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Analysis of the pressor response to the K^+ channel inhibitor 4-aminopyridine

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Abstract

The cardiovascular response to the K^+ channel inhibitor 4-aminopyridine in anaesthetized rats was analysed. 4-Aminopyridine produced a biphasic pressor response. First, it increased blood pressure, total peripheral vascular resistance, cardiac output and stroke volume. Nitric oxide synthase (NOS) inhibitor augmented the tension response; reserpine, phentolamine, propranolol, scopolamine, atropine, adrenalectomy, indomethacin, angiotensin AT_1 and endothelin ET_A receptor antagonists had no effect. Subsequently, heart rate increased, but total peripheral vascular resistance was no longer elevated. Reserpine and propranolol abolished the tachycardia. An elevated late tension occurred after propranolol and NOS inhibitor but not reserpine or phentolamine + NOS inhibitor. The peripherally acting 3,4-diaminopyridine produced similar responses. 4-Aminopyridine contracted isolated aortic rings also after denudation. These results are compatible with that the immediate tension response resulted from closure of vascular smooth muscle K^+ channels, and that closure of presynaptic K^+ channels in peripheral sympathetic nerves subsequently activated noradrenaline release, β-adrenoceptors and tachycardia, while nitric oxide counteracted a concomitant α-adrenergic vasoconstriction.

Keywords: Blood pressure; Vascular tension; K^+ channel; 4-Aminopyridine; Adrenergic system

1. Introduction

K^+ channels play an important role in blood pressure homeostasis by regulating the function of vascular smooth muscle cells and cardiomyocytes, and also indirectly by altering neuronal transmitter release. In a previous study (Berg and Koteng, 1997), it was noted that among several K^+ channel inhibitors, 4-aminopyridine in particular induced a marked and sustained pressor response. It is not known if the pressor response to 4-aminopyridine involves a rise in total peripheral vascular resistance and/or cardiac output. In vascular smooth muscle cells, 4-aminopyridine in mM concentration functions as an inhibitor of voltage-sensitive, Ca^{2+}-insensitive K^+ channels (K_V) with little effect on Ca^{2+}-sensitive or inward rectifier K^+ channels (Gelband and Hume, 1992; Ishikawa et al., 1993; Robertson and Nelson, 1994; Quayle et al., 1993; Knot and Nelson, 1995). However, it does not differentiate between different members of the numerous K_V family (Shieh et al., 2000). Small changes in the membrane potential in vascular smooth muscle cells, due to differences in K^+ conductance, will alter the influx of Ca^{2+} through voltage-gated Ca^{2+} channels and consequently the diameter and tension of resistance vessels. The rise in pressure following 4-aminopyridine may therefore be due to closure of vascular smooth muscle cell K_V participating in the control of resting mean blood pressure (Nelson et al., 1990; Nelson and Quayle, 1995; Cole et al., 1996; Martens and Gelband, 1998; Waldron and Cole, 1999). However, some of the K_V in vascular smooth muscle cells as well as other members of the K_V family are also found in other cells, which play a role in the blood pressure homeostasis (Shieh et al., 2000). Moreover, 4-aminopyridine has been reported in nonvascular cells to inhibit K^+ channels not belonging to the K_V family (Shieh et al., 2000). A rise in pressure following 4-aminopyridine may therefore also be due to activation of the adrenergic system, enhancing total peripheral vascular resistance and/or cardiac output. An increase in the adrenergic tone may result from a direct action on sympathetic nerve endings by that 4-aminopyridine inhibits presynaptic K^+ channels, thus favouring the influx of Ca^{2+} and consequently the release of noradrenaline (Kirpekar et al., 1977;

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Leander et al., 1977; Huang and Zhou, 1995). However, adrenergic activation may also occur indirectly by that 4-aminopyridine may stimulate, in the same fashion, the release of acetylcholine in the central nervous system and/or in the adrenals, thus enhancing sympathetic output and adrenal catecholamine release, respectively (Glover, 1982; Damsma et al., 1988; Sundaram and Sapru, 1988; Kirpekar et al., 1982). In addition, 4-aminopyridine-sensitive K_V have been demonstrated in cardiomyocytes, and 4-aminopyridine has been shown to have a positive inotropic effect (Northover, 1994). Moreover, 4-aminopyridine-sensitive K^+ channels have also been demonstrated in endothelial cells (Chen and Cheung, 1992). The activity of these channels may stimulate the secretion of endothelium-derived factors, such as nitric oxide (NO), prostacyclin or endothelin, and consequently influence vascular tension. In addition, endothelium-derived factors may function to regulate vascular smooth muscle cell K^+ channel open-state probability (Waldron and Cole, 1999; Shimoda et al., 1998; Betts and Kozlowski, 2000).

Many inhibitors, more selective for different channel subtypes than 4-aminopyridine, have been identified. However, none of these are organ-specific. The purpose of the present study was therefore to analyse the cardiovascular response to 4-aminopyridine, first by determining if the pressor response to 4-aminopyridine was due to a rise in cardiac output and/or total peripheral vascular resistance, and second by analysing the contribution due to activation of the adrenergic system and the influence of various endothelial-derived factors. The results will show that 4-aminopyridine induces an immediate and transient increase in total peripheral vascular resistance due to an effect on vascular smooth muscle cells. A second phase in the pressor response to 4-aminopyridine resulted from noradrenaline release from peripheral sympathetic nerve terminals, thus causing activation of β-adrenoceptors and tachycardia. The data will also show that a concomitant late α-adrenergic increase in total peripheral vascular resistance was prevented by NO synthesis.

2. Methods

2.1. Animals

Male Wistar Kyoto rats ($n = 261$, 12–14 weeks, 283 ± 2 g) on conventional rat chow diet (0.7% NaCl) were included in the present study in accordance with institutional guidelines and after approval by the institutional ethics committee.

2.2. In vivo experiments

The rats were anaesthetized (65 mg/kg Nembutal, i.p.), tracheotomized and placed on a Harvard Instruments Respirator. To record cardiac output (= minus coronary flow), the rats were thoracotomised at the third intercostal space, and a 2SB perivascular flowprobe was placed on the ascending aorta. The probe was connected to a T206 Ultrasonic Transit-Time Flowmeter (Transonic Systems, Ithaca, NY, USA). Systolic and diastolic blood pressures and heart rate were recorded through a heparinized catheter in the femoral artery, connected to a SensoNor 840 transducer (SensoNor, Horten, Norway). The flowmeter and pressure transducer were coupled to an amplifier and computer. Mean blood pressure ((systolic minus diastolic blood pressure)/3 + diastolic blood pressure), stroke volume and total peripheral vascular resistance were calculated. Drugs were dissolved in phosphate-buffered saline (PBS; 0.01 M Na-phosphate, pH 7.4, 0.14 M NaCl) and administered as bolus injections (0.6 ml/kg) through a catheter in the femoral vein, unless otherwise indicated. In some rats, arterial blood (100 μl) was sampled before and at the end of the experiment, and P_{CO_2}, P_{O_2}, pH and base excess were measured in an ABL 500 Radiometer (Radiometer Medical, Copenhagen, Denmark).

The pressor response to 4-aminopyridine (34.5 μmol/kg,) was analysed in rats given a prior injection of vehicle (0.6–1.3 ml/kg, −30 to −10 min). To evaluate differences due to central versus peripheral actions, 4-aminopyridine was substituted with an equimolar dose of 3,4-diaminopyridine. Contribution due to activation of the autonomic nervous system was elucidated by comparing the response to 4-aminopyridine in rats pretreated with PBS with that seen in rats after inhibition of components of the autonomic nervous system, i.e., (1) after depletion of noradrenaline from peripheral sympathetic nerve endings using reserpine (8 μmol/kg injected intraperitoneally −48 and −24 h prior to the experiment), (2) after inhibition of α- or β-adrenoceptors by substituting the PBS injection with phentolamine (6.3 μmol/kg, −10 min) or propranolol (44 μmol/kg, 1.3 ml/kg administered over a 10-min period, −20 min), respectively, (3) after inhibition of central or peripheral muscarinic receptors by scopolamine (Vargas and Ringdahl, 1990) (2.4 μmol/kg, −30 min) or atropine (6.9 μmol/kg, −20 min), respectively, and (4) after acute removal of the adrenal glands. Adrenalectomy was performed through flank incisions after the rats were anaesthetised, i.e., about 30 min before 4-aminopyridine. Similar to that in the controls, reserpinised and adrenalectomized rats received PBS 10 min prior to 4-aminopyridine. Reserpinised and adrenalectomized rats were also given 3,4-diaminopyridine instead of 4-aminopyridine. In the next protocol, the role of endothelial-derived factors were studied by pretreatment with a supramaximal dose (Rees et al., 1990) of NO synthase inhibitor (1.1 mmol/kg N^{ω}-nitro-L-arginine methyl ester, L-NAME, −30 min) alone or after additional pretreatment with phentolamine (6.3 μmol/kg, −10 min), by pretreatment with cyclooxygenase inhibitor (27.9 μmol/kg indomethacin, −10 min), endothelin ET_A receptor antagonist (1.1 μmol/kg ZD1611 ([3-?4-[3-(3-methoxy-5-methylpyrazin-2-ylsulfamoyl)-2-pyridyl]phenyl?-2,2-dimethylpropanoic acid]) (Wilson et al., 1999), −10 min), or angiotensin AT_1

receptor antagonist (79 μmol/kg Losartan, −10 min). The numbers of rats per group are shown in Tables 1 and 3. The efficacy of the antagonists was tested by the ability to inhibit the response to corresponding agonist.

2.3. In vitro experiments

Rats were killed with a blow on the head and bleeding. Thoracic aortic rings (3 mm) were mounted for isometric recording of tension in a modified Krebs–Henseleit buffer (in mM: Na^+ 143, K^+ 5.9, Ca^{2+} 0.25, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 1.2, HCO_3^- 24.9, SO_4^{2-} 1.2, D-glucose 11.1, 37 °C, gassed with 95% O_2/5% CO_2), washed 4 × over the next hour, and stretched to a preload of 4 g. Endothelium and vascular smooth muscle cell viability were confirmed by addition of 1 μM acetylcholine and 1.9 μM isoprenaline, respectively. These test was performed at the end of the experiment, i.e., after the 4-aminopyridine-induced contraction had been washed out and the rings re-contracted with 0.1 μM phenylephrine.

The 4-aminopyridine concentration response dependency was first established by addition of 4-aminopyridine (1–8 mM, noncumulatively), allowing contraction to develop over the next 5 h (6–12 rings per dose, eight rats). A concentration of 4 mM 4-aminopyridine and a 3-h observation period were chosen for further studies. The response to 4 mM 4-aminopyridine was first compared to that of an equimolar concentration of 3,4-diaminopyridine. The role of neural activation in the 4-aminopyridine-induced vasoconstriction was studied using rings from reserpinised rats, or after pre-incubation with 1 μM tetrodotoxin, 5 μM phentolamine, 1 μM propranolol, 5 μM scopolamine or 10 μM atropine. The role of endothelial-derived factors was studied by removal of the endothelium by gentle rubbing of the intimal surface with a roughened steel rod, or by pre-incubation with 100 μM L-NAME, 10 μM indomethacin, L-NAME combined with indomethacin or 3 μM ZD1611 (30 min). Control rings, pre-incubated with PBS, were included in all experiments. The efficacy of the antagonists was established in separate experiments by their ability to inhibit the response to agonist.

2.4. Reagents

Scopolamine hydrobromide, isoprenaline sulfate, propranolol hydrochloride and atropine sulphate were from The National Hospital, Oslo, Norway, phentolamine methanesulfonate (Regitine) from Ciba-Geigy, Basel, Switzerland, 3,4-diaminopyridine from MERCK, Schuchardt, Germany, and endothelin 1 from Phoenix Pharmaceuticals, Mountain View, CA, USA. The endothelin ET_A receptor antagonist ZD1611 was a kind gift from Zeneca Pharmaceuticals, Cheshire, UK, and Losartan from MSD Norge, Drammen, Norway. The remaining drugs were from Sigma, St. Louis, MO, USA.

2.5. Statistics

The results are expressed as means ± S.E.M. The in vivo data were averaged every minute throughout the experiments and every 7 beats at mean blood pressure peak/nadir to aminopyridines or agonists. Evaluation of the in vitro response to aminopyridines was carried out using the increase in tension expressed in gram force above the 4 g preload. The in vivo data were analysed by repeated measures including values before and after pretreatment(s), and immediately, 10, 15, 20 and 25 min after aminopyridine. The data were first analysed as overall tests, then between groups and for each group individually. The in vitro data were analysed by analysis of variance (ANOVA). When, after these tests, the presence of a significant response or group differences was indicated in the in vivo and in the in vitro data, these were located by one- and two-sample Student's *t*-tests, respectively. For the latter tests, the mean of values recorded at 10, 15, 20 and 25 min were used for the late in vivo response. Correlation factors (*r*) were determined with Pearson correlation tests. *P*-value limits were corrected according to Bonferroni.

3. Results

3.1. Analysis of the cardiovascular response to 4-aminopyridine in vivo

Typical recordings of the biphasic blood pressure and cardiac output response to 4-aminopyridine are shown in Fig. 1. During the immediate peak pressor response, which occurred after 1.3 ± 0.1 min, there was a 92 ± 4% increase in mean blood pressure with an increase in total peripheral vascular resistance (47 ± 5%), cardiac output (34 ± 5%) and stroke volume (46 ± 6%) ($P < 0.0001$), but no significant change in heart rate (−7 ± 3%) (Fig. 2). Baseline values are shown in Table 1. Significant correlations between the response to 4-aminopyridine and the corresponding baselines were not detected. The rise in systolic and diastolic blood pressures correlated with the rise in total peripheral

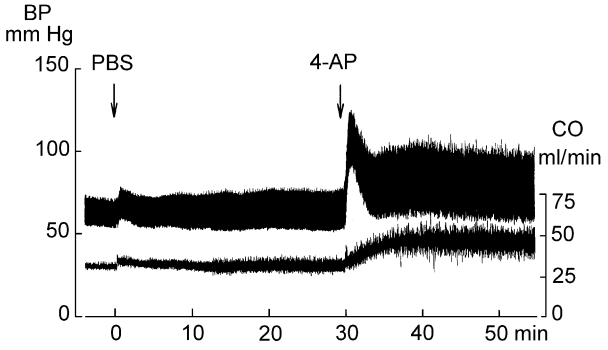

Fig. 1. A typical recording of the effect of 4-aminopyridine (4-AP) on blood pressure (BP) (upper tracing) and cardiac output (CO) (lower tracing) in control rats pre-injected with vehicle (PBS).

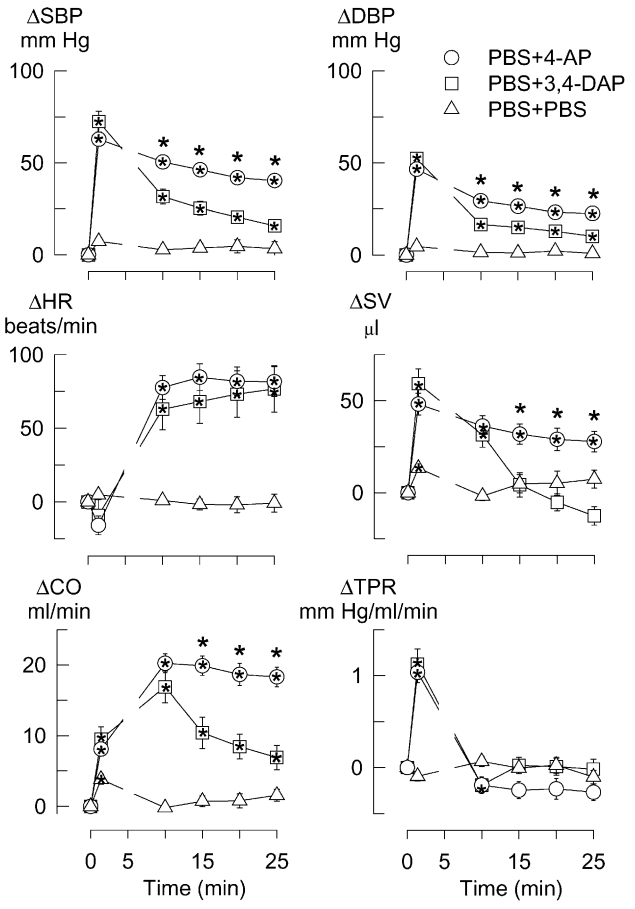

Fig. 2. The in vivo cardiovascular response to 34.5 μmol/kg 4-aminopyridine (4-AP) or 3,4-diaminopyridine (3,4-DAP) or a sham injection with PBS (means ± S.E.M.). All three groups were given PBS as pretreatment. Significant changes (* within symbol) and differences between the responses to 4-aminopyridine and 3,4-diaminopyridine (* above symbols) were located as indicated. Baselines prior to 4-aminopyridine did not differ between the groups (Table 1). SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance. *: $P < P$-value limit = 0.01.

vascular resistance ($r = 0.77$ and 0.70, respectively), whereas a negative correlation was observed between the change in total peripheral vascular resistance and cardiac output ($r = -0.82$) ($P < 0.001$). Administration of PBS instead of 4-aminopyridine in time controls (PBS + PBS) induced no or only minor changes (Fig. 2).

During the second phase (10–25 min), there was a 58 ± 5% rise in mean blood pressure, with a further increase in cardiac output (78 ± 8%), a continued, augmented stroke volume (32 ± 6%) and, in addition, tachycardia (36 ± 4%) ($P < 0.0001$) (Fig. 2). The increase in heart rate started 3–4 min after the injection of 4-aminopyridine. The late change in total peripheral vascular resistance (−8 ± 4%) was not significant (Fig. 2). A positive correlation was seen between the change in cardiac output and the change in stroke volume ($r = 0.64$), and the change in stroke volume was negatively correlated with the change in heart rate ($r = -0.59$) ($P \leq 0.002$).

4-Aminopyridine induced salivation in 90% of the rats. Salivation was first seen after 4 ± 1 min, and a total of 58 ± 18 μl was collected from the oral cavity. 4-Aminopyridine also induced strong muscular contractions, first observed 2.7 ± 0.3 min after the administration of 4-aminopyridine, however, in every rat later than the initial peak pressor response.

Arterial gas parameters measured at the beginning of the experiment and 25 min after 4-aminopyridine are shown in Table 2. During the experiment, pH, P_{O_2} and base excess decreased while P_{CO_2} increased, indicating a respiratory and metabolic acidosis. When the blood gas values (before, after and change from before to after the experiment) were correlated with the immediate cardiovascular response to 4-aminopyridine, a negative correlation was detected only between ΔP_{O_2} and the rise in total peripheral vascular resistance ($r = -0.85$, $P = 0.004$), i.e., the greater the decline in P_{O_2}, the greater was the immediate increase in total peripheral vascular resistance. For the late cardiovascular response, a correlation was found between the change in base excess and the change in heart rate as well as stroke volume ($r = 0.90$ and -0.81, respectively), and between P_{CO_2} after the changes in stroke volume and cardiac output ($r = 0.85$ and 0.90, respectively), with inverse values for pH_{after} correlated to the changes stroke volume and cardiac output ($r = -0.83$ and -0.80) ($P \leq 0.008$).

3.2. The cardiovascular response to 3,4-diaminopyridine in vivo

The immediate response to 3,4-diaminopyridine did not differ from that following 4-aminopyridine, whereas a decline was observed in the 3,4-diaminopyridine group in the late changes in systolic and diastolic blood pressures, cardiac output and stroke volume (Fig. 2). A correlation was observed between the immediate changes in total peripheral resistance and cardiac output ($r = -0.82$), and between the late changes in diastolic blood pressure and heart rate ($r = 0.85$, $P \leq 0.003$) following 3,4-diaminopyridine. ΔP_{CO_2} and ΔpH were less after 3,4-diaminopyridine than after 4-aminopyridine ($P < 0.0008$, pooled data for rats pretreated with PBS and reserpine) (Table 2). Salivation in response to 3,4-diaminopyridine (642 ± 112 μl) was greater than after 4-aminopyridine ($P = 0.0008$). Muscular twitches were not observed at all during the 25-min observation period in any of the rats given 3,4-diaminopyridine.

3.3. The in vivo response to 4-aminopyridine and 3,4-diaminopyridine after inhibition of components of the autonomic nervous system or endothelial cell function or angiotensin AT_1 receptor

The increase in systolic and diastolic blood pressures during the immediate response to 4-aminopyridine were

Table 1
Cardiovascular baseline values after inhibition of components of the autonomic nervous system, i.e., prior to 4-aminopyridine or 3,4-diaminopyridine

Groups	*N*	SBP (mm Hg)	DBP (mm Hg)	MBP (mm Hg)	HR (beats/min)	SV (μl)	CO (ml/min)	TPR (mm Hg/ml/min)
PBS + PBS (time control)	9	82 ± 3	51 ± 2	61 ± 2	224 ± 15	128 ± 10	28.2 ± 2.2	2.24 ± 0.16[a]
PBS + 4-AP (4-AP-control)	25	76 ± 3[a] (3 ± 1)	49 ± 2	58 ± 2	230 ± 7[a] (−21 ± 3)	116 ± 6[a] (8 ± 2)	26.9 ± 1.3	2.22 ± 0.05
Reserpine + PBS + 4-AP	6	73 ± 4	49 ± 2	57 ± 2	248 ± 18	118 ± 12	28.6 ± 1.7	2.01 ± 0.08
Phentolamine + 4-AP	10	56 ± 2[a] (− 12 ± 1)	36 ± 1[a] (− 11 ± 1)	42 ± 2[a] (− 11 ± 1)	202 ± 9[a] (− 50 ± 4)	110 ± 9	22.2 ± 1.6	1.98 ± 0.14[a] (− 0.33 ± 0.05)
Propranolol + 4-AP	9	72 ± 4	37 ± 4	49 ± 4	125 ± 7[a] (− 92 ± 3)	182 ± 18[a] (69 ± 10)	22.5 ± 2.1	2.32 ± 0.30
Scopolamine + 4-AP	10	75 ± 4	45 ± 3	54 ± 3	201 ± 10	123 ± 13	24.6 ± 2.6	2.37 ± 0.18
Atropine + 4-AP	7	77 ± 5	50 ± 3	59 ± 3	208 ± 10	134 ± 22	28.0 ± 3.5	2.27 ± 0.24
AdrX + PBS + 4-AP	10	65 ± 3[b]	44 ± 2	51 ± 2[b]	206 ± 13[a] (− 22 ± 2)	100 ± 14	20.6 ± 3.2	2.85 ± 0.34
PBS + 3,4-DAP (3,4-DAP-control)	10	76 ± 5	46 ± 2	56 ± 3	207 ± 7	148 ± 6	30.7 ± 1.3	1.84 ± 0.12
Reserpine + PBS + 3,4-DAP	6	76 ± 4	50 ± 2	58 ± 3	203 ± 4	143 ± 7	29.5 ± 2.0	2.02 ± 0.13
AdrX + PBS + 3,4-DAP	6	63 ± 3	40 ± 2[a] (− 5 ± 1)	48 ± 2	218 ± 10	87 ± 9[b]	18.5 ± 1.2[b]	2.64 ± 0.21[b]

The results represent means ± S.E.M. Changes in baselines due to the pretreatment given as a first injection during the experimental period were tested for using one-sample Student's *t*-tests. For those parameters where a significant change was detected ([a]: $P < P$-value limit = 0.0045), the Δ-values are indicated in parenthesis below. Baseline values in reserpinised and adrenalectomized (AdrX) rats, given PBS as their first injection during the experiment, were compared to their respective control groups (PBS + 4-AP and PBS + 3,4-DAP, respectively) using two-sample Student's *t*-tests. Differences were located as indicated ([b]: $P < P$-value limit = 0.025). 4-AP = 4-aminopyridine, 3,4-DAP = 3,4-diaminopyridine, N = number of rats per group, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance.

reduced in rats pretreated with phentolamine, propranolol and scopolamine, and the immediate increase in systolic and diastolic blood pressures following 3,4-diaminopyridine were reduced in reserpinised rats (Fig. 3). However, none of the procedures used to intervene with possible effects due to activation of the autonomic nervous system, i.e., central

Table 2
pH, P_{CO_2}, P_{O_2} and base excess at the start of the experiment and 25 min after 4-aminopyridine and 3,4-diaminopyridine

Group	*N*	At start				25 min after			
		pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Base excess (mM)	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Base excess (mM)
PBS + PBS (time control)	3	7.52 ± 0.04	3.65 ± 0.44	11.70 ± 1.89	0.57 ± 0.64	7.38 ± 0.01	5.23 ± 0.18	8.69 ± 1.01	− 1.77 ± 0.50
PBS + 4-AP	9	7.46 ± 0.02	4.13 ± 0.24	10.65 ± 1.03	− 1.29 ± 0.53	7.30 ± 0.02[a]	6.39 ± 0.34[a]	7.06 ± 0.57[a]	− 3.80 ± 0.87[a]
PBS + 3,4-DAP	4	7.47 ± 0.01	4.35 ± 0.17	9.23 ± 0.34	0.58 ± 0.45	7.37 ± 0.03[a]	5.23 ± 0.34	7.72 ± 0.57	− 2.58 ± 2.01
Reserpinise + PBS + 4-AP	5	7.50 ± 0.04	3.68 ± 0.26	11.35 ± 0.71	1.83 ± 0.42	7.38 ± 0.02	5.00 ± 0.46[a]	10.28 ± 1.12	− 3.32 ± 1.60
Reserpinised: PBS + 3,4-DAP	6	7.49 ± 0.01	4.13 ± 0.16	8.72 ± 0.27	0.95 ± 0.62	7.41 ± 0.01[a]	5.08 ± 0.15[a]	7.80 ± 0.28[a]	− 0.33 ± 0.61
PBS + L-NAME + 4-AP	6	7.52 ± 0.02	3.08 ± 0.23	13.27 ± 0.75	− 1.80 ± 0.32	7.28 ± 0.04[a]	4.73 ± 0.39[a]	8.86 ± 01.19	− 9.58 ± 1.80[a]
Phentolamine + L-NAME + 4-AP	7	7.50 ± 0.02	3.55 ± 0.31	11.09 ± 0.84	− 1.33 ± 0.73	7.27 ± 0.02[a]	5.53 ± 0.52[a]	9.59 ± 0.83	− 8.42 ± 0.64[a]
Indomethacin + 4-AP	7	7.46 ± 0.01	4.33 ± 0.20	9.32 ± 0.44	− 0.07 ± 0.62	7.30 ± 0.03[a]	6.54 ± 0.46[a]	6.74 ± 0.65[a]	− 3.73 ± 1.18
Losartan + 4-AP	6	7.44 ± 0.03	4.42 ± 0.36	9.25 ± 1.11	− 1.06 ± 0.47	7.33 ± 0.03[a]	5.54 ± 0.54[a]	8.88 ± 1.08	− 4.61 ± 0.67[a]
ZD1611 + 4-AP	6	7.47 ± 0.02	4.52 ± 0.17	8.59 ± 0.28	1.48 ± 0.37	7.27 ± 0.03[a]	7.43 ± 0.43[a]	6.33 ± 0.30[a]	− 3.65 ± 0.92[a]

The results represent means ± S.E.M. Significant differences in the blood gas parameters from start to 25 min after 4-aminopyridine (4-AP) or 3,4-diaminopyridine (3,4-DAP) were located as indicated ([a]: one-sample Student's *t*-tests, $P < P$-value limit = 0.0125). N = number of rats tested per group.

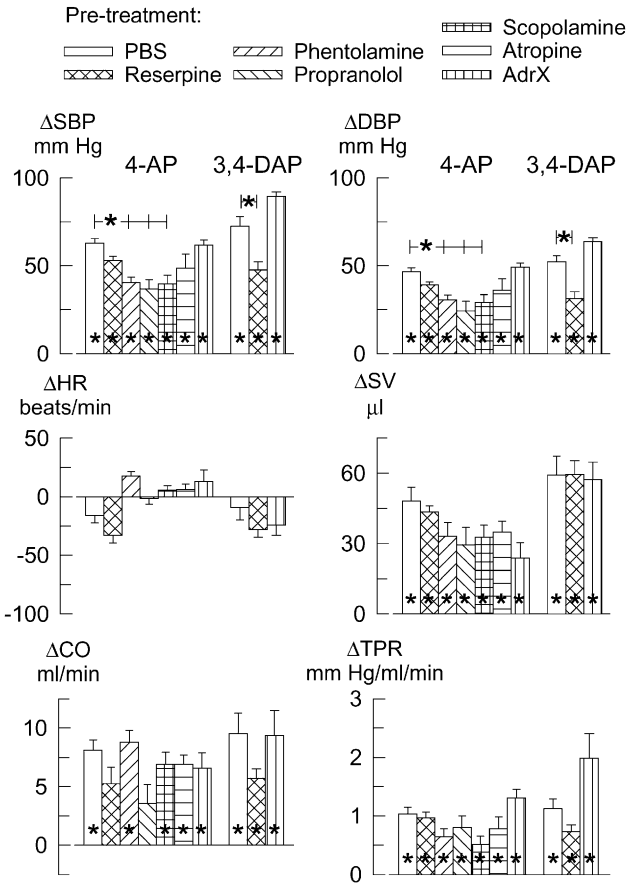

Fig. 3. The role of activation of the autonomic nervous system, including the adrenals, in the immediate in vivo response to 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP). The rats were pretreated as indicated by symbol legends. Significant responses (* within columns, *P*-value limit = 0.0071 and 0.0167 for 4-aminopyridine and 3,4-diaminopyridine, respectively) and differences from the control groups (* in brackets, *P*-value limit = 0.0083 and 0.025 for 4-aminopyridine and 3,4-diaminopyridine, respectively) were located as indicated. The values are means ± S.E.M. Baseline values after pretreatment, i.e., prior to 4-aminopyridine or 3,4-diaminopyridine, are shown in Table 1. SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance, AdrX = adrenalectomy.

or peripheral acetylcholine release, peripheral release of norepinephrine, or adrenal release of catecholamines, significantly altered the immediate change in heart rate, stroke volume, cardiac output or total peripheral vascular resistance in response to 4-aminopyridine or 3,4-diaminopyridine (Fig. 3). However, after L-NAME, the immediate rise in total peripheral vascular resistance was 102 ± 16% of baseline, which was 2.8 times greater than that in the control group ($P = 0.0015$) (Fig. 4), in spite of that L-NAME increased total peripheral vascular resistance baseline by 41 ± 3% (Table 3). Similarly, the increase in total peripheral vascular resistance was 2.6 times greater in rats pretreated with phentolamine + L-NAME as compared to phentolamine alone (Figs. 3 and 4, $P = 0.0056$). After L-NAME, bradycardia was observed during the immediate response to 4-aminopyridine, and the increase in cardiac output was totally abolished (Fig. 4). The increase in cardiac output was also greatly reduced after phentolamine + L-NAME (Figs. 3 and 4, $P < 0.0003$ compared to the phentolamine only group). The increase in total peripheral vascular resistance in response to L-NAME was inversely related to stroke volume baseline prior to L-NAME ($r = -0.91$, $P = 0.013$). The immediate response to 4-aminopyridine after pretreatment with Losartan, indomethacin or ZD1611 was not different from that in the controls, although the rise in total peripheral vascular resistance was not statistically significant in the Losartan group (Fig. 4).

During the late response, the increase in systolic and diastolic blood pressures following 4-aminopyridine were

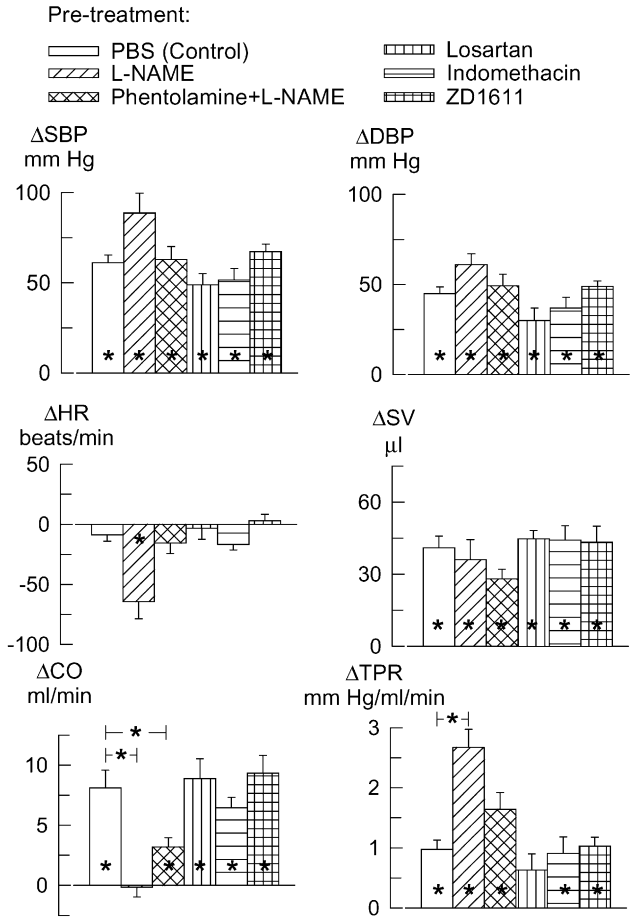

Fig. 4. The role of endothelial derived factors and angiotensin AT_1 in the immediate in vivo response to 4-aminopyridine in rats pretreated as indicated by symbol legends. Significant responses (* within columns, *P*-value limit = 0.0083) and differences between the controls and the other groups and between the L-NAME and the phentolamine + L-NAME groups (* in brackets, *P*-value limit = 0.0083) were located as indicated. The values represent means ± S.E.M. Baseline values after pretreatment, i.e., prior to 4-aminopyridine, are shown in Table 3. SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance.

Table 3
Cardiovascular baseline values prior to 4-aminopyridine after pretreatment with factors that influence endothelial function

Pretreatment	*N*	SBP (mm Hg)	DBP (mm Hg)	MBP (mm Hg)	HR (beats/min)	SV (μl)	CO (ml/min)	TPR (mm Hg/ml/min)
PBS	10	78 ± 4	48 ± 2	58 ± 3	243 ± 8	111 ± 9	27.1 ± 2.4	2.20 ± 0.16
PBS + L-NAME	6	102 ± 6	74 ± 6[b]	83 ± 6[b]	245 ± 11[a] (− 60 ± 9)	107 ± 12	25.5 ± 2.2[a] (− 5.2 ± 1.2)	3.52 ± 0.66[a] (1.12 ± 0.33)
Phentolamine + L-NAME	7	77 ± 2	56 ± 2	63 ± 2	199 ± 13[a] (− 64 ± 13)	117 ± 5	23.1 ± 0.9[a] (− 4.7 ± 1.1)	2.77 ± 0.07[ab] (0.55 ± 0.09)
Losartan	7	68 ± 5[a] (− 10 ± 1)	38 ± 3[a] (− 11 ± 1)	48 ± 4[a] (− 11 ± 1)	214 ± 15[a] (− 25 ± 3)	124 ± 14	25.8 ± 1.5	1.92 ± 0.16[a] (− 0.39 ± 0.08)
Indomethacin	6	75 ± 3	45 ± 1	55 ± 2	211 ± 7	132 ± 11	27.6 ± 1.6	2.02 ± 0.10
ZD1611	6	75 ± 3	47 ± 2	56 ± 3	205 ± 13[a] (− 36 ± 7)	128 ± 12	25.7 ± 1.6	2.20 ± 0.12

The results represent means ± S.E.M. For those parameters where a significant change ([a]: one-sample Student's *t*-tests, $P < P$-value limit = 0.0083) from before to after pretreatment was detected, the Δ-values are indicated in parenthesis below. Significant differences compared to the control group were detected as indicated ([b]: two-sample Student's *t*-tests, $P < P$-value limit = 0.01). *N* = number of rats per group, SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance.

reduced after both reserpine and phentolamine, whereas the same parameters in response to 3,4-diaminopyridine were increased in adrenalectomized rats (Fig. 5). Reserpine and propranolol totally abolished the 4-aminopyridine-induced tachycardia ($P \leq 0.0006$ and NS, two- and one-sample Student's *t*-tests, respectively), and propranolol also reduced ($P = 0.0006$) but did not eliminate the increase in cardiac output ($P = 0.0011$) (Fig. 5). Moreover, in propranolol-treated but not in reserpinised rats, total peripheral vascular resistance was elevated throughout the late phase ($P = 0.0038$ and 0.0004, one- and two-sample Student's *t*-tests, respectively). Reserpine also abolished the late tachycardia in response to 3,4-diaminopyridine (Fig. 5). The late increase in systolic blood pressure was somewhat reduced after phentolamine + L-NAME, and the increase in cardiac output was totally abolished after L-NAME, and reduced after phentolamine + L-NAME (Fig. 6). The heart rate response in the two L-NAME-treated groups was not significantly different from that in the controls but was greater in the phentolamine + L-NAME than in the L-NAME only group (Fig. 6). In L-NAME- but not in phentolamine + L-NAME-treated rats, a significantly augmented total peripheral vascular resistance ($P = 0.0068$) was seen during the late phase ($P = 0.0070$ compared to the control group) (Fig. 6).

L-NAME greatly increased the base deficit measured 25 min after 4-aminopyridine (the change in base excess was -2.78 ± 0.93 and -7.36 ± 0.74 mM in the controls and the two L-NAME groups (pooled), respectively, $P = 0.0014$). A difference was also seen in ΔpH (-0.160 ± 0.018 and -0.234 ± 0.020, respectively, $P = 0.011$), whereas there was no significant difference in ΔP_{CO_2} (2.19 ± 0.23 and 1.85 ± 0.25 kPa) or ΔP_{O_2} (-2.91 ± 0.46 and -2.88 ± 0.89 kPa).

When the response to 4-aminopyridine and 3,4-diaminopyridine in all groups were pooled, significant changes were observed for all parameters during both phases, also for the immediate bradycardia (-7 ± 2 beats/min) and the late reduction in total peripheral vascular resistance (-0.14 ± 0.05 mm Hg/ml/min) ($P \leq 0.004$). During the immediate phase, significant correlations between baseline and response were observed for diastolic blood pressure, heart rate and total peripheral vascular resistance ($r = 0.34$, -0.33 and 0.33, respectively), but only for total peripheral vascular resistance during the late phase ($r = -0.32$) ($P \leq 0.001$). The immediate rise in total peripheral vascular resistance correlated with the immediate increase in systolic and diastolic blood pressures, heart rate, and cardiac output ($r = 0.82$, 0.81, -0.41 and -0.52, respectively), but not with the change in stroke volume (P = NS). During the late response, the heart rate response correlated with the increase in systolic and diastolic blood pressures, stroke volume and cardiac output ($c = 0.43$, 0.44, -0.26 and 0.52), but not the change in total peripheral vascular resistance, whereas the latter was inversely related to the changes in stroke volume and cardiac output ($c = -0.62$ and -0.66, respectively) ($P \leq 0.004$).

3.4. The response to 4-aminopyridine and 3,4-diaminopyridine in isolated aortic rings

In isolated aortic rings, 4-aminopyridine induced a biphasic contractile response (Fig. 7). Both the first and second contractions were concentration-dependent ($P \leq 0.013$) (Fig. 7). A concentration of 4 mM was chosen for further studies, giving 58% and 79% of maximum contraction for the first and second responses, respectively (P = NS for first versus second contraction). Also, 3,4-diaminopyridine (4 mM) induced a biphasic contractile response, the first of which was less than that following 4-aminopyridine, whereas there was no significant difference for the second contractile response (Fig. 8). The response to 4-aminopyridine was reduced in rings from reserpinised

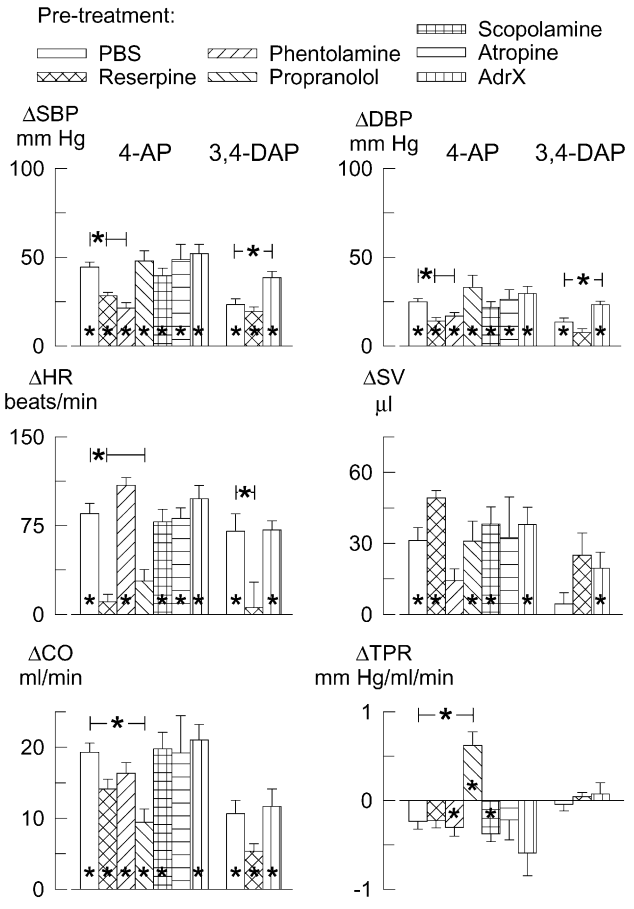

Fig. 5. The role of activation of the autonomic nervous system including the adrenals in the late in vivo response to 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP). The columns show the mean of values recorded at 10, 15, 20 and 25 min. The rats were pretreated as indicated by symbol legends. Significant responses (* within columns, *P*-value limit = 0.0071 and 0.0167 for 4-aminopyridine and 3,4-diaminopyridine, respectively) and differences from the control groups (* in brackets, *P*-value limit = 0.0083 and 0.025 for 4-aminopyridine and 3,4-diaminopyridine, respectively) were located as indicated. The values are means ± S.E.M. SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance, AdrX = adrenalectomy.

rats and after pre-incubation with phentolamine (Fig. 8), whereas phentolamine had no effect on rings from reserpinised rats (not shown). The second contraction was increased 1.3 times in the L-NAME + indomethacin group ($P = 0.01$) (Fig. 8). Propranolol, scopolamine, atropine, L-NAME and indomethacin alone, and ZD1611 had no effect on the response to 4-aminopyridine (Fig. 8). The pre-incubations did not alter tension baseline (data not shown).

3.5. Efficacy of antagonists

The efficacy of reserpine to deplete sympathetic nerve endings of noradrenaline was demonstrated by that the pressor response to tyramine in anaesthetized rats was abolished ($P = 0.0001$) (the change in mean blood pressure was 30 ± 3, 40 ± 3 and 55 ± 4 mm Hg in response to 0.6, 1.2 and 2.3 µmol/kg tyramine in the control group ($n = 8$, $P = 0.0001$), and 0 ± 3, 3 ± 2 and 10 ± 2 mm Hg in reserpinised rats ($n = 5$, P = NS). Also, the heart rate response was eliminated ($P = 0.0001$) (71 ± 9, 111 ± 14 and 103 ± 14 beats/min ($P = 0.0001$) and 2 ± 2, 4 ± 2 and 13 ± 5 beats/min (P = NS) in the control and reserpinised rats, respectively). In 4-aminopyridine-treated rats, the rise in mean blood pressure following 0.12 µmol/kg phenylephrine injected at the end of the experiments was totally eliminated by phentolamine, i.e., 47 ± 4, 3 ± 2

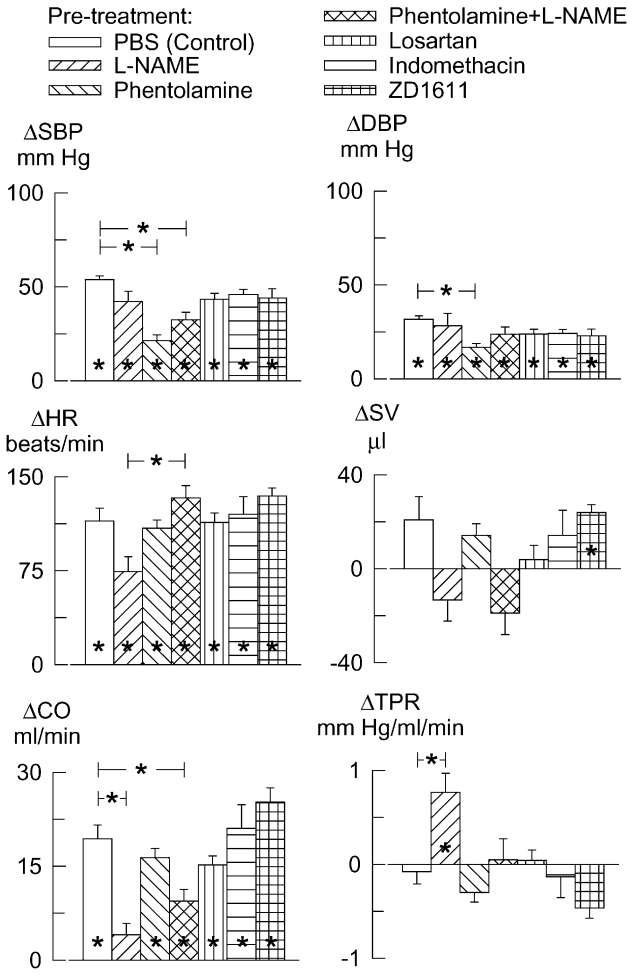

Fig. 6. The role of endothelial derived factors and angiotensin AT_1 receptor in the late in vivo response to 4-aminopyridine in rats pretreated as indicated by symbol legends. The columns show the mean of values recorded at 10, 15, 20 and 25 min. Significant responses (* within columns, *P*-value limit = 0.0083) and differences between the controls and the other groups and between the L-NAME and the phentolamine + L-NAME groups (* in brackets, *P*-value limit = 0.0083) were located as indicated. The values are means ± S.E.M. SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, SV = stroke volume, CO = cardiac output, TPR = total peripheral vascular resistance.

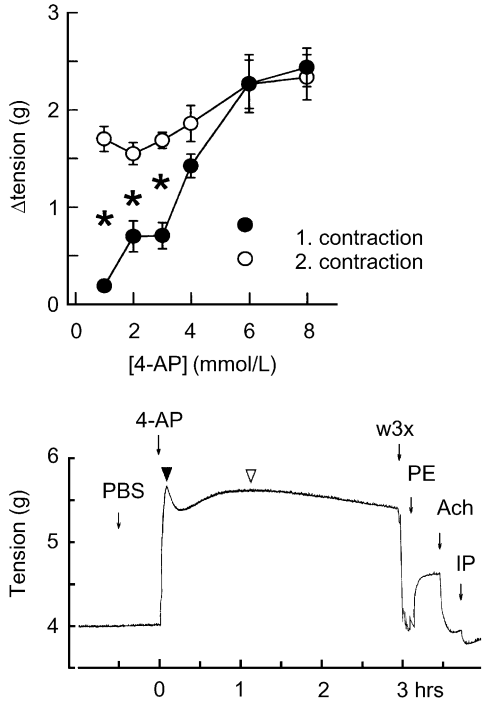

Fig. 7. Upper panel: Concentration–response curves (noncumulative) for the first and second contractile responses to 4-aminopyridine (4-AP) in isolated aortic rings (means ± S.E.M.). Significant differences were located at dose as indicated (*, *P*-value limit = 0.0083, 6–12 rings/dose, eight rats). Lower panel: A typical recording of the response to 4 mM 4-aminopyridine (4-AP). The time when the first (▼) and second (▽) contractions were recorded are indicated. After 3 h, the rings were washed (w3 ×), recontracted with phenylephrine (PE), and endothelial and vascular smooth muscle cell viability was subsequently tested with 1 μM acetylcholine (Ach) and 1.9 μM isoprenaline (IP), respectively. Control rings received vehicle (PBS) prior to 4-aminopyridine.

(P = NS, one-sample Student's t-test) and 40 ± 9 mm Hg in rats pretreated with PBS, phentolamine and propranolol, respectively (P = 0.0001 and NS compared to the PBS group, respectively). The rise in total peripheral vascular resistance was 1.23 ± 0.15 mm Hg/ml/min in the controls compared to −0.13 ± 0.04 and 2.51 ± 0.66 mm Hg/ml/min in the phentolamine and propranolol group, respectively (P = 0.0001 and NS, two-sample Student's t-tests). The efficacy of propranolol was demonstrated by that the fall in mean blood pressure in response to subsequent administration of 0.11 mmol/kg isoprenaline was reduced (P = 0.0001), i.e., −37 ± 2, −30 ± 2 and −14 ± 2 mm Hg in the PBS-, phentolamine- and propranolol-pretreated rats, respectively). The inhibitory effect of scopolamine and atropine in vivo was demonstrated by that both drugs abolished 4-aminopyridine-induced salivation. The efficacy of ZD1611 on endothelin ET_A receptors was verified in rats not given 4-aminopyridine by its ability to reduce the pressor response to 2.4 nmol/kg big endothelin 39 (rat) from 67 ± 3 mm Hg in controls to 26 ± 4 mm Hg in ZD1611-treated rats (P = 0.0072). The efficacy of Losartan was confirmed by that it inhibited the pressor response to 48 nmol/kg angiotensin II, i.e., 89 ± 4 and 7 ± 1 mm Hg, 10 min before and 10 min after Losartan, respectively, P = 0.0001).

The efficacy of the concentration of the antagonists in vitro was verified in rings not exposed to 4-aminopyridine by the following experiments: Tetrodotoxin inhibited the contractile response to 1 μM of the Na^+ channel opener veratridine (0.23 ± 0.05 and 0.01 ± 0.004 g in control and tetrodotoxin-pretreated rings, respectively, P = 0.0001). Phentolamine abolished contraction in response to 0.1 μM phenylephrine (1.7 ± 0.1 and 0.2 ± 0.1 g, respectively, P = 0.0001). In rings precontracted with phenylephrine to about 1.5 g above the 4 g preload tension, propranolol reduced the response to 1.9 μM isoprenaline (−49 ± 4 and −23 ± 2%, without and with propranolol, respectively, P = 0.0001), and scopolamine, atropine and L-NAME abolished the response to 1 μM acetylcholine (−52 ± 5, −1 ± 0.5, −1 ± 1 and −1 ± 2% in controls, scopolamine-, atropine- and L-NAME-treated rings, respectively, P = 0.0001). Moreover, ZD1611 totally abolished maximum constriction in response to endothelin 1, achieved at 10^{-8} M (0.99 ± 0.12 and 0.02 ± 0.01 g, respectively, P = 0.0002).

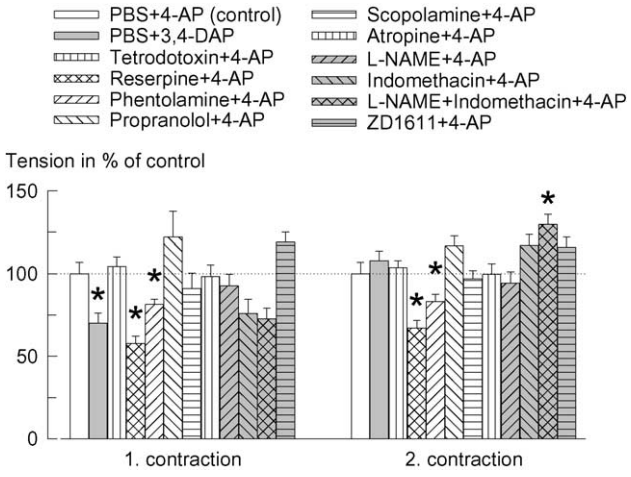

Fig. 8. The contractile response to 4 mM 4-aminopyridine (4-AP) in isolated aortic rings after inhibition of components of the autonomic nervous system or the endothelium as indicated by symbol legends. The 4-aminopyridine-induced contraction was also compared with that following 4 mM 3,4-diaminopyridine (3,4-DAP). The increase in tension in the experimental rings is expressed in percent of that seen in internal control rings (PBS + 4-AP) run within the same experiments. The values represent means ± S.E.M. (8–40 rings from 2 to 16 rats for each group). The response to 4-aminopyridine in all control rings pooled was 1.45 ± 0.04 and 1.47 ± 0.04 g above the 4 g preload for the first and second contractions, respectively (n = 162 rings). Significant effects of pretreatment were located as indicated with two-sample Student's t-tests (*: $P < P$-value limit after Bonferroni adjustment, i.e., 0.0083 for inhibition of components of the autonomic nervous system and 0.0125 for inhibition of endothelial factors).

4. Discussion

4.1. Role of a central stimulatory or peripheral action in the cardiovascular response to 4-aminopyridine

4-Aminopyridine crosses the blood–brain barrier readily (Damsma et al., 1988; Lemeignan et al., 1984), and microinjections of 4-aminopyridine into the rostral ventrolateral medulla have been shown to increase mean blood pressure and heart rate through activation of M_2 muscarinic receptors with a subsequent increase in the sympathetic output (Damsma et al., 1988; Sundaram and Sapru, 1988). In the present experiments, 4-aminopyridine evidently entered the central nervous system, since muscular twitches, caused by a central, cortical stimulatory action (Paskov et al., 1986), were observed in all rats. However, diaminopyridines cross the blood–brain barrier to a lesser extent than the monopyridine 4-aminopyridine, since, after peripheral administration, 2,4-DAP failed to increase central acetylcholine release (Damsma et al., 1988), and the concentration of 3,4-diaminopyridine in the cerebrospinal fluid was much less than that of 4-aminopyridine (Lemeignan et al., 1984). A low passage of 3,4-diaminopyridine across the blood–brain barrier was presently confirmed by that muscular twitches were not at all observed during the 25-min observation period, in spite of that a more ample salivation indicated it to be more efficient in activating peripheral acetylcholine release. Thus, based on the observation that the immediate increase in total peripheral vascular resistance and stroke volume and the late tachycardia in response to 4-aminopyridine did not differ from that following an equimolar dose of 3,4-diaminopyridine, it was concluded that these three responses depended on peripheral mechanisms. This conclusion was also supported by that these parameters were not altered by inhibition of central muscarinic receptors with scopolamine, although some reduction in the immediate blood pressure response was seen. Moreover, the first sign of the convulsive action of 4-aminopyridine was always seen later than the initial pressor peak. In addition, reserpine, which depletes sympathetic nerve endings of noradrenaline and, hence, would abolish effects due to a centrally dependent increase in the sympathetic tone, had no effect on the immediate response. In the isolated aorta, both aminopyridines induced contraction, although a difference in the time course for their action was suggested by that the first but not the second part of the response was less for 3,4-diaminopyridine than 4-aminopyridine. However, this difference may also reflect differences in affinity for different K^+ channels, since a similar difference was observed for 4-aminopyridine concentrations less than 4 mM.

In contrast, a central mechanism appeared to be involved in the late 4-aminopyridine-induced increase in stroke volume since this response was less after 3,4-diaminopyridine. However, this response was not significantly influenced by inhibition of various components of the autonomic nervous system, including adrenalectomy. On the other hand, the late 4-aminopyridine stroke volume response was strongly and positively correlated with P_{CO_2after} and inversely with pH_{after}, and the increase in P_{CO_2} and the fall in pH were greater after 4-aminopyridine than 3,4-diaminopyridine. The augmented stroke volume may therefore be connected to the increased muscular work due to the centrally dependent, convulsive action of 4-aminopyridine, which by increasing the muscle vein pump, may increase the venous return, and, hence, stroke volume.

4.2. The role of peripheral transmitter release, angiotensin AT_1 and endothelin ET_A receptors, and the endothelium in the immediate response to 4-aminopyridine

Neither depletion of sympathetic nerve endings with reserpine, nor inhibition of α- or β-adrenoceptors and peripheral muscarinic receptors, nor adrenalectomy significantly altered the immediate rise in total peripheral vascular resistance or stroke volume following 4-aminopyridine or 3,4-diaminopyridine. Thus, these responses were not caused by release of neuronal noradrenaline or acetylcholine or adrenal catecholamines. This conclusion was also supported by that all responses due to autonomic activation, i.e., the convulsive action of 4-aminopyridine, the adrenergically dependent tachycardia (discussed below) and the atropine- and scopolamine-sensitive salivation, all occurred later in time than the initial pressor response. In addition, a contractile response to the aminopyridines in the isolated aortic rings was only in part explained by release of noradrenaline from vascular wall neurons since reserpine and phentolamine in a non-additive manner reduced the contractile response to 4-aminopyridine by 18–42%. Activation of noradrenaline release was indeed also observed in vivo, but played a role first during the late response (discussed below). These observations were compatible with previous studies showing that phentolamine had no effect on vascular responses to 4-aminopyridine in the cat (Yen et al., 1985) or rabbit (Knot and Nelson, 1995), whereas it halved the initial tension response to 4-aminopyridine in isolated perfused rat lungs (Hasunuma et al., 1991).

A rise in tension due to activation of other vasoconstrictory receptors such as angiotensin AT_1 or endothelin ET_A receptors was also not indicated, since neither the immediate increase in total peripheral vascular resistance nor the in vitro aorta contractile response to 4-aminopyridine were altered after Losartan or ZD1611.

The vasoconstriction was also not due to a 4-aminopyridine-dependent inhibition of endothelial-derived relaxing factor secretion (Chen and Cheung, 1992), since neither L-NAME nor indomethacin reduced the increase in tension. Removal of the endothelium reduced the 4-aminopyridine-induced constrictions in the isolated aorta by about 30%, and a remaining contraction of about 50% was seen after reserpine and phentolamine in denuded as compared to intact rings (data not shown). However, the reduced re-

sponse in denuded rings was not consistent and was paralleled by a similar reduction in the response to subsequent incubation with phenylephrine. It therefore appeared that the reduced response in denuded rings was due to tissue damage introduced by the denudation, although a similar reduction was not seen in tissue without prior incubation with 4-aminopyridine. Still, these in vitro experiments identified vascular smooth muscle cells rather than endothelial cells as the main organ involved in the 4-aminopyridine-induced vasoconstriction. This conclusion is compatible with previous studies on isolated aorta smooth muscle cells (Gomez et al., 2000), where 4-aminopyridine in mM concentrations inhibited I_{K_V} and induced smooth muscle contraction. The I_{K_V} was responsible for the resting tension in the aorta smooth muscle cells. Moreover, the 4-aminopyridine-sensitive I_{K_V} in the aorta demonstrated electrophysiological properties similar to the delayed rectifier in other arteries (Gomez et al., 2000), and 4-aminopyridine in mM concentrations has little effect on vascular smooth muscle K^+ channels other than K_V (Gelband and Hume, 1992; Ishikawa et al., 1993; Robertson and Nelson, 1994; Quayle et al., 1993; Knot et al., 1996; Jackson, 2000). The 4-aminopyridine concentration used in the present in vivo experiments was at most 0.5 mM (calculated for a distribution exclusively in blood, i.e., about 20 ml). It was therefore deduced that the immediate increase in total peripheral vascular resistance in response to the aminopyridines was likely to result from closure of vascular smooth muscle cell K^+ channel(s), active in the control of resting smooth muscle tone. These may belong to the numerous K_V family; however, 4-aminopyridine is highly nonselective, and more selective inhibitors are required to identify the channel type(s) and subtype(s) involved.

The present experiments could not determine if the immediate increase in stroke volume represented an increased cardiac contractility and/or an increase in the venous return: 4-aminopyridine has been shown to have a direct positive inotropic effect on electrically paced rat atria (Northover, 1994). However, 4-aminopyridine has also been shown to constrict veins (Leander et al., 1977), and by that, it may influence the venous return and stroke volume.

4.3. NO and the immediate total peripheral vascular resistance response to 4-aminopyridine

L-NAME greatly increased the 4-aminopyridine-induced immediate rise in total peripheral vascular resistance. A similar increase was not observed in vitro, but the signal for NO release and, hence, the effect of L-NAME, is likely to be less in vitro. The augmented in vivo response did not represent an α-adrenoceptor activation, since it was also seen in rats pretreated in addition with phentolamine. NO synthesis therefore appeared to counter-act the 4-aminopyridine-induced vasoconstriction. However, the augmented response may also reflect a compensatory increase in the vascular smooth muscle cell 4-aminopyridine-sensitive K^+ channel open probability when the NO pathway was eliminated. On the other hand, it may also be due to the great base deficit following the L-NAME-induced peripheral vasoconstriction, since the rise in tension following 4-aminopyridine in the control group was strongly and inversely correlated with ΔP_{O_2}. This observation may suggest that the 4-aminopyridine-sensitive K^+ channel open probability increased when P_{O_2} declined. The effect may possibly be mediated through a decrease in intracellular pH since lowering of the intracellular pH has been shown to activate 4-aminopyridine-sensitive I_{K_V} in isolated coronary vascular smooth muscle cells but reduced I_{K_V} in pulmonary vascular smooth muscle cells (Berger et al., 1998). This tissue-specific regulation of K_V activity is compatible with that hypoxia dilates small arteries in the systemic circulation, contrary to the hypoxia-induced closure of the 4-aminopyridine-sensitive K_V responsible for hypoxic pulmonary vasoconstriction (Coppock et al., 2001).

4.4. Analysis of the mechanisms involved in the late aminopyridine-induced tachycardia

The mechanisms responsible for the late response to the aminopyridines clearly differed from that seen during the immediate phase: The late tachycardia following 4-aminopyridine was totally abolished after reserpine or propranolol, but not by any of the other pretreatments. Reserpine also abolished the tachycardia following 3,4-diaminopyridine. Since a central involvement in the late heart rate response was unlikely as discussed above, these results showed that the tachycardia was due to peripheral noradrenaline release and activation of β-adrenoceptors. This conclusion is compatible with previous studies showing that 4-aminopyridine inhibits presynaptic K^+ channels in peripheral sympathetic nerve endings and by that stimulates the release of noradrenaline (Kirpekar et al., 1977; Leander et al., 1977; Huang and Zhou, 1995). However, the late tachycardia was not significantly altered by inhibition of presynaptic noradrenaline release inhibiting receptors, i.e., α_2-adrenoceptors (phentolamine), muscarinic receptors (atropine) and NO receptors (L-NAME), or noradrenaline release stimulating receptors, i.e., angiotensin AT_1 receptors (Losartan) (Langer, 1997).

Pretreatment with propranolol resulted in an elevated total peripheral vascular resistance during the late phase, evidently dependent on noradrenaline release since it was not present in reserpinised rats. This observation also demonstrated that the inhibitory effect of propranolol on the late tachycardia was due to inhibition of postsynaptic β_1-adrenoceptors rather than inhibition of presynaptic noradrenaline release stimulating β_2-adrenoceptors (Schmidt et al., 1984). Since an elevated late total peripheral vascular resistance was not seen in the controls, it appeared that activation of β-adrenoceptors prevented noradrenaline in concomitantly to cause an α-adrenoceptor-dependent vasoconstriction. However, an elevated late total peripheral

vascular resistance was also seen after L-NAME but not after phentolamine + L-NAME, indicating that the down-regulation of an α-adrenergic vasoconstriction involved NO synthesis. The strong negative correlation between the late changes in total peripheral vascular resistance and stroke volume as well as cardiac output but not heart rate (all groups pooled) suggested that this mechanism was augmented by the late increase in stroke volume, causing a greater stretch of the arteriolar wall during each pulse, and, hence, NO synthesis and counter-action of the α-adrenergic vasoconstriction. A stretch-induced NO release (Franchi-Micheli et al., 2000) is fully compatible with the strong, positive correlation detected between stroke volume but not heart rate prior to L-NAME and the rise in total peripheral vascular resistance in response to L-NAME in the PBS + L-NAME group.

4.5. Conclusion

The present study demonstrated that the K^+ inhibitor 4-aminopyridine elicited a complex cardiovascular response. The response involved an immediate increase in total peripheral vascular resistance, due to a direct action on the vascular smooth muscle cells, compatible with an inhibitory effect on K^+ channels in these cells. A later increase in heart rate resulted from increased noradrenaline release from peripheral sympathetic nerves, most likely due to closure of presynaptic K^+ channels in the nerve terminals. A concomitant increase in total peripheral vascular resistance due to activation of vasoconstrictory α-adrenoceptors in the vascular smooth muscle cells was prevented by NO synthesis. This observation suggested that upon a general sympathetic discharge, a rise in cardiac output was allowed for, while the same cardiac response suppressed a simultaneous peripheral vasoconstriction by stimulating NO synthesis.

Acknowledgements

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