, which inhibits endothelial NO synthesis, is shown in Fig. 1. The application of 2×10^{-4} M L-NNA produced a slight depolarization of the resting E_m (mean value 2.5 ± 0.7 mV, $n = 8$). After pre-exposure to L-NNA, the hyperpolarization evoked by 3×10^{-8} M acetylcholine (3.7 ± 0.3 mV, $n = 4$) was significantly inhibited (Fig. 1A). On the other hand, the initial peak hyperpolarization caused by 10^{-5} M acetylcholine (10.9 ± 1.5 mV) was not significantly affected by L-NNA (9.1 ± 1.3 mV). However, consistent with earlier findings in which the other NO synthase (NOS) inhibitors L-NAME and L-NMMA were tested (Vanheel et al. 1994), we found that the recovery of the membrane potential in the presence of acetylcholine was faster after inhibition of endogenous NO synthesis. After 10 min, the E_m reached a level significantly less negative than in the absence of L-NNA (Fig. 1B). Thus, at least part of the slow, maintained phase of the hyperpolarization induced by application of higher concentrations of acetylcholine is due to action of endogenous NO liberated by the endothelium.

Influence of glibenclamide

In five experiments, we compared the hyperpolarization elicited by 10^{-5} M acetylcholine in the absence and the presence of glibenclamide. Glibenclamide (10^{-5} M) was superfused for at least 10 min before investigating its influence on the acetylcholine-induced hyperpolarization. The mean hyperpolarizations produced by acetylcholine in the absence and the presence of glibenclamide, obtained from all experiments in which both readings were obtained during the same cell impalement, are shown in Fig. 2. (For a typical experiment, see Fig. 4B.) The K_{ATP} channel inhibitor had no significant effect on the resting E_m (Fig. 2). In the presence of glibenclamide, the mean peak hyperpolarization was slightly but not significantly decreased. In addition, the later phase of the acetylcholine-induced hyperpolarization, in which EDNO participates, was not significantly affected.

Fig. 1. Original traces of the rat aortic smooth muscle membrane potential (E_m) response to acetylcholine (ACh) (A) 3×10^{-8} M and (B) 10^{-5} M in the absence and the presence of nitro-L-arginine (L-NNA, 2×10^{-4} M).



Influence of glibenclamide on exogenous NO induced hyperpolarization

Influence of sodium nitroprusside

SNP caused a concentration-dependent hyperpolarization of the rat aortic smooth muscle cells, which was endothelium independent (Fig. 3A). Maximal hyperpolarization was observed with 10^{-6}–10^{-5} M SNP (5.4 ± 0.8 mV, $n = 11$, and 5.7 ± 0.4 mV, $n = 13$, respectively). On repeated application of SNP, the membrane electrical response decreased (Fig. 3B), the second exposure to 10^{-5} M of the NO donor provoking on average 3.4 ± 0.3 mV ($n = 4$) hyperpolarization, or 77% of the magnitude of the first E_m change in this group of preparations.

Influence of glibenclamide

The hyperpolarization in response to 10^{-6} or 10^{-5} M SNP still persisted after 10 min pre-exposure to 10^{-5} M glibenclamide (Figs. 4A, 4B). The mean values for the hyperpolarization in response to a second application of 10^{-6} M SNP in the absence and the presence of glibenclamide were 2.1 ± 0.3 and 2.9 ± 0.6 mV ($n = 4$), respectively. For 10^{-5} M SNP, these respective values were 3.4 ± 0.3 and 2.5 ± 0.6 mV ($n = 4$). Pre-exposure to glibenclamide therefore had no significant effect on the hyperpolarization induced by both concentrations of SNP. On the other hand, the same concentration of glibenclamide completely prevented the hyperpolarization induced by 5×10^{-7} M levcromakalim, a K_{ATP} channel opener ($n = 3$, not shown).

Discussion

Nitric oxide is released from the endothelium both under basal conditions and after stimulation with various vasodilators. EDNO activates muscular guanylyl cyclase, resulting in increased cGMP levels and relaxation. In the guinea-pig uterine artery, it has been shown that NO also causes membrane hyperpolarization (Tare et al. 1990). In the rat small mesenteric artery, NO applied either as NO gas or acidified $NaNO_2$ caused hyperpolarization as well (Garland and McPherson 1992). A small-amplitude hyperpolarization has also been shown to occur in response to high concentrations of NO gas (15×10^{-6} M)

Fig. 2. Mean endothelium-dependent hyperpolarization of the membrane potential (E_m) in response to acetylcholine (ACh, 10^{-5} M) in the absence (continuous lines) and the presence (broken lines) of glibenclamide (10^{-5} M). Mean values (boldface lines) ± SE (lightface lines) from 5 experiments are shown in which E_m traces in control conditions and in the presence of glibenclamide were obtained during the same cell impalement. Neither mean peak hyperpolarization nor the sustained component of the E_m change in the presence of acetylcholine is significantly affected by glibenclamide pre-exposure.

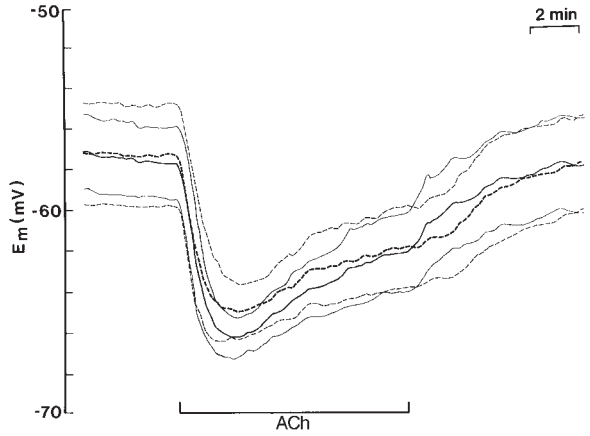
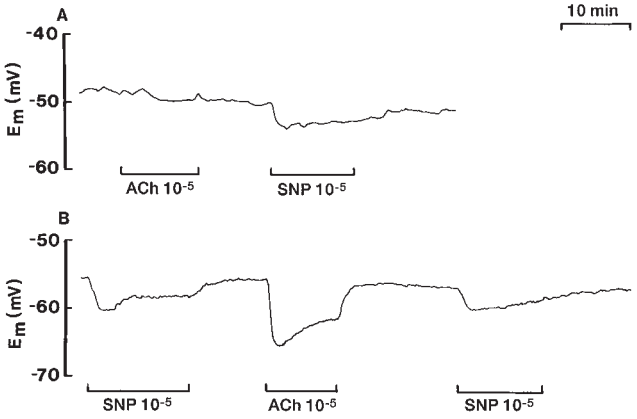


Fig. 3. Influence of sodium nitroprusside (SNP) on the membrane potential (E_m) of the rat aorta. (A) After removal of the endothelium, acetylcholine (ACh) induced but not SNP-induced hyperpolarization is greatly suppressed. (B) During repeated exposure to SNP (10^{-5} M), the membrane hyperpolarization decreases.

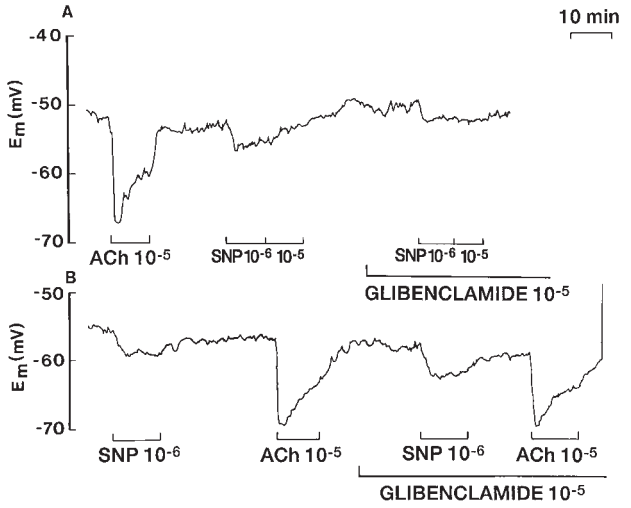


in the rabbit basilar artery (Rand and Garland 1992). While these findings seemed at first to be in contrast with earlier studies (Komori et al. 1988; Brayden 1990) in which no effect of NO on E_m was found, a more recent study on coronary arteries from several species showed that the membrane electrical response to NO could be dependent on the degree of passive membrane stretch (Parkington et al. 1993). In the latter study, acetylcholine caused a membrane hyperpolarization consisting of an initial peak E_m change, as a result of the release of EDHF, followed by two slower components caused by NO (sensitive to L-NAME) and prostacyclin (sensitive to indomethacin), respectively. The third component could be mimicked by addition of iloprost, a stable analogue of prostacyclin. In the cited study the latter phases of the acetylcholine-induced E_m change were present only in stretched preparations, and became progressively larger at increasing muscle tension. However, in vessels from the rat, iloprost had no effect on E_m, possibly as a result of an absence of prostacyclin receptors on coronary artery smooth muscle cells in this species (Parkington et al. 1993). We showed earlier that, in the aorta of the rat, part of the maintained hyperpolarization induced by larger concentrations of acetylcholine is inhibited by L-NAME and L-NMMA (Vanheel et al. 1994). In the present experiments, we found a similar effect of another inhibitor of NO synthase, L-NNA, which lacks the antimuscarinic activity as reported for L-NAME. This clearly indicates that the endothelial release of NO is responsible for part of the smooth muscle E_m change in response to higher concentrations of acetylcholine. In addition, the present results show that lower concentrations of acetylcholine ($1–3 \times 10^{-8}$ M), which already induce endothelium-dependent relaxation in preconstricted aortic rings (Van de Voorde et al. 1992), apparently mainly produce the slower component of hyperpolarization (Fig. 1A), which is sensitive to inhibition of endogenous NO production.

Since the resting E_m is within the range in which current through Ca^{2+} channels shows strong voltage dependence (Nelson et al. 1990), the small hyperpolarization to low concentrations of acetylcholine is expected to contribute to the endothelium-dependent relaxation of preconstricted vessels. Indeed, a hyperpolarization of similar magnitude induced by the endothelium-independent K_{ATP} channel opener cromakalim induces some (about 10%) relaxation of norepinephrine-preconstricted aortic rings (Vanheel and Van de Voorde 1996). Higher acetylcholine concentrations presumably stimulate the release of an additional, distinct, hyperpolarizing factor (EDHF), of which either the release or the effect is more transient. This is in agreement with other studies. In the perfused rat mesenteric bed, hemoglobin (which inactivates endogenous NO) and methylene blue (which both inhibits activation of guanylyl cyclase and inactivates NO) were found to be more effective in blocking the relaxation to low concentrations of acetylcholine (near complete inhibition) than to higher concentrations of the vasodilator (only about 40% inhibition) (Khan et al. 1992). Thus, in the mesenteric bed, acetylcholine-evoked relaxation is mediated by NO and, at higher concentrations, additionally by a hyperpolarizing factor distinct from NO and inhibited by 25 mM K^+ perfusion (Parsons et al. 1994). In the rat coronary microcirculation, L-NNA diminished the duration but not the maximum of the dilator response to bradykinin, indicating that the release of EDNO causes the sustained rather than the initial phase of the observed decrease in coronary perfusion pressure (Bauersachs et al. 1994).

In the present experiments on the aortic muscle strip, high concentrations of SNP ($10^{-6}–10^{-5}$ M) induced a limited hyperpolarization, which was endothelium independent. A small E_m change by large concentrations (10^{-4} M) of SNP was also noted in the rabbit basilar artery (Rand and Garland 1992). Given the small magnitude of the E_m changes obtained with these SNP concentrations, and the uncertain amount of stretch exerted on the muscle strip when mounting the preparation, our findings are not necessarily inconsistent with earlier reports, in which no effect of (lower concentrations of) SNP on

Fig. 4. Membrane potential (E_m) traces of rat aorta showing the influence of pre-exposure to 10^{-5} M glibenclamide on the hyperpolarization induced by sodium nitroprusside (SNP). (A) SNP 10^{-6} and 10^{-5} M were cumulatively applied. (B) SNP 10^{-6} M and acetylcholine 10^{-5} M were sequentially applied.



E_m of the rat aorta (Taylor et al. 1988) has been described. In addition, we found the magnitude of hyperpolarization to decrease with repeated exposures to SNP.

In the present study, it was found that in the rat aorta pre-exposure to glibenclamide, in concentrations that completely inhibited the hyperpolarization by levcromakalim, had no significant effect on the E_m response to SNP (10^{-6} and 10^{-5} M). These findings suggest that the activation of K_{ATP} channels is unlikely to be involved in the hyperpolarizing response to NO in this preparation. Similarly, the later component of the acetylcholine-induced hyperpolarization, mediated at least partly by EDNO as indicated by the L-NNA experiments (Fig. 1), was not significantly affected by 10^{-5} M of the K_{ATP} channel inhibitor (Fig. 2).

Both acetylcholine and SNP cause an increase in aortic smooth muscle cGMP levels (Taylor et al. 1988). In cells of the rat aorta, increased cGMP levels have been shown to enhance both ATP-regulated and Ca-regulated K^+ channel activity (Kubo et al. 1994). Our experiments with glibenclamide indicate that the hyperpolarization caused by SNP or endogenous NO is unlikely to be mediated by K_{ATP} channel activation. In smooth muscle cells from the rabbit cerebral artery, NO and a membrane permeable analogue of cGMP have been shown to activate K_{Ca} channels (Robertson et al. 1993). In the rabbit mesenteric artery, SNP, nitroglycerin, and NO induced a relaxation that was sensitive to the specific K_{Ca} channel blockers charybdotoxin and iberiotoxin (Khan et al. 1993). Also in the rat pulmonary artery, NO and cGMP have been shown to enhance K_{Ca} channel activity and induce a charybdotoxin-sensitive relaxation (Archer et al. 1994). Thus, K_{Ca} channel rather than K_{ATP} channel activation might possibly be responsible for the NO-induced hyperpolarization in the rat aorta. In this respect, it can be noted that stretching the membrane patch of patch-clamped rabbit mesenteric artery cells in the cell attached or the inside-out configuration increased the activity of the large conductance K_{Ca} channels (Dopico et al. 1994). Moreover, a direct K_{Ca} channel activity enhancing influence of NO, not requiring the cGMP pathway, has been shown (Bolotina et al. 1994). On the other hand, it has been shown in rabbit thoracic aorta that NO and SNP cause a time- and concentration-dependent stimulation of ouabain-sensitive ^{86}Rb uptake (Gupta et al. 1994). The mechanism for this NO-induced stimulation of Na^+–K^+ ATPase was independent of the ability of NO to increase the intracellular cGMP concentration but might involve Na–H exchange activation (Gupta et al. 1994). The relatively slowly developing hyperpolarization observed with low concentrations of acetylcholine in the present study (Fig. 1) might equally be explained by a similar action of NO in the rat aorta.

In conclusion, the major new finding in this study is that in the rat aorta both endogenous and exogenous NO produce a small membrane hyperpolarization which is not inhibited by glibenclamide (10^{-5} M), an inhibitor that completely antagonizes the effect on the membrane potential of the K_{ATP} channel opener levcromakalim. The lack of inhibition of the NO-induced hyperpolarization by glibenclamide contrasts with the results obtained in the mesenteric artery from the same species (Garland and McPherson 1992). These observations suggest that NO-induced membrane hyperpolarization can result from different mechanisms in different vessel types, the physiological significance of which has yet to be determined.

Acknowledgements

The present work was supported by the National Fund for Scientific Research (NFWO, Belgium, grant 3.0016.94). B.Vanheel is a senior research associate of the NFWO.

References

Archer, S.L., Huang, J.M., Hampl, V., Nelson, D.P., Shultz, P.J., and Weir, E.K. 1994. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. Proc. Natl. Acad. Sci. U.S.A. **91**: 7573–7587.

Bauersachs, J., Hecker, M., and Busse, R. 1994. Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic acid metabolite in the coronary microcirculation. Br. J. Pharmacol. **113**: 1548–1553.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., and Cohen, R. 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature (London), **368**: 850–853.

Brayden, J.E. 1990. Membrane hyperpolarization is a mechanism of endothelium-dependent vasodilation. Am. J. Physiol. **259**: H668–H673.

Chen, G., and Suzuki, H. 1989. Some electrical properties of the endothelium-dependent hyperpolarization recorded from rat arterial smooth muscle cells. J. Physiol. (London), **410**: 91–106.

Chen, G., Yamamoto, Y., Miwa, K., and Suzuki, H. 1991. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am. J. Physiol. **260**: H1888–H1892.

Corriu, C., Félétou, M., Canet, E., and Vanhoutte, P.M. 1996. Inhibitors of the cytochrome P450 mono-oxygenase and endothelium-dependent hyperpolarizations in the guinea-pig isolated carotid artery. Br. J. Pharmacol. **117**: 607–610.

Dopico, A.M., Kirber, M.T., Singer, J.J., and Walsh, J.V., Jr. 1994. Membrane stretch directly activates large conductance Ca^{2+}-activated K^+ channels in mesenteric artery smooth muscle cells. Am. J. Hypertens. **7**: 82–89.

Eckman, D.M., Frankovich, J.D., and Keef, K.D. 1992. Comparison

of the actions of acetylcholine and BRL 38227 in guinea-pig coronary artery. Br. J. Pharmacol. **106**: 9–16.

Ganitkevich, V.Y., and Isenberg, G. 1993. Membrane potential modulates inositol 1,4,5-triphosphate-mediated Ca^{2+} transients in guinea-pig coronary myocytes. J. Physiol. (London), **470**: 35–44.

Garland, C.J., and McPherson, G.A. 1992. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br. J. Pharmacol. **105**: 429–435.

Gupta, S., McArthur, C., Grady, C., and Ruderman, N.B. 1994. Stimulation of vascular Na^+–K^+-ATPase activity by nitric oxide: a cGMP independent effect. Am. J. Physiol. **266**: H2146–H2151.

Hecker, M., Bara, A., Bauersachs, J., and Busse, R. 1994. Characterization of endothelium-dependent hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. J. Physiol. (London), **481**: 407–414.

Khan, M.T., Jothianandan, D., Matsunaga, K., and Furchgott, R.F. 1992. Vasodilation induced by acetylcholine and by glyceryl trinitrate in rat aortic and mesenteric vasculature. J. Vasc. Res. **29**: 20–28.

Khan, S.A., Mathews, W.R., and Meisheri, K.D. 1993. Role of calcium-activated K^+ channels in vasodilation induced by nitroglycerine, acetylcholine and nitric oxide. J. Pharmacol. Exp. Ther. **267**: 1327–1335.

Komori, K., Lorenz, R.R., and Vanhoutte, P.M. 1988. Nitric oxide, acetylcholine, and electrical and mechanical properties of canine arterial smooth muscle. Am. J. Physiol. **255**: H207–H212.

Kubo, M., Nakaya, Y., Matsuoka, S., Saito, K., and Kuroda, Y. 1994. Atrial natriuretic factor and isosorbide dinitrate modulate the gating of ATP-sensitive K^+ channels in cultured vascular smooth muscle cells. Circ. Res. **74**: 471–476.

Nelson, M.T., Patlak, J.B., Worley, J.F., and Standen, N.B. 1990. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am. J. Physiol. **259**: C3–C18.

Parkington, H.C., Tare, M., Tonta, M.A., and Coleman, H.A. 1993. Stretch revealed three components in the hyperpolarization of guinea-pig coronary artery in response to acetylcholine. J. Physiol. (London), **465**: 459–476.

Parkington, H.C., Tonta, M.A., Coleman, H.A., and Tare, M. 1995. Role of membrane potential in endothelium-dependent relaxation of guinea-pig coronary arterial smooth muscle. J. Physiol. (London), **484**: 469–480.

Parsons, S.J.W., Hill, A., Waldron, G.J., Plane, F., and Garland, C.J. 1994. The relative importance of nitric oxide and nitric oxide-independent mechanisms in acetylcholine-evoked dilatation of the rat mesenteric bed. Br. J. Pharmacol. **113**: 1275–1280.

Plane, F., and Garland, C.J. 1993. Differential effects of acetylcholine, nitric oxide and levcromakalim on smooth muscle membrane potential and tone in the rabbit basilar artery. Br. J. Pharmacol. **110**: 651–656.

Quast, U. 1993. Do the K^+ channel openers relax smooth muscle by opening K^+ channels? Trends Pharmacol. Sci. **14**: 332–337.

Rand, V.E., and Garland, C.J. 1992. Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarization. Br. J. Pharmacol. **106**: 143–150.

Robertson, B.E., Schubert, R., Hescheler, J., and Nelson, M.T. 1993. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am. J. Physiol. **265**: C299–C303.

Tare, M., Parkington, H.C., Coleman, H.A., Neild, T.O., and Dusting, G.J. 1990. Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. Nature (London), **346**: 69–71.

Taylor, S.G., Southerton, J.S., Weston, A.H., and Baker, J.R.J. 1988. Endothelium-dependent effects of acetylcholine in rat aorta: a comparison with sodium nitroprusside and cromakalim. Br. J. Pharmacol. **94**: 853–863.

Van de Voorde, J., Vanheel, B., and Leusen, I. 1992. Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. Circ. Res. **70**: 1–8.

Vanheel, B., and Van de Voorde, J. 1996. Impaired endothelium-dependent hyperpolarization and relaxation in the aorta from the renal hypertensive rat. *In* Endothelium-derived hyperpolarizing factor. *Edited by* P.M. Vanhoutte. Harwood Academic Publishers, Amsterdam, The Netherlands. pp. 235–245.

Vanheel, B., and Van de Voorde, J. 1997. Evidence against the involvement of cytochrome P-450 metabolites in endothelium-dependent hyperpolarization of the rat main mesenteric artery. J. Physiol. (London). **501**: 331–341.

Vanheel, B., Van de Voorde, J., and Leusen, I. 1994. Contribution of nitric oxide to the endothelium-dependent hyperpolarization in rat aorta. J. Physiol. (London), **475**: 277–284.