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The vascular response to the K^+ channel inhibitor 4-aminopyridine in hypertensive rats

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Abstract

The K^+ channel inhibitor 4-aminopyridine induced an immediate increase in blood pressure and tension in spontaneously hypertensive rats (SHR). Further analysis strongly suggested this to be due to closure of vascular smooth muscle K^+ channels, as previously concluded for normotensive rats (WKY). The tension response was greater in SHR than WKY, suggesting an increased channel activity in order to compensate for the high total peripheral vascular resistance in SHR. The response was enhanced after nitric oxide (NO) synthase inhibitor in both strains, probably reflecting increased channel activity after elimination of the NO–cGMP pathway. The response in SHR but not WKY was increased after α_1-adrenoceptor inhibition and adrenalectomy but not sympathetic nerve transmitter depletion. It increased also after angiotensin AT_1 and endothelin ET_A receptor antagonists and protein kinase C inhibitor. These results indicated an increased adrenal catecholamine, angiotensin AT_1 and endothelin ET_A activation of the phospholipase C-protein kinase C pathway in SHR, inhibiting the 4-aminopyridine-sensitive K^+ channels.

Keywords: Hypertension; K^+ channel; Sympathetic system; Vascular tension; Angiotensin AT_1 receptor; Endothelin ET_A receptor; Protein kinase C

1. Introduction

Outward-directed K^+ currents induce hyperpolarization, which in turn causes voltage-dependent Ca^{2+} channels to close. In vascular smooth muscle cells, the resulting reduction in intracellular Ca^{2+} will induce relaxation (Waldron and Cole, 1999). Opening of vascular smooth muscle K^+ channels is therefore an important antihypertensive mechanism. In a recent study on anaesthetized, normotensive rats (Berg, 2002), the K^+ channel inhibitor 4-aminopyridine was observed to cause a biphasic pressor response. The first pressor response involved a rise in total peripheral vascular resistance, which further analysis revealed compatible with an effect due to closure of vascular smooth muscle K^+ channels. The second pressor response, however, involved noradrenaline release due to closure of presynaptic K^+ channels in peripheral sympathetic nerves, with subsequent activation of cardiac β-adrenoceptors and tachycardia. A dysfunction in arterial smooth muscle 4-aminopyridine-sensitive K^+ channels has been suggested as the cause of primary pulmonary hypertension, since reduced currents through these channels were demonstrated in pulmonary artery smooth muscle cells from patients with primary but not secondary pulmonary hypertension (Yuan et al., 1998). Little is known about the activity of 4-aminopyridine-sensitive K^+ channels or their role in determining vascular tension in spontaneous hypertension. However, renal arteriolar cells from the spontaneously hypertensive rat (SHR) have been shown to be more depolarized and express less 4-aminopyridine sensitive K^+ current than cells from normotensive rats (Martens and Gelband, 1996). This observation may indicate a decreased expression of these K^+ channels in the SHR, however, the levels of Kv mRNA have been demonstrated to be increased in arteries from SHR (Cox et al., 2001). However, vasoconstrictors such as endothelin and angiotensin II inhibit 4-aminopyridine-sensitive K^+ currents through the formation of protein kinase C (Betts and Kozlowski, 2000; Clement-Chomienne et al., 1996). Also, α_1-adrenoceptors stimulate the phospholipase C–protein kinase C pathway and inhibit outward K^+ currents in arteries (Mistry and Garland, 1999). An excessive inhibition of arterial smooth muscle K^+ currents may contribute to the development of the hypertensive condition.

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The purpose of the present study was therefore first to investigate if 4-aminopyridine induced an immediate pressor response in SHR, similarly to that previously demonstrated in normotensive rats (Berg, 2002), and, by further analysis, to evaluate if this response was due to a rise in total peripheral vascular resistance caused by closure of vascular smooth muscle K^+ channels. When this had been confirmed, the next goal was to evaluate if channel activity, visualized by the immediate tension response to 4-aminopyridine, differed in magnitude between SHR and their normotensive controls and, subsequently, to study if channel activity was differently regulated in the two strains. The data will show that the response to 4-aminopyridine was not incapacitated in SHR, on the contrary, it was greater than in WKY and, in addition, unlike that in WKY, the rise in total peripheral vascular resistance was augmented after inhibition of α_1-adrenoceptors, angiotensin AT_1 and endothelin ET_A receptors, and protein kinase C.

2. Materials and methods

2.1. Animals

Seven- and 12–14-week-old, male SHR (Okamoto, SHR/NHsd strain, $n=195$) and their age-matched, normotensive control (Wistar Kyoto, WKY, $n=48$) were included in the present study in accordance with institutional guidelines and after approval by the institutional ethics committee. The rats were allowed food (conventional rat chow diet, 0.7% NaCl) and water ad lib until the time of the experiments.

2.2. In vivo experiments

Systolic, diastolic and mean arterial blood pressures, heart rate, stroke volume, cardiac output (=minus coronary flow, i.e., ascending aorta flow) and total peripheral vascular resistance were recorded in anaesthetized (65 mg/kg Nembutal, i.p.), artificially ventilated, open-chest rats as previously described (Berg, 2002). Drugs were dissolved in PBS (0.01 M Na-phosphate, pH 7.4, 0.14 M NaCl) and administered as 0.6-ml bolus injections through the femoral vein (unless otherwise indicated). Arterial blood was sampled at the beginning and at the end of the experiment. Arterial P_{CO_2}, P_{O_2}, pH and base excess were measured in an ABL 500 Radiometer (Radiometer Medical, Copenhagen, Denmark).

The cardiovascular response to 34.5 μmol/kg 4-aminopyridine in SHR was compared to that in age-matched WKY. In the 13-week-old SHR, the response to 4-aminopyridine was also compared to that in a time control group, given PBS instead of 4-aminopyridine, and to that of 3,4-diaminopyridine, which unlike 4-aminopyridine does not enter the central nervous system (Damsma et al., 1988; Lemeignan et al., 1984). These rats all received an injection containing PBS 10 min before 4-aminopyridine. To investigate the role of the autonomic nervous system, the response to 4-aminopyridine in the 13-week-old SHR was in the next protocol compared to that seen after pretreatment with (1) reserpine (8 μmol/kg, 2 ml/kg, i.p. −48 and −24 h) to deplete peripheral sympathetic nerve endings of noradrenaline, (2) adrenoceptor antagonists, i.e., phentolamine (α_{1+2}, 6.3 μmol/kg, −10 min), prazosin (α_1, 0.24 μmol/kg, −10 min), yohimbine (α_2, 5 μmol/kg, −10 min) and propranolol (β, 44 μmol/kg, 1.3 ml/kg administered over a 10-min period, −20 min), (3) peripheral (atropine, 6.9 μmol/kg, 2 ml/kg, −20 min) or central (scopolamine, 2.4 μmol/kg, −30 min) muscarinic receptor antagonists and (4) adrenalectomy, performed through flank incisions about 30 min before administration of 4-aminopyridine. Reserpinized and adrenalectomized rats received PBS 10 min before 4-aminopyridine. In the third protocol, to study the role of endothelial-derived factors, the rats were pretreated with (1) a supramaximal dose of nitric oxide (NO) synthase inhibitor (1.1 mmol/kg N^{ϖ}-nitro-L-arginine methyl ester, L-NAME, −30 min) given 10 min after an injection with PBS, (2) L-NAME following a prior injection with phentolamine, (3) cyclooxygenase inhibitor (27.9 μmol/kg indomethacin, −10 min) or (4) 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). In addition, to study the role of angiotensin AT_1 receptors and protein kinase C, SHR were pretreated with losartan (79 μmol/kg, −10 min) and staurosporine (214 nmol/kg, Tanno et al., 2000, −10 min), respectively. Prazosin, yohimbine, adrenalectomy and staurosporine were also given as pretreatment to 13-week-old WKY. The tension response to 4-aminopyridine in 13-week-old WKY pretreated with L-NAME, indomethacin, ZD1611 and losartan were obtained from a previous study (Berg, 2002), performed intermittently with the present. The number of rats per group is shown in Table 1. Antagonist concentrations were as previously established in WKY (Berg, 2002) and inhibitory efficacy was confirmed in SHR (data not shown).

2.3. In vitro experiments

Isometric recording of tension in isolated aortic rings from 51 SHR (12–14 weeks old) were used as previously described (Berg, 2002) to study the response to 4-amino-

Notes to Table 1:
For those parameters where a significant change from before to after pretreatment was detected ($P<0.05$), the changes are indicated in parenthesis below. Differences between the SHR 4-aminopyridine controls (PBS + 4-AP, 13 weeks) and reserpinized and adrenalectomized (AdrX) SHR or WKY (13 weeks) ([a]), between 7-week-old WKY and 13-week-old WKY or 7-week-old SHR ([b]) were located as indicated. Differences between control and adrenalectomized WKY were not detected. N = number of rats per group. [a,b]$P<0.05$.

Table 1
Cardiovascular baselines in 7- and 13-week-old WKY and SHR controls, and after inhibition of components of the autonomic nervous system, endothelial-derived factors, angiotensin AT_1 receptor or protein kinase C, i.e., prior to 4-aminopyridine (4-AP) or 3,4-diaminopyridine (3,4-DAP)

Strain and age	Treatment	*N*	SBP (mm Hg)	DBP (mm Hg)	MBP (mm Hg)	HR (beats/min)	SV (μl)	CO (ml/min)	TPR (mm Hg/ml/min)
WKY 7 weeks	PBS + 4-AP	6	95 ± 3	60 ± 2	72 ± 2	286 ± 11	105 ± 8	29.9 ± 1.9	2.44 ± 0.12
						(− 30 ± 8)			
WKY 13 weeks	PBS + 4-AP	11	77 ± 4[a,b]	48 ± 2[a,b]	57 ± 2[a,b]	240 ± 8[a]	111 ± 8[a]	26.8 ± 2.1[a]	2.22 ± 0.14[a]
						(− 42 ± 6)			
	Prazosin + 4-AP	6	74 ± 3	53 ± 3	60 ± 3	276 ± 10	91 ± 6	25.1 ± 2.2	2.46 ± 0.19
			(− 15 ± 3)	(− 9 ± 3)	(− 11 ± 3)	(− 29 ± 10)			(− 0.24 ± 0.07)
	Yohimbine + 4-AP	6	61 ± 3	37 ± 7	45 ± 3	213 ± 15	118 ± 9	24.9 ± 1.8	1.83 ± 0.13
			(− 28 ± 5)	(− 23 ± 3)	(− 25 ± 4)	(− 71 ± 13)	(19 ± 6)	(− 2.7 ± 0.7)	(− 0.73 ± 0.17)
	AdrX: PBS + 4-AP	7	66 ± 4	44 ± 2	51 ± 3	212 ± 17	94 ± 20	20.1 ± 4.7	3.09 ± 0.46
						(− 22 ± 1)		(− 2.0 ± 0.5)	
	Staurosporine + 4-AP	6	90 ± 10	62 ± 7	72 ± 8	295 ± 23	116 ± 15	33.9 ± 4.6	2.21 ± 0.23
						(− 20 ± 8)	(15 ± 5)		
SHR 7 weeks	PBS + 4-AP	6	71 ± 6[a,b]	51 ± 5[a]	58 ± 5[a]	286 ± 11	37 ± 5[b]	10.3 ± 1.0[a,b]	5.65 ± 0.33[b]
SHR 13 weeks	PBS + PBS	10	102 ± 6	81 ± 5	88 ± 6	306 ± 11	47 ± 4	14.3 ± 1.4	6.47 ± 0.56
							(3 ± 1)		
	PBS + 4-AP	30	96 ± 5	71 ± 4	80 ± 4	289 ± 7	59 ± 4	17.1 ± 1.2	5.11 ± 0.35
	PBS + 3,4-DAP	9	91 ± 8	68 ± 7	76 ± 7	309 ± 10	48 ± 5	14.8 ± 1.9	5.68 ± 0.87
						(− 12 ± 4)			
	Reserpine: PBS + 4-AP	6	90 ± 8	64 ± 6	73 ± 6	267 ± 17	81 ± 8[a]	21.2 ± 1.7	3.47 ± 0.26[a]
						(− 35 ± 5)	(7 ± 2)		
	Phentolamine + 4-AP	6	52 ± 6	37 ± 4	42 ± 4	271 ± 9	39 ± 5	10.5 ± 1.3	4.21 ± 0.52
			(− 44 ± 7)	(− 36 ± 6)	(− 38 ± 6)	(− 26 ± 7)	(− 7 ± 3)	(− 3.2 ± 0.6)	(− 2.09 ± 0.58)
	Prazosin + 4-AP	7	56 ± 4	43 ± 3	47 ± 4	320 ± 12	28 ± 4	8.7 ± 1.0	5.55 ± 0.34
			(− 35 ± 11)	(− 28 ± 10)	(− 30 ± 10)	(− 39 ± 4)	(− 8 ± 2)	(− 3.9 ± 0.6)	
	Yohimbine + 4-AP	6	81 ± 7	63 ± 7	69 ± 6	342 ± 14	37 ± 2	12.8 ± 1.1	5.38 ± 0.38
			(− 16 ± 5)	(− 14 ± 5)	(− 14 ± 5)				(− 1.16 ± 0.21)
	Propranolol + 4-AP	7	85 ± 7	56 ± 4	65 ± 5	150 ± 6	84 ± 13	12.8 ± 2.1	5.76 ± 0.71
				(− 13 ± 4)	(− 10 ± 4)	(− 145 ± 15)	(34 ± 10)		
	Scopolamine + 4-AP	6	85 ± 9	60 ± 6	68 ± 7	222 ± 9	53 ± 5	12.2 ± 1.1	5.63 ± 0.46
						(− 39 ± 6)			
	Atropine + 4-AP	6	84 ± 8	63 ± 8	70 ± 8	281 ± 23	53 ± 8	15.0 ± 1.8	4.97 ± 0.67
	AdrX: PBS + 4-AP	8	78 ± 4[a]	62 ± 4	67 ± 4	306 ± 7	29 ± 2[a]	8.8 ± 0.6[a]	7.95 ± 0.76[a]
			(− 10 ± 2)	(− 9 ± 2)	(− 9 ± 2)	(− 17 ± 4)	(− 3 ± 1)	(− 1.3 ± 0.3)	
	PBS + L-NAME + 4AP	6	152 ± 9	128 ± 8	136 ± 9	285 ± 20	31 ± 4	8.9 ± 1.4	16.47 ± 1.69
			(51 ± 7)	(50 ± 5)	(50 ± 6)	(− 28 ± 9)	(− 14 ± 1)	(− 5.2 ± 0.3)	(10.16 ± 1.10)
	Pha + L-NAME + 4AP	6	85 ± 5	69 ± 4	74 ± 5	276 ± 22	30 ± 2	8.1 ± 0.8	9.51 ± 0.92
			(18 ± 3)	(13 ± 4)	(15 ± 4)	(− 61 ± 6)		(− 5.8 ± 1.3)	(2.45 ± 0.77)
	Indomethacin + 4AP	6	91 ± 5	69 ± 4	77 ± 4	288 ± 16	48 ± 5	13.5 ± 1.3	5.71 ± 0.40
						(− 28 ± 10)			
	ZD1611 + 4AP	6	106 ± 8	83 ± 5	90 ± 6	327 ± 10	36 ± 3	11.7 ± 1.0	7.88 ± 0.57
						(− 27 ± 4)			
	Losartan + 4AP	7	59 ± 3	42 ± 2	48 ± 2	277 ± 9	30 ± 3	8.2 ± 0.7	5.99 ± 0.60
			(− 32 ± 3)	(− 29 ± 3)	(− 29 ± 3)	(− 56 ± 4)	(− 6 ± 1)	(− 3.7 ± 0.2)	
	Staurosporine + 4-AP	6	108 ± 10	82 ± 8	90 ± 9	297 ± 12	45 ± 4	13.4 ± 1.3	6.77 ± 0.05
						(− 29 ± 5)			

pyridine in vitro. The concentration–response curve (1–8 mM 4-aminopyridine, noncumulatively) was established, and a 4 mM concentration and 3-h incubation time was used for further studies. The response to 4 mM 4-aminopyridine in SHR rings was compared to that in rings from six age-matched WKY and in SHR rings to that of an equimolar concentration of 3,4-diaminopyridine, and after inhibition of neural and endothelial factors by using the same antagonists as above. The inhibitory efficacy of these antagonists has previously been established in rings from WKY (Berg, 2002) and was in the present study confirmed in rings from SHR (data not shown).

2.4. Reagents

Scopolamine hydrobromide, isoprenaline sulfate, propranolol hydrochloride and atropine sulfate were from The National Hospital, Oslo, Norway; phentolamine methanesulphonate (Regitine) from Ciba-Geigy, Basel, Switzerland; 3,4-diaminopyridine from MERCK, Schuchardt, Germany. The endothelin ET_A receptor antagonist Zeneca 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

Results are expressed as means ± S.E.M. The in vivo data were averaged every minute throughout the experiments and every seven beats at blood pressure-peak/nadir to aminopyridines or agonists. The data were for each protocol analyzed as overall tests, then between groups and for each group individually by repeated measures. Values before and after pretreatment(s), and immediately, 10, 15, 20 and 25 min after aminopyridine were included. The three SHR control groups did not differ and were pooled. Evaluation of the in vitro response to aminopyridines was carried out by analysis of variance (ANOVA) using the increase in tension expressed in gram force above a 4-g preload. When, these tests indicated the presence of a significant response or group differences; these were located by one- and two-sample Student's *t*-tests, respectively, alternatively by Wilcoxon signed rank and Kruskal–Wallis nonparametric tests to correct for the presence of outlayers. Correlation factors (*r*) were determined with Pearson correlation tests. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Analysis of the components responsible for the in vivo pressor response to 4-aminopyridine in 13-week-old SHR

As previously seen in 13-week-old WKY (Berg, 2002), 4-aminopyridine induced a biphasic pressor response also in 13-week-old SHR, an immediate peak with a 76 ± 8% rise in mean arterial blood pressure after 1.4 ± 0.1 min, followed shortly after by a decline in pressure before reaching a stable plateau phase after about 10 min. The first pressor response was followed by an increase in total peripheral vascular resistance of 39 ± 5% ($P = 0.0001$), whereas a stable tachycardia dominated the second plateau phase, i.e., from 10 to 25 min. The bradycardia (−4 ± 2%, $P = 0.019$) and increase in stroke volume (35 ± 6%, $P = 0.0001$) and cardiac output (28 ± 5%, $P = 0.0001$) during the immediate phase did not differ from that following a sham injection with PBS in the time control group (−1 ± 1%, P = NS; 22 ± 2%, $P = 0.0001$; 22 ± 2%, $P = 0.001$, respectively).

After reserpine, the immediate bradycardia was enhanced (−44 ± 10 and −12 ± 5 beats/min in reserpinized and control SHR, respectively, $P = 0.014$), whereas a reduced pressor response and abolished tachycardia were observed during the late phase ($P \leq 0.005$) (data not shown). However, reserpine had no effect on the immediate increase in total peripheral vascular resistance (Fig. 1).

Also, the peripherally acting 3,4-diaminopyridine induced a biphasic pressor response with an immediate peak after 1.5 ± 0.1 min. The immediate change in systolic (105 ± 14 mm Hg) and diastolic blood pressure (73 ± 9 mm Hg), heart rate (−62 ± 14 beats/min), stroke volume (29 ± 3 μl) and total peripheral vascular resistance (3.51 ± 0.56 mm Hg/ml/min) ($P \leq 0.01$) was greater than that seen after 4-aminopyridine (66 ± 5 and 50 ± 4 mm Hg, −12 ± 5 beats/min, 18 ± 3 μl and 1.97 ± 0.30 mm Hg/ml/min, respectively, $P \leq 0.028$), whereas there was no significant difference in the rise in cardiac output (4.24 ± 0.97 and 3.95 ± 0.57 ml/min, respectively). Muscular twitches, which are due to a central cortical stimulation, were not

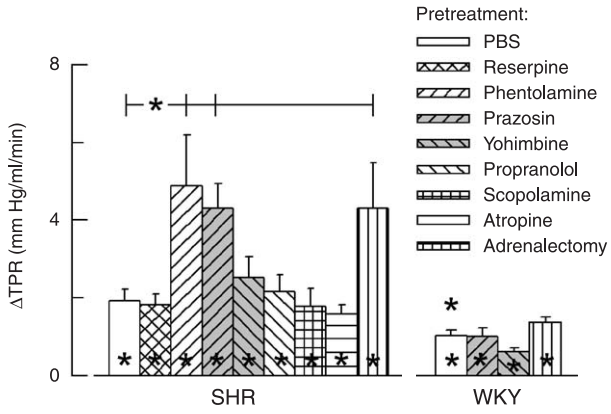

Fig. 1. The immediate in vivo tension response to 4-aminopyridine in 13-week-old SHR and WKY after of inhibition of various components of the autonomic nervous system. The rats were pretreated as indicated by symbol legends. The values represent means ± S.E.M. Significant responses (*within columns) and differences compared to the corresponding PBS controls (*in brackets) were located as indicated. The difference between the SHR and WKY controls is indicated (*above WKY). Baseline values after pretreatment, i.e., prior to aminopyridine, are shown in Table 1. TPR = total peripheral vascular resistance. *$P < 0.05$.

seen in rats given 3,4-diaminopyridine, but were seen in all rats receiving 4-aminopyridine.

3.2. The immediate pressor response to 4-aminopyridine in 7- and 13-week-old SHR compared to that in age-matched WKY

Baseline systolic and diastolic blood pressures were greater in SHR than in WKY in the 13-week-old rats but not in the 7-week-old (Table 1). Stroke volume and cardiac output were less in SHR in both age groups, whereas total peripheral vascular resistance was greater (Table 1). In addition, a greater base deficit was observed in SHR ($P=0.003$, Table 2). Strain-related differences in the pressor response to 4-aminopyridine were not detected at either age (Fig. 2), whereas an age-related increase in the systolic pressure and stroke volume (data not shown) and cardiac output (Fig. 2) response was seen in both strains. The increase in the latter two was markedly less in SHR, hence, the rise in stroke volume (not shown) and cardiac output (Fig. 2) was clearly less in SHR than in WKY in the older rats. Significant group differences were not detected in the immediate heart rate response (-17 ± 5 ($P=0.015$) and 6 ± 6 ($P=$NS) beats/min in the 7-week-old WKY and SHR, respectively, and -10 ± 5 ($P=$NS) and -12 ± 5 ($P=0.015$) beats/min in the 13-week-old WKY and SHR). Furthermore, the rise in total peripheral vascular resistance was about two times greater in SHR than in WKY, whereas age-related differences were not detected (Fig. 2).

In the 13-week-old SHR, the immediate rise in systolic and diastolic blood pressure correlated with the increase in total peripheral vascular resistance ($r=0.50$ and 0.57, respectively, $P=0.001$). The rise in stroke volume was inversely related to the changes in heart rate ($r=-0.84$, $P=0.001$) and total peripheral vascular resistance ($r=-0.36$, $P=0.05$). A correlation between the response to 4-aminopyridine and corresponding baseline was detected only for the increase in total peripheral vascular resistance ($r=0.49$, $P=0.006$). The increase in resistance was positively correlated with P_{O_2} ($r=0.74$ and 0.77 for P_{O_2} at the start and end of the experiment, respectively), whereas the increase in cardiac output was negatively correlated with arterial pH (after) and the fall in P_{O_2} ($r=-0.72$ and -0.78, respectively) ($P\leq0.009$).

3.3. The immediate response to 4-aminopyridine after adrenoceptor or muscarinic receptor antagonists or adrenalectomy in 13-week-old SHR and WKY

Phentolamine, prazosin, yohimbine, propranolol, scopolamine and atropine had no effect on the immediate pressor response to 4-aminopyridine in SHR (data not shown), whereas adrenalectomy augmented the rise in diastolic pressure (67 ± 5 and 50 ± 4 mm Hg in the adrenalectomized and control groups, respectively, $P=0.01$). The bradycardia was significantly eliminated by scopolamine

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

Strain and age	Group	At start					25 min after			
		N	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Base excess (mmol/l)	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Base excess (mmol/l)
WKY 7 weeks	PBS+4-AP	6	7.43 ± 0.03	4.78 ± 0.37	11.87 ± 0.53	− 0.10 ± 0.70	7.29 ± 0.02[a]	7.23 ± 0.35[a]	8.31 ± 0.51[a]	− 1.97 ± 0.87[a]
WKY 12 weeks	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]
SHR 7 weeks	PBS+4-AP	6	7.40 ± 0.04	4.09 ± 0.65	13.69 ± 1.26	− 5.50 ± 0.87	7.19 ± 0.05[a]	7.43 ± 1.01[a]	10.24 ± 1.34[a]	− 9.17 ± 1.24[a]
SHR 12 weeks	PBS+PBS	4	7.51 ± 0.03	3.03 ± 0.36	12.98 ± 1.03	− 2.50 ± 0.51	7.38 ± 0.03[a]	3.44 ± 0.52	13.65 ± 1.67	− 8.45 ± 0.89[a]
	PBS+4-AP	12	7.38 ± 0.03	4.31 ± 0.42	9.98 ± 0.70	− 5.16 ± 0.98	7.25 ± 0.03[a]	4.66 ± 0.61[a]	11.00 ± 1.28	− 11.85 ± 1.55[a]
	AdrX: PBS+4-AP	5	7.41 ± 0.03	3.37 ± 0.36	11.96 ± 1.44	− 6.95 ± 1.25	7.30 ± 0.02[a]	2.65 ± 0.51	15.81 ± 1.59[a]	− 15.61 ± 2.02[a]
	PBS+3,4-DAP	6	7.42 ± 0.05	3.34 ± 0.31	11.58 ± 1.39	− 6.30 ± 1.71	7.39 ± 0.03	2.78 ± 0.36	14.61 ± 1.18[a]	− 10.09 ± 1.72[a]
	PBS+L-NAME+4-AP	6	7.46 ± 0.02	3.57 ± 0.17	10.35 ± 0.95	− 2.93 ± 0.43	7.13 ± 0.04[a]	4.03 ± 0.22[a]	12.57 ± 1.38	− 19.52 ± 2.26[a]
	Phentolamine+L-NAME+4-AP	6	7.48 ± 0.02	3.51 ± 0.36	11.78 ± 1.49	− 2.50 ± 0.58	7.15 ± 0.03[a]	4.24 ± 0.46[a]	14.30 ± 1.60[a]	− 18.43 ± 1.37[a]
	Indomethacin+4-AP	7	7.45 ± 0.02	3.79 ± 0.15	9.88 ± 0.25	− 2.75 ± 0.80	7.28 ± 0.04[a]	5.31 ± 0.36[a]	9.17 ± 0.76	− 8.40 ± 1.70[a]
	ZD1611+4-AP	6	7.49 ± 0.01	3.34 ± 0.12	10.85 ± 0.44	− 2.03 ± 0.53	7.30 ± 0.01[a]	4.40 ± 0.28[a]	10.99 ± 0.51	− 9.53 ± 0.67[a]
	Losartan+4-AP	6	7.46 ± 0.02	3.32 ± 0.21	11.75 ± 0.79	− 4.77 ± 0.77	7.33 ± 0.02	4.13 ± 0.27[a]	11.70 ± 0.73	− 8.54 ± 1.39[a]
	Staurosporine+4-AP	6	7.44 ± 0.04	3.55 ± 0.49	14.16 ± 0.92	− 4.72 ± 1.03	7.34 ± 0.02[a]	4.10 ± 0.21[a]	14.97 ± 1.10	− 5.30 ± 0.51

Significant differences in the blood gas parameters from start to 25 min after 4-aminopyridine or 3,4-diaminopyridine were located as indicated. AdrX = adrenalectomy, N = number of rats per group.
[a] $P<0.05$.

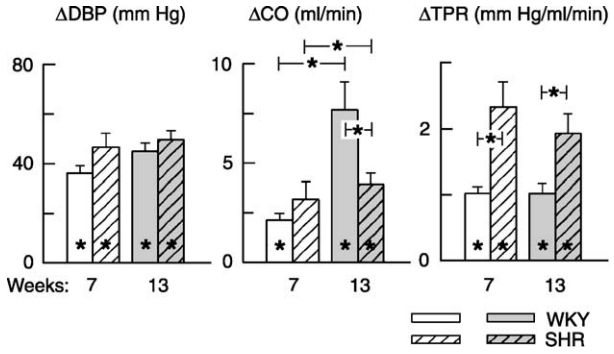

Fig. 2. The immediate in vivo response to 4-aminopyridine in 7- and 13-week-old WKY and SHR. Significant responses (*within symbol) and strain- and age-related differences (*in brackets) were located as indicated. The values represent means ± S.E.M. Baseline values prior to 4-aminopyridine are shown in Table 1. DBP = diastolic blood pressure, CO = cardiac output, TPR = total peripheral vascular resistance. *$P < 0.05$.

(10 ± 6 and − 12 ± 5 beats/min in the SHR scopolamine and control groups, respectively, $P = 0.014$). However, the immediate rise in total peripheral vascular resistance in SHR was clearly augmented after phentolamine, prazosin and adrenalectomy, but was not altered after reserpine as mentioned above, or after yohimbine, propranolol, scopolamine or atropine (Fig. 1). A similar increase in the tension response after prazosin or adrenalectomy was not seen in WKY (Fig. 1).

3.4. The immediate response to 4-aminopyridine after inhibition of endothelial-derived factors, angiotensin AT_1 receptors or protein kinase C in 13-week-old SHR and WKY

In SHR, L-NAME, alone or combined with phentolamine, totally abolished the increase in stroke volume and cardiac output (not shown). L-NAME also clearly enhanced the rise in total peripheral vascular resistance (Fig. 3). An augmented total peripheral vascular resistance response was also seen in SHR after pretreatment with phentolamine + L-NAME, indomethacin, losartan, ZD1611 and staurosporine (Fig. 3). Losartan also eliminated the bradycardia (6 ± 2 and − 12 ± 5 beats/min in the losartan and control groups, respectively, $P = 0.04$). An enhanced tension response was seen in WKY after L-NAME, but not after indomethacin, ZD1611, losartan or staurosporine (Fig. 3).

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

In isolated aortic rings from 13-week-old SHR, 4-aminopyridine induced a dose-dependent ($P < 0.001$), biphasic vasoconstriction, with a first transient peak followed by a second sustained plateau phase. There was no difference between the first and second concentration–tension–response curves in rings from SHR. A close to maximum response was reached with 4 mM (2.06 ± 0.15 and 1.54 ± 0.21 g for the first and second contraction) and the response at this concentration was not significantly different from that using 8 mM (1.65 ± 0.18 and 1.85 ± 0.16 g, respectively). With increased concentrations of 4-aminopyridine, the time to reach the first peak in SHR rings was prolonged, whereas that to the second maximum was achieved more rapidly. At 4 mM, the two peaks were reached after 8 ± 1 and 131 ± 16 min, respectively. When compared to the concentration–response curve in rings from WKY (Berg, 2002), no strain-related difference was detected, except for that the second contraction was 4.5 times greater in WKY rings for the lowest concentration of 4-aminopyridine, i.e., 1 mM (1.70 ± 0.13 and 0.38 ± 0.12 g in WKY and SHR rings, respectively, $P = 0.0001$). No strain-related difference was detected in the tension response to 4 mM 4-aminopridine (Fig. 4).

The response to 4 mM 4-aminopyridine in SHR rings did not differ from that following an equimolar concentration of 3,4-diaminopyridine (Fig. 4). Pre-incubation with phentolamine reduced the second but not the first contractile response to 4 mM 4-aminopyridine by 42% ($P = 0.0001$), whereas none of the other antagonists had any significant effect (Fig. 4). Removal of the endothelium reduced the first and second contraction by 36 and 47%, respectively ($P = 0.0001$). However, a 37% reduction was also seen in the denuded rings in the response to subsequent addition of phenylephrine, i.e., after the 4-aminopyridine-induced contraction had been washed out.

When SHR rings were contracted with 4-aminopyridine to 1.61 ± 0.07 g, almost no relaxation was observed in response to acetylcholine (− 0.07 ± 0.01 g) or subsequent application of isoprenaline (− 0.03 ± 0.004 g) ($P \leq 0.0001$, one-sample Student's *t*-tests). This was in contrast ($P < 0.0001$) to the marked relaxation observed when rings were

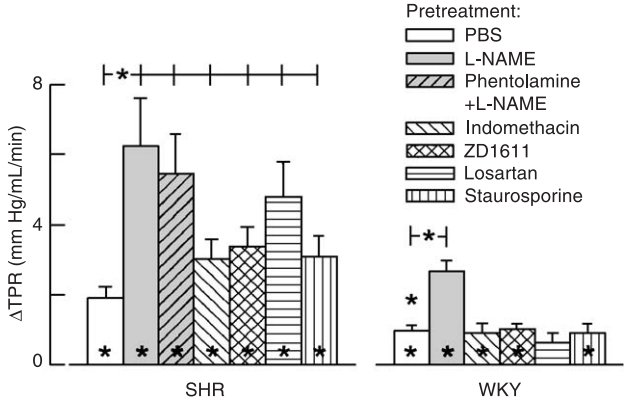

Fig. 3. The immediate in vivo tension response to 4-aminopyridine in 13-week-old SHR and WKY after inhibition of various endothelial-derived factors, angiotensin AT_1 receptors or protein kinase C. The rats were pretreated as indicated by symbol legends. The values represent means ± S.E.M. Significant responses (*within columns) and group differences (*above columns) were located as indicated. The difference between the SHR and WKY controls is indicated (*above WKY). Baseline values after pretreatment, i.e., prior to 4-aminopyridine, are shown in Table 1. TPR = total peripheral vascular resistance. *$P < 0.05$.

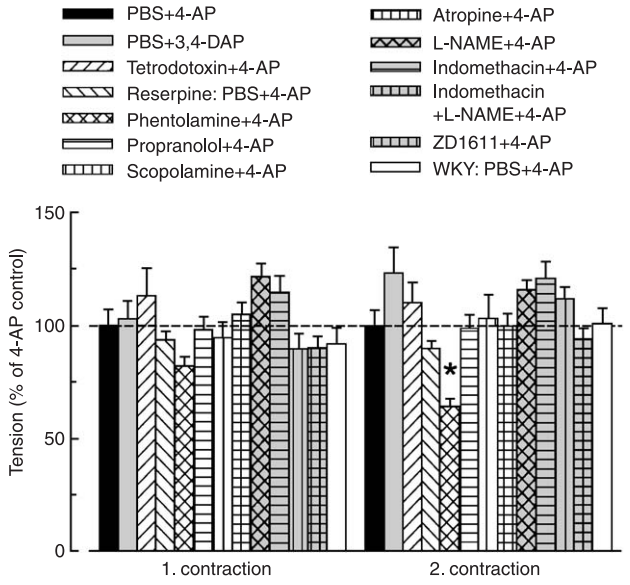

Fig. 4. The first and second contractile response in rings from 13-week-old SHR following 4 mM 4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP), and the effect of inhibition of autonomic nervous system components or endothelial-derived factors on the response to 4-aminopyridine. The figure also shows the response to 4 mM 4-aminopyridine in rings from age-matched WKY. The rings were pretreated as indicated by the symbol legends. The following antagonist concentrations were used: 1 μM tetrodotoxin (TTX), 5 μM phentolamine, 1 μM propranolol, 5 μM scopolamine, 10 μM atropine, 100 μM L-NAME, 10 μM indomethacin and 3 μM ZD1611 (30 min pre-incubation). Rings from reserpinized rats were used to evaluate the effect of noradrenaline depletion. The contraction 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. (8–47 rings from 2–13 rats for each group). The response to 4-aminopyridine was significant in all groups ($P \leq 0.001$, not shown). The response to 4-aminopyridine in all SHR control rings pooled was 1.58 ± 0.04 and 1.37 ± 0.03 g above the 4-g preload for the first and second contraction, respectively ($n = 137$ rings from 27 rats). A response significantly different from that in the SHR 4-aminopyridine control rings was located as indicated. *$P < 0.05$.

contracted to 0.98 ± 0.12 g with phenylephrine (− 0.71 ± 0.08 and − 0.21 ± 0.04 g for acetylcholine and isoprenaline, respectively).

4. Discussion

In the first part of the study, the immediate pressor response to 4-aminopyridine in SHR was shown to depend on a rise in total peripheral vascular resistance. This response was apparently due to a peripheral action since an immediate increase in tension was also observed in response to 3,4-diaminopyridine which, unlike 4-aminopyridine, has little ability to cross the blood brain barrier (Damsma et al., 1988; Lemeignan et al., 1984). This difference was supported by that muscular twitches, which are caused by a central cortical stimulation (Paskov et al., 1986), were not observed after 3,4-diaminopyridine but were present in all rats given 4-aminopyridine. A role of 4-aminopyridine-induced central acetylcholine release with subsequent increased sympathetic output (Damsma et al., 1988; Varagic et al., 1991; Sundaram and Sapru, 1988) was also contradicted by that the central muscarinic receptor antagonist scopolamine did not reduce the tension response. Furthermore, the increase in tension was not due to activation of peripheral noradrenaline release (Kirpekar et al., 1977; Leander et al., 1977; Huang and Zhou, 1995), since the response to 4-aminopyridine was not reduced after depletion of sympathetic nerve transmitter by reserpine, or after any of the α-adrenoceptor antagonists, i.e., phentolamine, prazosin and yohimbine. It was also not dependent on adrenal catecholamine release since the immediate increase in tension was not reduced by acute adrenalectomy. Furthermore, both aminopyridines induced a biphasic contraction in the isolated aorta, which was not different in rings from reserpinized rats, although the second contraction was in part reduced by phentolamine. This may be due a structural inhibitory effect of phentolamine unrelated to its α-adrenoceptor antagonistic effect as previously demonstrated for K_{ATP} channels (McPherson and Angus, 1989). Hence, a 4-aminopyridine-induced increase in adrenergic activity was not the reason for the early rise in tension in vivo, and a direct effect on resistance vessels was therefore indicated. This effect may result from a reduced release of endothelium-derived relaxing factors such as nitric oxide or prostacyclin due to inhibition of 4-aminopyridine-sensitive K^+ channels in the endothelial cells (Chen and Cheung, 1992). However, this did not seem to be a likely explanation, since L-NAME and indomethacin did not reduce the tension response to 4-aminopyridine in vivo or in vitro. On the other hand, the in vitro contractile response was decreased after removal of the endothelium. However, a similar reduction was also seen in the response to subsequent incubation with phenylephrine, as in WKY (Berg, 2002), and the reduction in the tension response to 4-aminopyridine in denuded rings was likely to be due to tissue damage introduced by the denudation procedure. Furthermore, activation of angiotensin AT_1 or endothelin ET_A receptors was also not responsible for the in vivo tension response to 4-aminopyridine since it was not reduced after losartan or ZD1611. From these results, it was concluded that the immediate increase in peripheral vascular resistance in response to 4-aminopyridine in SHR was likely to be due to closure of K^+ channels in the vascular smooth muscle cells. This conclusion was in full agreement with that previously found in WKY (Berg, 2002).

On the other hand, the immediate aminopyridine-induced minor reduction in heart rate appeared to be due to activation of peripheral release of acetylcholine, since it was not observed after muscarinic receptor antagonists, with a significant difference compared to the controls after scopolamine. The bradycardia was significantly greater after 3,4-diaminopyridine than 4-aminopyridine, in accordance with that the former was also more efficient in causing an

atropine-sensitive salivation in SHR (data not shown) and WKY (Berg, 2002), and with other studies showing 3,4-diaminopyridine to be about six to seven times more potent than 4-aminopyridine in inducing transmitter release (Molgo et al., 1980). An aminopyridine-induced muscarinic inhibitory effect on heart rate was further supported by that the bradycardia was clearly increased after the cardiac sympathetic drive had been eliminated by reserpine. Furthermore, bradycardia was also absent after losartan, possibly due to inhibition of presynaptic angiotensin AT_1 receptors, which enhance parasympathetic nerve transmitter release (Du et al., 1998). However, the release of acetylcholine did not appear to have any effect on the immediate tension response, since neither atropine nor scopolamine altered the immediate rise in total peripheral vascular resistance.

When the immediate response to 4-aminopyridine in SHR was compared to that in WKY, differences were detected both in the magnitude of the response as well as in the factors influencing it. In agreement with previous in vitro studies on rat aorta (Gomez et al., 2000), there was no age-related difference in the 4-aminopyridine-induced rise in total peripheral vascular resistance in either strain. However, the immediate tension response in SHR was found to be clearly greater than that in WKY in both 7- and 13-week-old rats, in agreement with an increased expression in SHR of some but not all subtypes of the Kv1.X family present in arterial tissue (Cox et al., 2001). Furthermore, the rise in tension in SHR was positively correlated with baseline and with P_{O_2}. In WKY (Berg, 2002), it increased with decreases in P_{O_2} during the experiment and was independent of baseline. These findings may suggest that vascular smooth muscle 4-aminpyridine sensitive K^+ channels were activated, directly or indirectly, by a reduction in the oxygen tension in WKY, whereas a higher activity was present in SHR to compensate for the increased peripheral vascular resistance in SHR.

Moreover, the age-related augmented stroke volume and cardiac output response to 4-aminopyridine was clearly less in SHR than in WKY. This difference may be due to the development of cardiac hypertrophy in SHR, since there was no parallel difference in the afterload, based on that there was no age-related difference in resistance baseline or the tension response to 4-aminopyridine.

In both strains, the immediate rise in total peripheral vascular resistance was greatly enhanced after L-NAME. This effect was apparently not due to an L-NAME-dependent increased adrenergic activity (Sander and Victor, 1999) or by an altered tension baseline, since a similar increase was also seen after additional pretreatment with phentolamine in WKY (Berg, 2002) as well as in SHR. The enhanced tension response after L-NAME is likely to reflect increased channel activity as observed after inhibition of the NO–cGMP pathway in rat isolated, small arteries (Sampson et al., 2001). The augmented response may also at least in part be due to that a greater rise in tension response may be permitted when the counteracting vasodilatory NO-synthesis had been eliminated. An increased tension response was detected in SHR, but not in WKY, also after indomethacin, although to a much lesser degree than that after L-NAME. As for L-NAME, this may be due to increased channel activity and/or elimination of a counter-acting vasodilatory arachidonic acid metabolite.

On the other hand, a clear difference between the two strains was detected by the inhibitory action of α_1-adrenoceptors and angiotensin AT_1 and endothelin ET_A receptors on the immediate rise in total peripheral vascular resistance after 4-aminopyridine in SHR but not in WKY. These observations were compatible with that the hypertensive condition in SHR, as well as in man, is associated with an increased adrenergic activity (Lee et al., 1991; Mark, 1996; Rahn et al., 1999; Esler, 1993), and that the hypertension was reversed after angiotensin AT_1 receptor antisense gene therapy in adult SHR (Katovich et al., 1999). The inhibitory action of α_1-adrenoceptors was demonstrated by the augmented tension response in SHR pretreated with phentolamine or prazosin but not yohimbine. An inhibitory, although modest, effect of α_1-adrenoceptors on outward aminopyridine-sensitive K^+ currents has been demonstrated in rabbit mesenteric artery smooth muscle cells (Mistry and Garland, 1999) and the present results suggested this mechanism to be clearly enhanced in SHR. However, the adrenergic inhibitory influence appeared to depend on the adrenal glands rather than peripheral nerves, since an augmented rise in tension was seen after acute adrenalectomy but not after reserpine. Why the 4-aminopyridine tension response was not augmented also after muscarinic receptor antagonists may be explained by that adrenal catecholamine secretion stimulated by extracellular K^+ has been shown to occur independently of muscarinic receptor activation (Kirpekar et al., 1982). The tension response to 4-aminopyridine in SHR, unlike WKY (Berg, 2002), was increased also after ZD1611 and losartan, indicating that the K^+ channels in SHR were also inhibited by endothelin ET_A and angiotensin AT_1 receptor activity. This conclusion is supported by the observed inhibitory action of endothelin through endothelin ET_A receptors on 4-aminopyridine-sensitive K^+ currents in rat renal arterial smooth muscle cells (Betts and Kozlowski, 2000) and that of angiotensin II on 4-aminopyridine-sensitive K^+ currents in rabbit vascular myocytes (Clement-Chomienne et al., 1996). Moreover, the tension response to 4-aminopyridine was also enhanced after staurosporine, which is in agreement with that α_1-adrenoceptors, endothelin ET_A and angiotensin AT_1 receptors all inhibit the outward directed, 4-aminopyridine-sensitive K^+ currents by stimulating the phospholipase C–protein kinase C pathway (Betts and Kozlowski, 2000; Clement-Chomienne et al., 1996; Mistry and Garland, 1999).

The open-state probability of vascular smooth muscle cell 4-aminopyridine sensitive K^+ channels has previously been shown to be stimulated by β-adrenoceptor agonist through protein kininase A in isolated rabbit portal vein myocytes (Aiello et al., 1998). A role of β-adrenoceptors in

maintaining channel activity, as indicated by the tension response to 4-aminopyridine, was not detected, since the response was not reduced by reserpine or propranolol. However, the decrease in tension following the β-adrenoceptor agonist isoproterenol in vitro was blocked by 4-aminopyridine. A role of acetylcholine, which may stimulate the release of endothelial-derived hyperpolarizing factor (Nagao and Vanhoutte, 1991) and cause relaxation in part through 4-aminopyridine-sensitive K^+ channels (Zygmunt et al., 1997), was also not evident, since the muscarinic receptor antagonists influenced neither the tension response to 4-aminopyridine nor baseline. These results were similar to that previously seen in WKY (Berg, 2002).

In conclusion, 4-aminopyridine induced an immediate increase in blood pressure and total peripheral vascular resistance. Further analysis strongly suggested that the increase in tension was due to closure of K^+ channels in the vascular smooth muscle cells. The tension response was markedly augmented in SHR compared to WKY. These results show that the high peripheral resistance in SHR is not due to a failure in the 4-aminopyridine-sensitive K^+ channels, on the contrary, the contribution of channel activity in the tension homeostasis appeared to be elevated, possibly to compensate for the excessive peripheral vasoconstriction. Furthermore, as in WKY, the tension response to 4-aminopyridine was enhanced after L-NAME, most likely due to an increase in K^+ channel activity when the NO–cGMP pathway was interrupted. However, unlike that in WKY, the response in SHR increased after α_1-adrenoadrenoceptor inhibition and adrenalectomy but not reserpine, suggesting an inhibitory action due to adrenal rather than neuronal catecholamines. The tension response was augmented also after angiotensin AT_1 and endothelin ET_A receptor antagonists and protein kinase C inhibitor. These results suggested the presence of an increased agonist activation of the phospholipase C–protein kinase C pathway in SHR, with an inhibitory effect on the vasodilatory, outward directed 4-aminopyridine-sensitive K^+ channels.

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