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Inhibition of Vascular Smooth Muscle Tone by the H^+, K^+-ATPase Inhibitor SCH 28080

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Abstract

Vascular smooth muscle is thought to possess an H^+-K^+ ATPase that contributes to the regulation of intracellular K^+ concentration and pH. We have examined the effect of the H^+, K^+-ATPase inhibitor SCH 28080 on vascular smooth muscle tone in guinea-pig and human isolated arteries, and on $^{86}Rb^+$ uptake in cultured guinea-pig aortic smooth muscle cells.

SCH 28080 (0·1–300 μM) produced relaxation of isolated guinea-pig aorta, guinea-pig pulmonary artery and human pulmonary artery. Relaxation occurred in tissues pre-contracted with phenylephrine, histamine or the thromboxane mimetic U44069. Relaxation was reversible, and was not affected by tetrodotoxin, indomethacin, nordihydroguiaretic acid (NDGA), 1-aminobenzotriazole (1-ABT), N^G-nitro-L-arginine methyl ester (L-NAME), removal of the endothelium or removal of extracellular K^+. SCH 28080 had no effect on $^{86}Rb^+$ uptake in cultured guinea-pig aortic smooth muscle cells.

In conclusion, SCH 28080 relaxes vascular smooth muscle at concentrations known to inhibit the H^+-K^+ ATPase. The persistence of relaxation in a K^+-free medium and the failure of SCH 28080 to inhibit $^{86}Rb^+$ uptake suggest that relaxation may be unrelated to H^+, K^+-ATPase inhibition, and indicate that this agent may not be considered as a selective H^+, K^+-ATPase inhibitor in vascular preparations.

SCH 28080 (3-cyanomethyl-2-methyl-8-(phenylmethoxy)-imidazo[1,2-*a*]pyridine) is considered to be a potent and selective inhibitor of gastric H^+, K^+-ATPase (Scott & Sundell 1985; Beil et al 1986; Wallmark et al 1987). This H^+, K^+-ATPase is an ion-motive phosphorylating ATPase that catalyses the exchange of intracellular H^+ for extracellular K^+ at the expense of ATP. It is particularly abundant in the stomach, and early studies demonstrate that SCH 28080 inhibits gastric acid secretion in-vivo and in-vitro (Chiu et al 1983; Long et al 1983; Kaminski et al 1985), as well as K^+-dependent ATPase activity and H^+ transport in gastric microsomes (Scott & Sundell 1985; Beil et al 1986; Wallmark et al 1987). Kinetic studies have shown SCH 28080 to be a K^+-competitive reversible inhibitor of the gastric H^+, K^+-ATPase with a potency in the micromolar concentration range (Beil et al 1986; Wallmark et al 1987; Keeling et al 1988).

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Functional and molecular evidence suggests the presence of an H^+, K^+-ATPase in vascular smooth muscle (McCabe & Young 1992; Marrelli et al 1997). Transport studies demonstrate that SCH 28080 and other H^+, K^+-ATPase inhibitors reduce the uptake of $^{86}Rb^+$ (used as a marker for K^+) into cultured vascular smooth muscle cells (McCabe & Young 1992). This component of $^{86}Rb^+$ uptake is not affected by ouabain or bumetanide (inhibitors of the Na^+, K^+-ATPase and Na^+-K^+-$2Cl^-$ co-transporter, respectively), and is potentiated by intracellular acidification. H^+, K^+-ATPase inhibitors also produce an intracellular acidification in vascular smooth muscle that is mimicked by K^+-removal (McCabe & Young 1992). Molecular studies suggest that the vascular H^+, K^+-ATPase may be identical to the gastric enzyme. Canine carotid artery and cultured carotid artery smooth muscle cells express mRNA for the H^+, K^+-ATPase, and a polymerase chain reaction (PCR) product generated from the carotid artery possesses a high sequence identity (91%) to the gastric enzyme (Marrelli et al 1997).

The presence of an H^+, K^+-ATPase in vascular smooth muscle could have functional implications for the regulation of vascular tone. K^+ is one of the principal determinants of the membrane potential, and changes in intracellular pH are known to affect vascular contractility (Wray 1988). Leminoprazole, another H^+, K^+-ATPase inhibitor, has been found to inhibit contractile responses of isolated arteries to a number of agonists, but the mechanism appears to be unrelated to H^+, K^+-ATPase inhibition (Okabe et al 1996). This study was designed to investigate the effect of SCH 28080 on vascular smooth muscle tone in isolated arteries. Furthermore, the effect of SCH 28080 on $^{86}Rb^+$ uptake was examined in cultured guinea-pig aortic smooth muscle cells to establish the presence of the H^+, K^+-ATPase in these cells.

Materials and Methods

Drugs

ATP, 1-aminobenzotriazole (1-ABT), histamine, indomethacin, nordihydroguiaretic acid (NDGA), N^G-nitro-L-arginine methyl ester (L-NAME), phenylephrine, tetrodotoxin and U44069 were purchased from Sigma (St Louis, MO). SCH 28080 was obtained as a gift from Schering-Plough Research Institute (Kenilworth, NJ). Indomethacin and NDGA were dissolved in ethanol, SCH 28080 in dimethylsulphoxide (DMSO) and all other drugs in distilled water.

Animal and human tissues

Adult male guinea-pigs, 600–1000 g (Camm Animals, Wayne, NJ and Covance Research Products, Denver, PA), were killed by an overdose of sodium pentobarbital (100–150 mg kg^{-1}, i.p.) and sections of the descending thoracic aorta and pulmonary artery were excised. Human pulmonary arteries were obtained as surgical specimens from subjects undergoing resection for carcinoma of the lung at Yale-New Haven Hospital (New Haven, CT) and were macroscopically normal. Protocols were approved by the Pierce Animal Care and Use Committee and the Yale University Human Investigations Committee.

Force generation

Force generation was measured in freshly isolated guinea-pig aortas, guinea-pig pulmonary arteries and human pulmonary arteries. Vessels were cut into 3–4-mm wide rings and mounted in 4-mL organ baths (Kent Scientific, Litchfield, CT) containing modified Krebs-Henseleit solution (composition, mM, NaCl, 117·5, KCl, 5·37, $MgSO_4$, 0·57, $CaCl_2$, 2·5, NaH_2PO_4, 1·2, $NaHCO_3$, 15·5 and glucose, 5·0). The bathing medium was maintained at 37°C and aerated with 5% CO_2 and 95% O_2 (pH 7·4). Changes in tension were measured with isometric force transducers (Kent Scientific, Litchfield, CT) and recorded with Workbench PC data aquisition software (Strawberry Tree, Sunnyvale, CA). Tissues were placed under their optimal resting tension (2 g for guinea-pig arteries; 2·5 g for human arteries) and equilibrated for 1 h. The effect of SCH 28080 on vascular smooth muscle tone was examined on resting tension or following pre-contraction with an EC50 (concentration producing 50% of maximum contraction) of phenylephrine, histamine or the stable thromboxane A_2 mimetic U44069. The effect of the endothelium on SCH 28080-induced relaxation was examined in adjacent preparations by gently rubbing the lumen of one preparation before mounting. The presence or absence of endothelium was verified by assessing the effect of adenosine 5′-triphosphate (ATP) on tissues contracted with phenylephrine. ATP (10 μM) relaxed intact preparations (49·7 ± 10·2% inhibition of tone, n = 4) but had no significant effect on endothelium-denuded preparations (4·1 ± 4·5% inhibition of tone, n = 4). The effect of K^+-free medium on SCH 28080-induced relaxation was examined by replacing 5·37 mM KCl in the Krebs-Henseleit solution with equimolar NaCl. Since exposure to a K^+-free medium causes contraction by inhibiting the Na^+, K^+ pump, we also measured SCH 28080-induced relaxation following pre-contraction with the Na^+, K^+ pump inhibitor ouabain (10 μM) in the presence of KCl. SCH 28080 was added to the baths at the peak of contraction.

Cell culture

Cell culture was established from explants of freshly isolated guinea-pig aorta dissected clear of connective tissue. The medial layer was finely minced, and maintained in culture dishes containing Dulbecco's Modified Eagle Medium supplemented with 10% foetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Dishes containing explants were placed in a humidified CO_2 incubator at 37°C. Within 1 week cells migrated out of explants and were allowed to grow to confluence before passaging. The identity of cells was confirmed by immunohistochemistry, with 95–100% of cells staining positively for smooth muscle α-actin and smooth muscle myosin.

$^{86}Rb^{+}$ uptake

$^{86}Rb^{+}$ uptake was measured in cultured guinea-pig aortic smooth muscle cells (passage 1) grown to confluence in 24-well plates. Cells were maintained at 37°C in a balanced salt solution (BSS), pH 7·4 (composition, mM, NaCl, 140, KCl, 5·0, $MgCl_2$, 1·0, $CaCl_2$, 2·0, HEPES, 10·0 and glucose, 10·0). Cells were incubated for 10 min with BSS containing 0·5 μCi mL^{-1} $^{86}Rb^{+}$ in the presence or absence of ouabain (100 μM), bumetanide (10 μM), SCH 28080 (1–100 μM) or vehicle. Uptake was stopped by 6 washes with ice-cold BSS containing RbCl instead of KCl, to displace $^{86}Rb^{+}$ from extracellular sites. Cells were solubilized in Lowry reagent and total cellular protein measured by the Lowry method (Sigma protein assay kit P5656). Cellular radioactivity was measured by liquid scintillation counting. Within each experiment, uptake was measured in triplicate wells.

Data analysis

Results are expressed as means ± s.e.m. Statistical comparisons were performed by Student's *t*-test or by one-way analysis of variance with the Bonferroni post-test for multiple comparisons. $P < 0.05$ was considered significant. Concentrations producing 50% of maximum contraction (EC50) or 50% inhibition of induced tone (IC50) were determined by non-linear regression analysis with log concentration–response data fitted to a sigmoidal curve with GraphPad Prism (GraphPad Software, San Diego, CA).

Results

SCH28080-induced relaxation

SCH 28080 (1–300 μM) had no effect on resting tone but induced a concentration-dependent relaxation of guinea-pig aorta pre-contracted with an EC50 of phenylephrine, histamine or U44069 (Figure 1). Relaxation occurred with log IC50 values of −4·53 ± 0·05 (n = 9), −4·59 ± 0·19 (n = 8) and −4·88 ± 0·09 (n = 6) for tissues contracted with phenylephrine, histamine and U44069, respectively. At the highest concentration used, SCH 28080 induced the complete reversal of agonist-induced tone. Relaxation was slow in onset and was maximal 20–30 min after the addition of SCH 28080. The vehicle for SCH 28080 (DMSO, highest bath concentration 0·15%) produced a small relaxation (~5%), but this response was transient and tone returned to the pre-application level within 10 min.

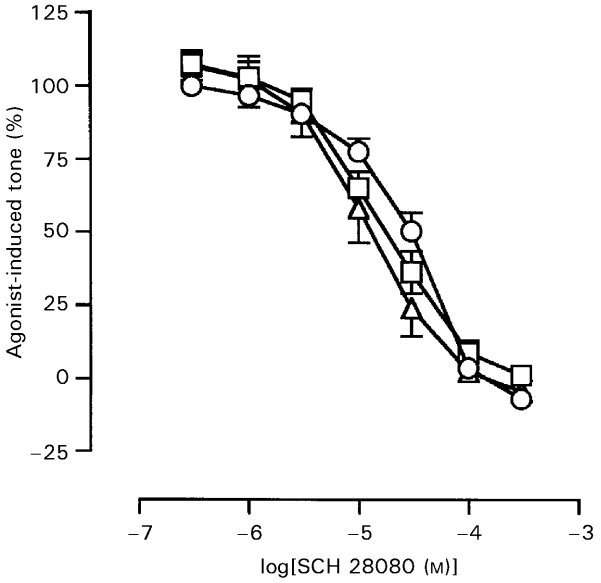

Figure 1. Effect of SCH 28080 on agonist-induced tone of the guinea-pig aorta. Tissues were contracted with an EC50 of phenylephrine (○), histamine (□) or U44069 (△) before the addition of SCH 28080. Relaxation induced by SCH 28080 is expressed as % inhibition of agonist-induced tone. Points represent mean ± s.e.m., n = 9 (phenylephrine), n = 8 (histamine) and n = 6 (U44069).

To determine whether SCH 28080-induced relaxation occurs in other species and vascular beds, the effect of SCH 28080 was also examined on guinea-pig and human pulmonary arteries pre-contracted with phenylephrine. SCH 28080 relaxed both preparations with similar log IC50 values of −4·79 ± 0·05 (n = 4) for guinea-pig and −4·84 ± 0·49 (n = 3) for human pulmonary arteries (Figure 2).

To determine whether the effect of SCH 28080 on tone is reversible, tissues were exposed to SCH 28080 in the presence of phenylephrine, rinsed with fresh KH solution and allowed to recover for 1 h. Tissues were then re-contracted with phenylephrine and exposed to the same concentration of SCH 28080. Not only did phenylephrine produce a similar contractile response, but SCH 28080 also induced a similar relaxant response upon second exposure (Figure 3) suggesting that SCH 28080-induced relaxation is both reversible and reproducible in the same preparation.

The mechanism by which SCH 28080 relaxes vascular smooth muscle was investigated by examining the effects of various pharmacologic interventions on relaxation of guinea-pig aorta pre-contracted with phenylephrine. Pretreatment with 1 μM tetrodotoxin, 3 μM indomethacin, 10 μM NDGA, 1 mM 1-ABT, 10 μM L-NAME or removal

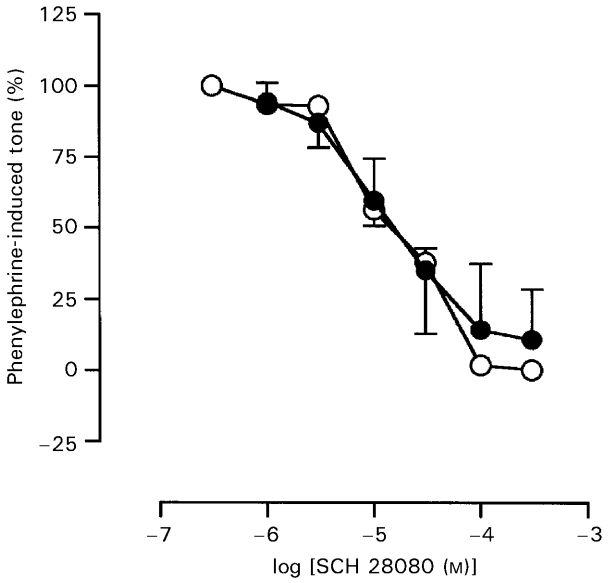

Figure 2. Effect of SCH 28080 on agonist-induced tone of guinea-pig and human pulmonary arteries. Guinea-pig (○) and human (●) pulmonary arteries were contracted with an EC50 of phenylephrine before the addition of SCH 28080. Relaxation induced by SCH 28080 is expressed as % inhibition of phenylephrine-induced tone. Points represent mean ± s.e.m., n = 4 (guinea-pig arteries) and n = 3 (human arteries).

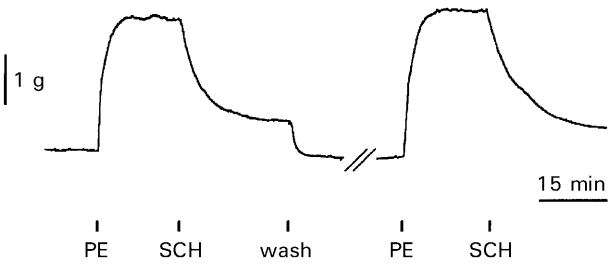

Figure 3. Reversibility of SCH 28080-induced relaxation of guinea-pig aorta. Tissues were contracted with 3·6 μM phenylephrine (PE), before the addition of 100 μM SCH 28080 (SCH). When maximal relaxation was reached, tissues were washed with Krebs-Henseleit solution and allowed to recover for 1 h. Reapplication of phenylephrine and SCH 28080 induced similar contractile and relaxant responses respectively. The figure is a representative tracing of n = 4 experiments.

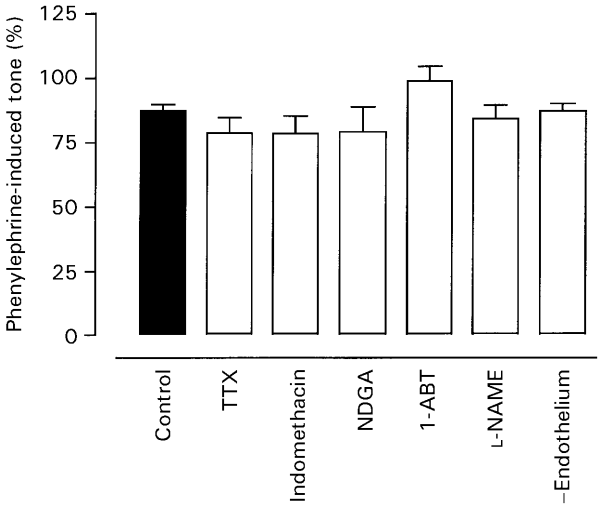

Figure 4. Mechanism of SCH 28080-induced relaxation of guinea-pig aorta. Relaxation induced by 100 μM SCH 28080 was measured in tissues pretreated for 20 min with 1 μM tetrodotoxin (TTX), 3 μM indomethacin, 10 μM NDGA, 1 mM 1-ABT, 10 μM L-NAME or in endothelium-denuded preparations (− endothelium). Relaxation is expressed as % inhibition of tone induced by 3·6 μM phenylephrine. Bars represent mean ± s.e.m., n = 4.

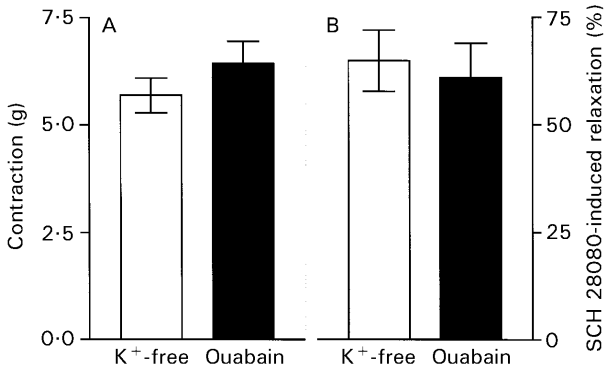

Figure 5. Effect of K^+-removal and ouabain on resting tone (A) and SCH 28080-induced relaxation (B) of guinea-pig aorta. Tissues were contracted by exposure to a K^+-free medium (□) or 10 μM ouabain (■), before the addition of 100 μM SCH 28080. Contraction is expressed in grams, and relaxation as % inhibition of tone induced by K^+-removal or ouabain. Points represent mean ± s.e.m., n = 4.

of the endothelium had no significant effect on relaxation induced by 100 μM SCH 28080 (Figure 4).

H^+, K^+-ATPase activity is dependent on the presence of extracellular K^+, therefore the effect of removing extracellular K^+ on the ability of SCH 28080 to relax guinea-pig aorta was examined. Since K^+ removal will also inhibit the Na^+, K^+-ATPase, the effect of SCH 28080 on ouabain-induced tone was also examined. Exposure to a K^+-free solution resulted in a slow contraction that was similar in magnitude to that induced by ouabain (Figure 5A). SCH 28080 (100 μM) relaxed tissues in a K^+-free medium, and this effect was similar to that induced in the presence of ouabain (Figure 5B).

$^{86}Rb^+$ uptake

SCH 28080 (1–100 μM) had no effect on $^{86}Rb^+$ uptake into cultured guinea-pig aortic smooth muscle cells in the absence or presence of 100 μM ouabain and 10 μM bumetanide (Figure 6). Ouabain and bumetanide together caused a 92·0 ± 0·7% inhibition of $^{86}Rb^+$ uptake.

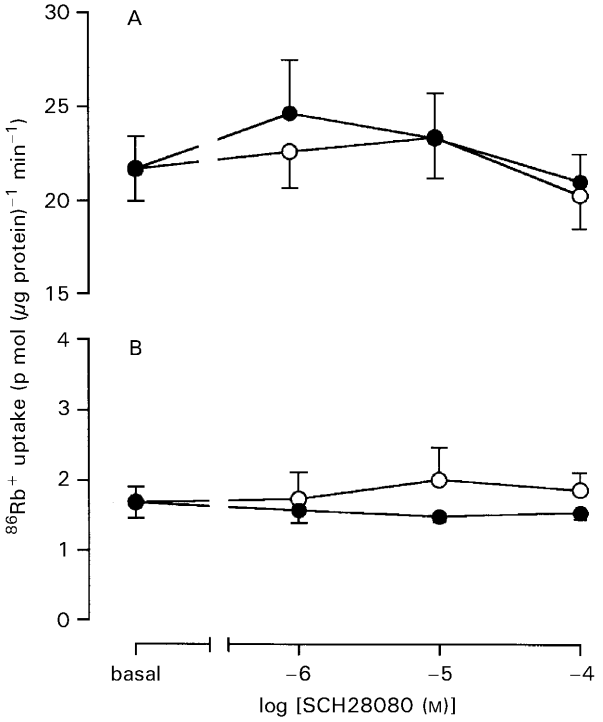

Figure 6. Effect of SCH 28080 on $^{86}Rb^+$ uptake in cultured guinea-pig aortic smooth muscle cells. Cells were exposed to SCH 28080 (●) or vehicle (○) for 10 min. Uptake was measured in the absence (A, total uptake) or presence (B; ouabain- and bumetanide-insensitive uptake) of 100 μM ouabain and 10 μM bumetanide. Points represent mean ± s.e.m., n = 4.

Discussion

SCH 28080 is the most widely used H^+, K^+-ATPase inhibitor in pharmacologic and physiologic studies of ion transport, and sensitivity to this agent is often used as a criterion for the identification of the H^+, K^+-ATPase. In this study we demonstrated that SCH 28080 can exert additional effects on vascular smooth muscle which may be unrelated to H^+, K^+-ATPase inhibition. SCH 28080 induced relaxation of vascular smooth muscle pre-contracted with three different contractile agonists, and in three different preparations. SCH 28080 has also been found to relax airway smooth muscle with a similar potency as vascular smooth muscle (Rhoden et al 1996).

Two lines of evidence suggest that SCH 28080-induced relaxation may be independent of H^+, K^+-ATPase inhibition. First, relaxation persisted in the absence of extracellular K^+ whereas H^+, K^+-ATPase activity requires K^+ binding (Wallmark et al 1980). K^+ removal would be expected to inhibit not only the H^+, K^+-ATPase but also the Na^+, K^+-ATPase, and the resulting changes in resting tension would be expected to reflect a balance of the two effects. In this study, K^+ removal produced the same degree of contraction as did ouabain, and had no effect on SCH 28080-induced relaxation, suggesting that the effect of SCH 28080 on vascular tone is independent of extracellular K^+.

The second line of evidence against a role for the H^+, K^+-ATPase in SCH 28080-induced relaxation is that SCH 28080 failed to inhibit $^{86}Rb^+$ uptake in cultured vascular smooth muscle cells from the guinea-pig aorta. This is in contrast to cultured rat aortic and canine coronary artery cells, in which SCH 28080 caused a 21–25% inhibition of $^{86}Rb^+$ uptake (McCabe & Young 1992), albeit at a high concentration of 300 μM. In this study, ouabain and bumetanide reduced $^{86}Rb^+$ uptake by 92%, suggesting that the Na^+, K^+-ATPase and the Na^+, K^+-Cl^- co-transporter are the principal pathways of K^+ transport into guinea-pig aortic smooth muscle cells. Ouabain- and bumetanide-insensitive uptake was also unaffected by SCH 28080, and may therefore represent passive diffusion of $^{86}Rb^+$ into cells along its electrochemical gradient. Thus, under the conditions used in our experiments, H^+-K^+ ATPase activity could not be measured in cultured guinea-pig aortic smooth muscle cells, at least through measurements of $^{86}Rb^+$ uptake. Although cells undergo phenotypic changes in culture, only cells in the first passage were used, and cells stained positively for smooth muscle α-actin and smooth muscle myosin, confirming their identity as differentiated smooth muscle cells.

Leminoprazole, another H^+, K^+-ATPase inhibitor, has been repored to relax isolated rat aortic rings pre-contracted by phenylephrine and KCl (Okabe et al 1996). Relaxation was inhibited by removal of the endothelium, and by pretreatment with N^G-monomethyl-L-arginine (a nitric oxide synthase inhibitor) and nifedipine, suggesting that relaxation is mediated in part by endothelial nitric oxide and in part by inhibition of voltage-operated Ca^{2+} channels. In our study, SCH 28080-induced relaxation of the guinea-pig aorta was not affected by L-NAME (another nitric oxide synthase inhibitor) or by removal of the endothelium, suggesting that it is not mediated by the generation of nitric oxide or any other endothelial factor. Thus, leminoprazole and SCH 28080 relax vascular smooth muscle by different mechanisms. This is not entirely surprising since the two inhibitors are structurally unrelated, leminoprazole being a substituted benzimidazole and SCH 28080 a substituted imidazo[1,2-*a*]pyridine.

The mechanism by which SCH 28080 relaxes vascular smooth muscle remains unclear. Relaxation was not affected by tetrodotoxin suggesting that it is not mediated by release of an inhibitory neurotransmitter from nerves present in the pre-

paration. Relaxation was also unaffected by indomethacin, NDGA and 1-ABT, suggesting that it is not mediated by cyclooxygenase, lipoxygenase or monooxygenase metabolites of arachidonic acid. Effects of SCH 28080 unrelated to H^+-K^+ ATPase inhibition have also been reported in other systems. SCH 28080 inhibits a phospholipid flippase in gastric vesicles (Suzuki et al 1997), bafilomycin A_1-sensitive H^+-ATPase in the turtle bladder (Graber & Devine 1993; Kohn et al 1993) and a K^+-dependent ATPase encoded by the human ATP1AL1 gene (Grishin et al 1996). Alternative pathways of relaxation that remain to be investigated include activation of adenylate or guanylate cyclase, inhibition of cyclic nucleotide phosphodiesterases, modulation of Ca^{2+} mobilization and sequestration, activation of K^+ channels, inhibition of Ca^{2+} calmodulin kinase, modulation of myosin light-chain phosphorylation and direct interaction with cytoskeletal proteins.

In conclusion, SCH 28080 induces relaxation of vascular smooth muscle by a mechanism that may be independent of H^+, K^+-ATPase inhibition. These results suggest that caution is needed in the use of SCH 28080 as an H^+, K^+-ATPase inhibitor, and that inhibitors with a greater selectivity are required to study the functional role of the H^+, K^+-ATPase in vascular preparations.

Acknowledgements

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References

Beil, W., Hackbarth, I., Sewing, K. F. (1986) Mechanism of gastric antisecretory effect of SCH 28080. Br. J. Pharmacol. 88: 19–23

Chiu, P. J. S., Casciano, C., Tetzloff, G., Long, J. F., Barnett, A. (1983) Studies on the mechanisms of the antisecretory and cytoprotective actions of SCH 28080. J. Pharmacol. Exp. Ther. 226: 121–125

Graber, M. L., Devine, P. (1993) Omeprazole and SCH 28080 inhibit acid secretion by the turtle urinary bladder. Renal Physiol. Biochem. 16: 257–267

Grishin, A. V., Bevensee, M. O., Modyanov, N. N., Rajendran, V., Boron, W. F., Caplan, M. J. (1996) Functional expression of the cDNA encoded by the human *ATP1AL1* gene. Am. J. Physiol. 271: F539–F551

Kaminski, J. J., Bristol, J. A., Puchalski, C., Lovey, R. G., Elliott, A. J., Guzik, H., Solomon, D. M., Conn, D. J., Domalski, M. S., Wong, S. C., Gold, E. H., Long, J. F., Chiu, P. J. S., Steinberg, M., McPhail, A. T. (1985) Antiulcer agents. 1. Gastric antisecretory and cytoprotective properties of substituted imidazo[1,2-*a*]pyridines. J. Med. Chem. 28: 876–892

Keeling, D. J., Laing, S. M., Senn-Bilfinger, J. (1988) SCH 28080 is a lumenally acting, K^+-site inhibitor of the gastric $(H^+ + K^+)$-ATPase. Biochem. Pharmacol. 37: 2231–2236

Kohn, O. F., Mitchell, P. P., Steinmetz, P. R. (1993) Sch-28080 inhibits bafilomycin-sensitive H^+ secretion in turtle bladder independently of luminal $[K^+]$. Am. J. Physiol. 265: F174–F179

Long, J. F., Chiu, P. J. S., Derelanko, M. J., Steinberg, M. (1983) Gastric antisecretory and cytoprotective activities of SCH 28080. J. Pharmacol. Exp. Ther. 226: 114–120

Marrelli, S. P., Zhao, X., Allen, J. C. (1997) Molecular evidence for a vascular smooth muscle H^+-K^+-ATPase. Am. J. Physiol. 272: H869–H874

McCabe, R. D., Young, D. B. (1992) Evidence of a K^+-H^+-ATPase in vascular smooth muscle cells. Am. J. Physiol. 262: H1955–H1958

Okabe, S., Amagase, K., Fujita, H., Iwata, K., Satake, N., Shibata, S. (1996) Vasoinhibitory effect of leminoprazole, a H^+, K^+-ATPase inhibitor, on rat aortic rings. Gen. Pharmacol. 27: 117–121

Rhoden, K. J., Tallini, G., Douglas, J. S. (1996) H^+-K^+ ATPase inhibitors cause relaxation of guinea-pig and human airway smooth muscle in vitro. J. Pharmacol. Exp. Ther. 276: 897–903

Scott, C. K., Sundell, E. (1985) Inhibition of H^+ K^+ ATPase by SCH 28080 and SCH 32651. Eur. J. Pharmacol. 112: 268–270

Suzuki, H., Kamakura, M., Morii, M., Takeguchi, N. (1997) The phospholipid flippase activity of gastric vesicles. J. Biol. Chem. 272: 10429–10434

Wallmark, B., Stewart, H. B., Rabon, E., Saccomani, G., Sachs, G. (1980) The catalytic cycle of gastric $(H^+ + K^+)$-ATPase. J. Biol. Chem. 255: 5313–5319

Wallmark, B., Briving, C., Fryklund, J., Munson, K., Jackson, R., Mendlein, J., Rabon, E., Sachs, G. (1987) Inhibition of gastric H^+, K^+-ATPase and acid secretion by SCH 28080, a substituted pyridyl-(1,2*a*)imidazole. J. Biol. Chem. 262: 2077–2084

Wray, S. (1988) Smooth muscle intracellular pH: measurement, regulation, and function. Am. J. Physiol. 254: C213–C225