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ARTICLE

5-hydroxytryptamine is a mediator of 4-aminopyridine induced contractions in porcine and human isolated coronary arteries

Attila Kun[1]*, János Pataricza[1], Irén Krassói[2], Miklós Opincariu[3], János Szécsi[3], Julius Gy. Papp[2]

[1] Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary, [2] Research Unit for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary, [3] Department of Cardiac Surgery, University of Szeged, Szeged, Hungary

ABSTRACT The effect of 4-aminopyridine (4-AP), a known inhibitor of voltage-dependent K^+ channels (K_V), was studied on the resting vasomotor tone in porcine and human coronary arteries. Isolated coronary artery preparations were suspended in organ bath for isometric tension recordings. 4-AP ($1.25x10^{-8}$ - $5.8x10^{-4}$ M) caused concentration-dependent contractions both in porcine and human coronary arteries. The EC_{50} values obtained with 4-AP did not differ significantly in porcine and human coronary preparations (-4.54 logM and -4.37 logM, respectively). Loading the amine stores of the coronary tissues with 1 mM noradrenalin or blocking the beta-receptors with 2 μM propranolol did not change the contractile effects of 4-AP. In contrast, loading the isolated blood vessels with 1 mM 5-hydroxytryptamine (5-HT) resulted in significantly higher contractions, when induced by micromolar concentrations of 4-AP. The 5-HT receptor blocker, methysergide, almost completely inhibited these contractions. Our present observation provides evidence for the functional role of K_V in setting the vasomotor tone of coronary arteries through the release of 5-HT. In the light of these findings we suggest that the porcine coronary artery preparation can serve as a model for studying the functional effect of drugs on K_V-type potassium channels. **Acta Biol Szeged 44(1-4):39-44 (2000)**

KEY WORDS

4-aminopyridine
voltage-dependent potassium channel
porcine coronary artery
human coronary artery

Potassium channels are the most widely distributed ion channels in vascular tissues and these ion channels have been shown to play important roles in the regulation of the cell membrane potential (Nelson and Quayle 1995). In the arterial smooth muscle four main types of K^+ channels have been identified: the voltage-dependent K^+ channel (K_V), the Ca^{2+} activated K^+ channel (K_{Ca}), the inward rectifier K^+ channel (K_{IR}) and the ATP-sensitive K^+ channel (K_{ATP}) (Nelson and Quayle 1995). The functional role of these potassium channels in the modulation of coronary vasomotor tone has not yet been characterised.

Diameter of blood vessels is largely determined by the interactions between the smooth muscle and endothelial cells as well as the medio-adventitial neurons through the liberation of vasoactive mediators. All of these sites in the blood vessel wall have been shown to contain K_V channels (Hara et al. 1980; Takeda et al. 1987; Adams 1994; Halliday et al. 1995; Knot and Nelson 1995; Gollasch et al. 1996; O'Rourke 1996; Fryer and Glover 1997; Ishikawa et al. 1997). K_V channels have been presented to be responsible for maintaining the resting membrane potential of coronary smooth muscles and the activation of K_V channels was inhibited by 4-aminopyridine (4-AP) (O'Rourke 1996; Ishikawa et al. 1997). Blocking of K_V channels located on the membrane of nerve endings was suggested to affect the tone of blood vessels via the release of vasoconstrictor neurotransmitters (Hara et al. 1980; Fryer and Glover 1997). The involvement of vasoactive mediators in the function of K_V channels, however, is not known in coronary arteries. In porcine coronary artery 5-HT is an important modulator of the blood vessel tone (Cushing and Cohen 1992). In the same species, the role of some vasoactive mediators in the contractile effect of 4-AP was excluded but a possible mediation through the liberation of 5-HT was not studied (O'Rourke 1996).

In this study we investigated the effect of the K_V blocker, 4-AP, on the resting tone of porcine and human coronary arteries and the possible role of noradrenalin and 5-HT in the mechanism of the contractile effect of the K_V-type potassium channel blocker.

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*Corresponding author. Phone: 36(62)545-674, Fax: 36(62)544-565, E-mail: kuna@phcol.szote.u-szeged.hu

Materials and methods

Tissue preparation and experimental protocol

Human coronary arteries were obtained from heart transplants without coronary artery disease with the permission of the Ethical Committee of the Albert Szent-Györgyi Medical University (51-57/1997.O.E.j., 16.05.1997.). The hearts were stored in cardioplegic solution at 4°C and used for the experiments within 12 hours. Porcine hearts of either sex were obtained from a local slaughterhouse and were transported to the laboratory in Krebs-Hensenleit solution (KHS) at 4°C. The epicardial coronary arteries of human and porcine hearts were carefully removed, cleaned from the adhering connective tissue and the blood vessels were cut into 5 mm ring segments.

Rings were placed into an organ bath filled with KHS and were aerated with a mixture of 95% O_2 and 5% CO_2 at 37°C. The isometric tension was recorded with a force-displacement transducer (Type F30, Hugo Sachs Elektronic, Germany). Mechanical responses of arterial rings were displayed by means of a pen recorder (Type 175, KUTESZ, Hungary). Strips were stretched passively (porcine coronary artery rings 30 mN, human coronary artery rings 10 mN), and equilibrated for 45 min, during which the medium was changed every 15 min. Endothelium of porcine coronary arteries was removed mechanically, *i.e.* thin glass stick covered with cotton wool was pulled through the rings. The absence of endothelium was confirmed by the lack of relaxation in response to the endothelium dependent vasodilator bradykinin (10^{-6} M).

Concentration-response curves to 4-aminopyridine (4-AP, $1.25x10^{-8}$ - $5.8x10^{-4}$M) were obtained by adding the drug in a cumulative manner to the organ bath at the end of the equlibration period. After contraction induced by 4-AP the preparation was washed three times and incubated with either 1 mM noradrenalin (NA) or 1 mM 5-hydroxytryptamine (5-HT) for 10-30 min. After this treatment coronary artery rings were washed again, and the changes of basal tone by repeated administration of 4-AP were detected. In some experiments, contractile responses of 4-AP were investigated in the presence of S-propranolol (2 μM, before and after NA treatment) and methysergide (100 μM, after 5-HT treatment) to inhibit β-adrenergic receptors and 5-HT receptors, respectively.

Drugs

Drugs used in the present study were 4-AP, 5-HT, creatinine sulfate complex, S-propranolol hydrochloride (Sigma), L-Noradrenalin-L-tartarat (Merck) and methysergide (Sandoz). These drugs were diluted in distilled water. Cardioplegic solution had the following composition (in mM): NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 1.2, $NaHCO_3$ 10 (REANAL, Hungary). The composition of the KHS was as follows (in mM): NaCl 120, $NaHCO_3$ 20, KCl 4.1, KH_2PO_4 1.2, $MgCl_2x6H_2O$ 1.2, $CaCl_2$ 1.5, glucose 11 (pH 7.4) (REANAL, Hungary).

Statistics

Contractile responses of human and porcine coronary arteries were expressed as milliNewton (mN). The concentrations and EC_{50} values (the concentration having half of the maximum effect) were expressed in -log molar (logM) concentration. Results are presented as means ± SEM (standard error of the mean). n = the number of animals from which vessels were taken. Values of corresponding concentrations were compared by one-way ANOVA, followed by Bonferroni post-hoc test and were considered as significantly different at $p<0.05$. Nonparametric Wilcoxan-test was used for the evaluation of the data in the case of methysergide treatment and were considered as significantly different at $p<0.05$.

Results

4-AP, an inhibitor of K_V, increased the basal tone of porcine coronary artery preparations concentration dependently in low micromolar concentrations (Fig. 1, filled circle, panel A, before NA). Pretreatment of the same preparations with 1 mM noradrenalin (NA) did not result in an enhanced contraction to 4-AP (before NA: 16.2 ± 6.4 mN, filled circle, panel A; after NA: 8 ± 3.1 mN, filled circle, panel B, not significant, n = 9, Fig. 1). The β-adrenoceptor blocker S-propranolol did not modulate the contraction induced by 4-AP either before or after NA treatment (Fig. 1, open circle, panel A and B, n = 9).

In contrast to this observation, after pretreatment of coronary arteries with 1 mM 5-hydroxytryptamine (5-HT) contraction to 4-AP became significantly higher compared to non-treated ones (before 5-HT: 12.2 ± 3.8 mN, after 5-HT: 24 ± 3.5 mN, $p<0.05$, n = 7, Fig. 2). The nonselective 5-HT receptor blocker, methysergide, almost completely abolished the contractions (Fig. 3, n = 7).

4-AP also could induce dose-dependent contraction in the human coronary artery (Fig. 4, n = 5). In human coronary preparations, uptake of 5-HT resulted in an enhanced contraction — similar to porcine coronary artery — and methysergide was able to reverse the effect of 4-AP (Fig. 5, representative figure).

As shown in Table 1, neither the treatment with NA nor presence of S-propranolol influenced the EC50 values of 4-AP in porcine coronary arteries. Treatment with 5-HT significantly increased the sensitivity to 4-AP of porcine coronary preparations. The EC_{50} values were similar in untreated porcine and human coronary arteries (-4.54 ± 0.1 logM and -4.37 ± 0.18 logM, respectively).

Discussion

The results of the present investigations demonstrate that the K_V channel blocker, 4-aminopyridine (4-AP), induces contraction of porcine and human coronary arteries via the release of vasoconstrictor 5-HT. These results are in agreement with previous studies that regulation of membrane potential through activation or inhibition of K_V channel activity provides an important mechanism to dilate or constrict arteries. Indeed, the importance of potassium conductance in maintaining the resting vasomotor tone has suggested that potassium channel blockers are able to cause contractions of smooth muscles. This contractile effect of 4-

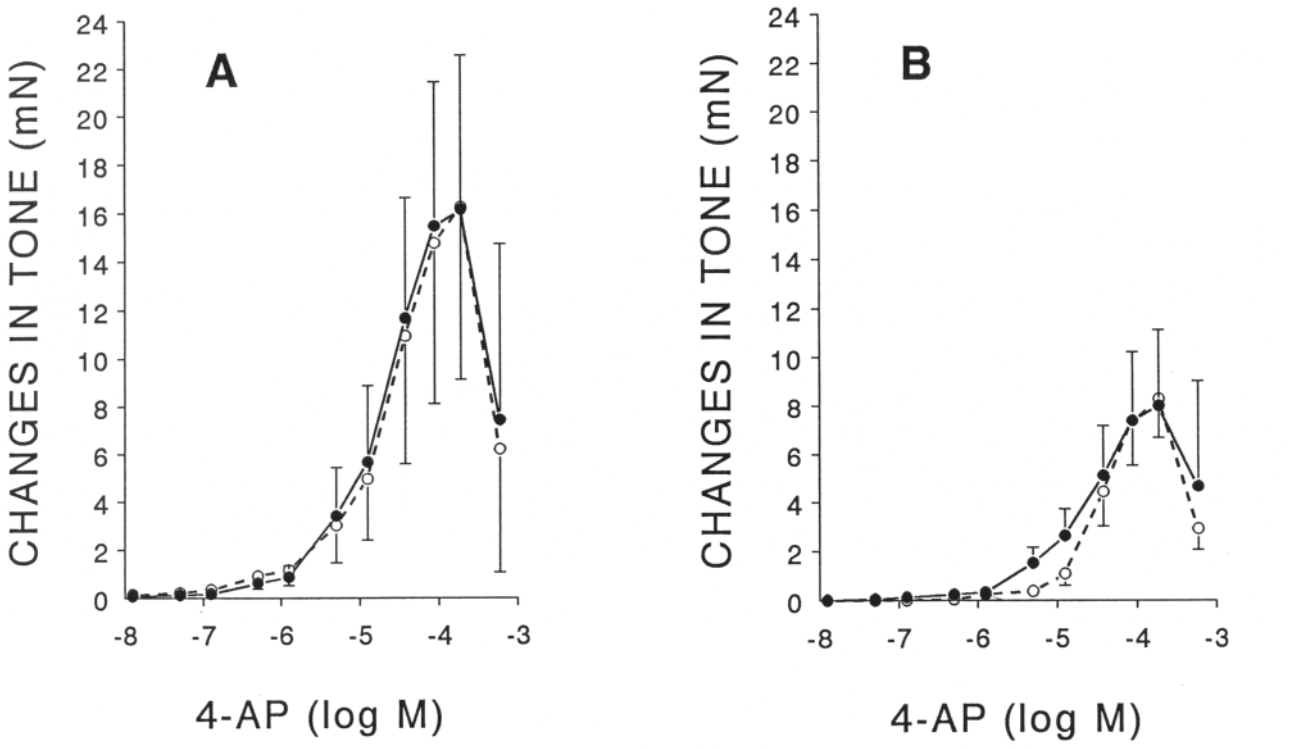
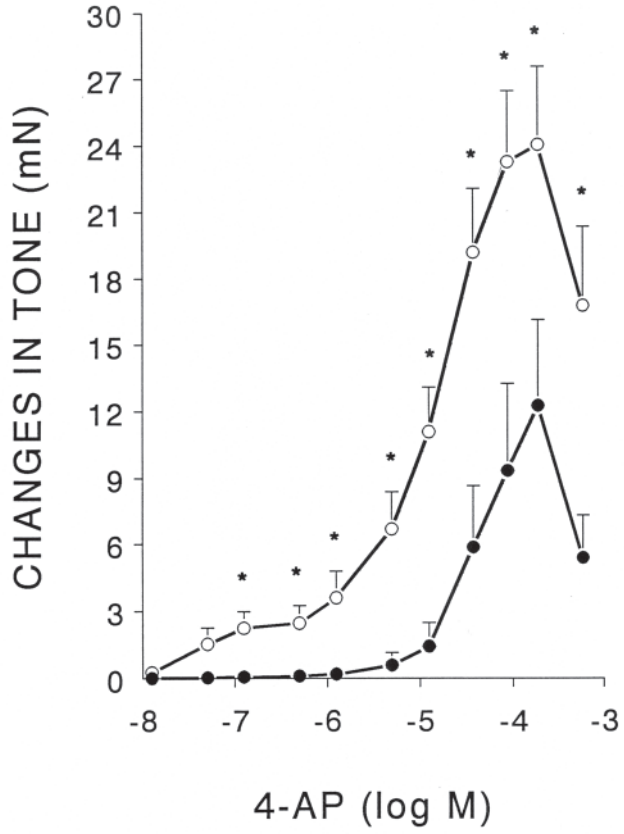


Figure 1. Effect of 4-aminopyridine (4-AP) on the basal tone and the influence of the loading of coronary tissue with noradrenalin (NA) on the elevated tone induced by 4-AP. S-propranolol (S-prop) did not change the 4-AP induced tone in pig coronary artery. **A** represents before, and **B** after pretreatment the coronary preparations with NA. Data represent mean ± SEM. n=9; • = 4-AP, o = 4-AP + S-prop.

AP on some blood vessels have been recently characterized (Hara et al. 1980; Halliday et al. 1995; Knot and Nelson 1995; O'Rourke 1996; Fryer and Glover 1997; Ishikawa et al. 1997). The role of K_V channels in setting the coronary tone of some animal species is also well studied (O'Rourke 1996; Ishikawa et al. 1997). Vascular smooth muscle cells of human coronary artery have also been shown to contain K_V channels (Gollasch et al. 1996). In an earlier study 4-AP was demonstrated to enhance the basal tone of human coronary artery as well (Uchida et al. 1986). Our observation provides evidence that the K_V channel is a functionally important channel not only in the porcine but in human coronary artery.

In our experiments, the contraction induced by blocking of K_V by 4-AP depends on the release of vasoconstrictor 5-hydroxytryptamine (5-HT). Inhibition of K_V channel by 4-AP present on the sympathetic nerve fibers induces membrane depolarisation, and this change of membrane potential causes transmitter release and thus smooth muscle contraction (Hara et al. 1980; Fryer and Glover 1997). The arteries are innervated by adrenergic nerves and these nerves are able to accumulate 5-HT. From these perivascular sympathetic nerve endings 5-HT, as vasoconstrictor co-transmitter with noradrenalin (NA) were shown to be released (Cohen 1985; Saito and Lee 1987; Szabó et al. 1991). Under our experimental conditions, the role of adrenergic innervation is not likely to be important in the contractile effect of 4-AP, because the presence of S-propranolol (both before and after NA treatment) did not modulate the contraction induced by 4-AP. Moreover, loading the isolated porcine coronary preparation with noradrenalin (NA) could not increase the tone after administration of the potassium channel blocker. This consists with the previous observation, that in porcine coronary artery neither alpha-receptor blocker phentolamine nor beta-receptor blocker propranolol modulated the contraction induced by 4-AP (O'Rourke 1996). Moreover, alpha-1 adrenoreceptor appears to have no significant role in the modulation of the tone of this blood vessel. It is well known that NA released from sympathetic nerve endings can cause vasorelaxation via beta adrenoreceptor stimulation (Horst and Robinson 1985; Quillen et al. 1992). In our preliminary experiments, in human coronary artery either these adrenerg antagonist agents or NA treatment also did not modulate the contractile effect of 4-AP (unpublished data). This corresponds with observation that in human coronary artery contraction induced by 4-AP was not eliminated by phentolamine or yohimbine (Uchida et al. 1986).



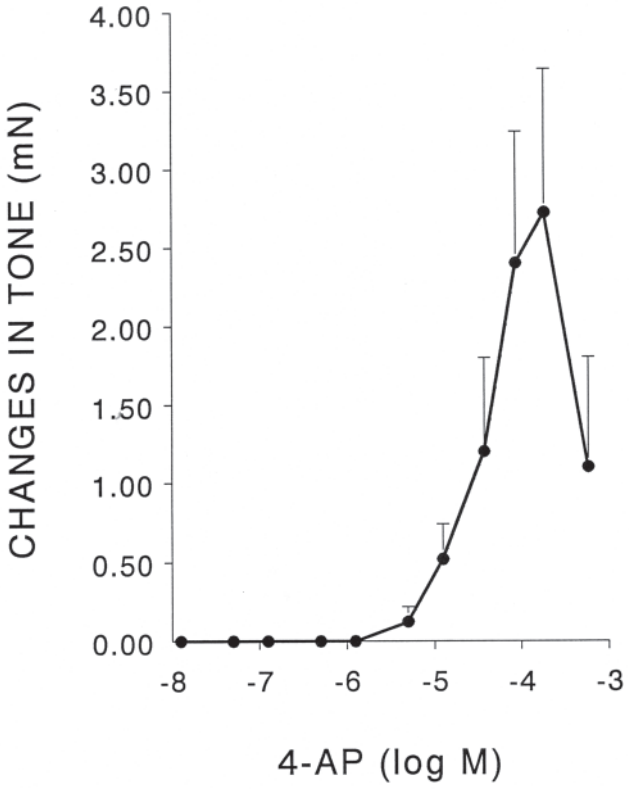
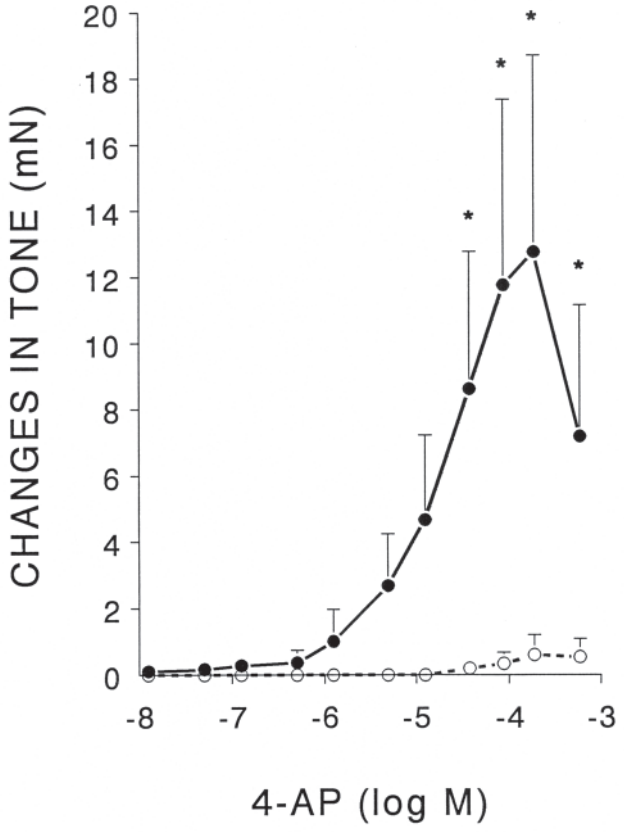


Figure 4. Effect of 4-aminopyridine (4-AP) on the basal tone of human coronary artery. Data represent mean ± SEM. n = 5.



Concerning the role of 5-HT, pretreatment of coronary arteries with 1 mM 5-HT, 4-AP induced significantly higher contraction and methysergide abolished this contraction both in porcine and in human preparations. This suggested an intact 5-HT storage capacity and intact uptake mechanism of coronary arteries under our experimental conditions. It should be emphasized that the source of 5-HT would be not only the perivascular nerves but extraneuronal stores, too. In earlier study it has been demonstrated that 5-HT accumulation in the rat coronary circulation is mainly extraneuronal and the mechanism of this uptake of 5-HT is similar to that of NA (Bryan et al. 1989). Thus, extraneuronal 5-HT stores may also be responsible for the contraction by 4-AP.

Figure 2. Effect of pretreatment with 5-hydroxytryptamine (5-HT) on the elevated tone by 4-aminopyridine (4-AP) in pig coronary artery. Data represent mean ± SEM. n = 7; * $p<0.05$; • = before 5-HT, o = after 5-HT.

Figure 3. Effect of 100 μM Methysergide (Meth) on the elevated tone by 4-aminopyridine (4-AP) in pig coronary artery after pretreatment with 5-hydroxytryptamine. Data represent mean ± SEM. n = 7, * $p<0.05$, • = 4-AP, o = 4-AP+Meth.

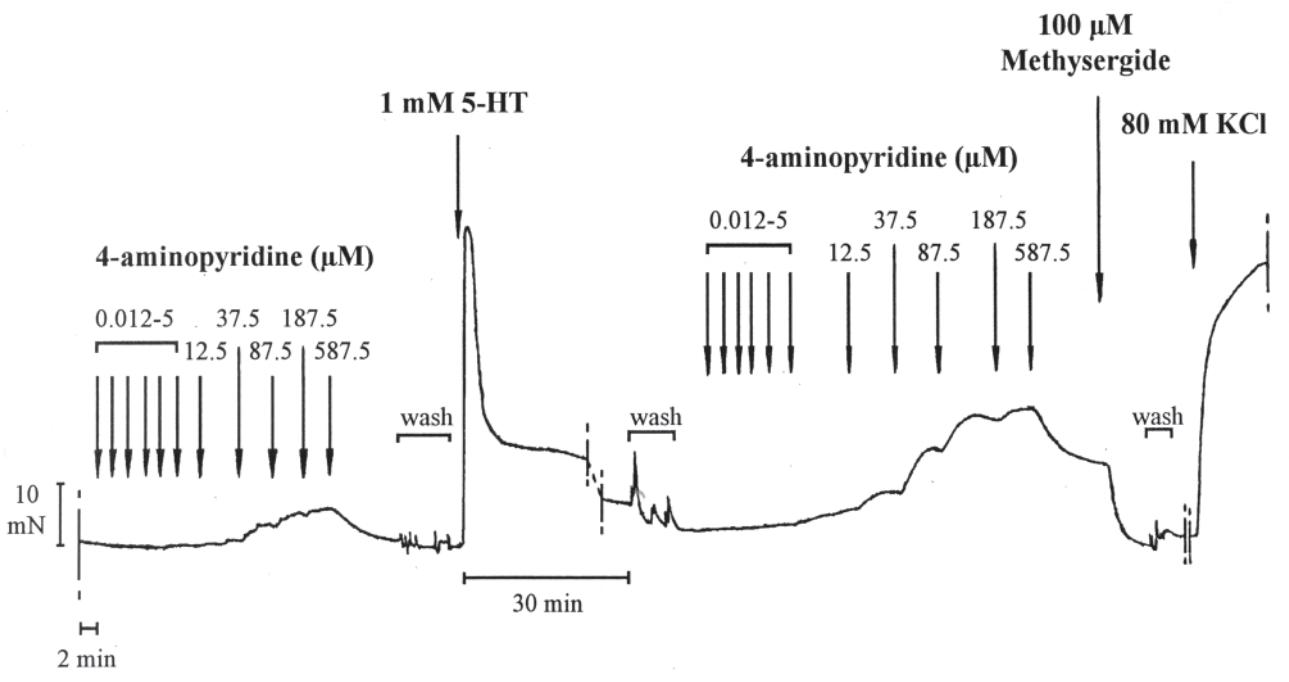


Figure 5. Effect of Methysergide on 5-hydroxytryptamine (5-HT) loaded human coronary artery in the presence of 4-aminopyridine. Data represent mean ± SEM.

Based on the present findings we propose that under pathological conditions the neuronal and/or extraneuronal stores of coronary arteries are filled with platelet-derived 5-HT (Cohen 1985). This serotonin may subsequently contribute to an abnormal contractile responsiveness, *i.e.* vasospasm of the coronary artery. The 5-HT stores of the blood vessel wall may be strongly affected by drugs acting on K_V-type potassium channels and, conseqently influence the coronary tone.

In our experiments, 4-AP induced 5-HT release was effective even at low micromolar concentrations. However, 4-AP was shown to exert smooth muscle contractions in millimolar concentrations (Uchida et al. 1986; Halliday et al. 1995; Knot and Nelson 1995; Ishikawa et al. 1997). In central and peripheral nerves, 4-AP blocked K_V also in low micromolar concentrations (Kumamoto and Kuba 1985; Hu and Fredholm 1991) which corresponds to the potency of the drug in the present study. The EC_{50} value of 4-AP was lower in the absence of 5-HT loading compared to the value obtained after pretreatment of the coronary preparations with 5-HT. We do not know the exact explanation of this change in EC_{50}, but we think that a larger mass of 5-HT released from the coronary artery after loading masks the direct contractile effect of 4-AP on the smooth muscle. It is well known that 5-HT is a very potent vasoactive mediator with EC_{50} of lower than 1 µM (Cushing and Cohen 1992).

Table 1. Sensitivities of coronary arteries obtained from porcine and human coronary artery preparations to 4-AP.

	-log (M) EC_{50}						
	before NA		after NA		before 5-HT	after 5-HT	untreated
Coronary preparation	4-AP	4-AP+S-prop	4-AP	4-AP+S-prop			
Porcine	4.7 ± 0.1	4.47 ± 0.15	4.51 ± 0.14	4.39 ± 0.13	4.43 ± 0.14	4.92 ± 0.06*	4.54 ± 0.1
Human	—	—	—	—	—	—	4.37±0.18

*Value after 5-HT treatment is significantly different from value before 5-HT treatment in porcine coronary artery ($p<0.05$). Untreated = before 5-HT + before NA (4-AP). Each value represents the mean ± SEM. 4-AP: 4-aminopyridine, S-prop: S-propranolol, 5-HT: 5-hydroxytryptamine.

Recently, the role of K_V has been demonstrated in different pathological conditions. Inhibition of K_V was found to be responsible for hypoxic pulmonary vasoconstriction (Post et al. 1995), for Long QT-Syndrome and overexpression of K_V in pancreatic islet cells might cause diabetes (Grissmer 1997). These K^+ channel dysfunctions have been termed as "K^+ channelopathies" (Grissmer 1997). This is the reason why the exact mechanism of the K_V channel blocker, 4-AP, requires considerable attention. It has also been demonstrated that pharmacological modulations of K_V ion channels may have potential value in the cardiac inotropic medication (Varró and Papp 1995). The current findings suggest that 4-AP induces spasm of the coronary artery with consequent ischemic damage of heart muscle. However, this potential side effect of the K_V channel inhibition on vascular tone would be eliminated by introducing subtype selective inhibition of K_V channels in the heart (Iost et al. 1998).

In summary, the present findings provide pharmacological evidences that the K_V channel plays a crucial role in setting the resting vasomotor tone of porcine and human coronary arteries via the regulation of the release of the vasoconstrictor mediator, 5-HT. The pharmacodynamic effect of 4-AP in the human coronary artery resembles that obtained in the porcine coronary artery. This suggests that coronary artery preparations obtained from the porcine heart can serve as a model for studying the functional effect of drugs on K_V-type potassium channels.

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