Systemic and regional haemodynamic interactions between K⁺ channel openers and the sympathetic nervous system in the pithed SHR

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1 The interactions between two K⁺ channel openers, cromakalim and SR 44866 (infused i.v. at equi-hypotensive doses), and the sympathetic nervous system at the systemic and regional (mesentery, kidney, hindlimb) vascular levels were investigated in the pithed spontaneously hypertensive rat (SHR) by use of the pulsed Doppler technique.

2 The two K⁺ channel openers did not affect postsynaptic α_1 - but slightly reduced postsynaptic α_2 -adrenoceptor mediated systemic pressor and regional vasoconstrictor responses.

3 Both drugs significantly decreased the systemic pressor and regional vasoconstrictor responses elicited by spinal cord stimulation. These sympathoinhibitory effects were not homogeneously distributed among the different vascular beds, the decreasing rank order being: mesentery > kidney > hindlimb. Simultaneously, the spinal cord stimulation-induced tachycardia remained unaffected.

4 After treatment with K⁺ channel openers, restoration of initial blood pressure and vascular tone values by infusion of prostaglandin F_{2a} (PGF_{2a}) and vasopressin respectively did not affect and abolished the sympathoinhibitory effects of cromakalim and SR 44866.

5 We conclude that in SHRs the two K⁺ channel openers that we investigated exert similar sympathoinhibitory effects which affect some vascular beds more than others. These effects are not dependent upon the arterial blood pressure level and are most likely prejunctionally located.

Introduction

Activation of K⁺ channels has recently been considered as a very attractive mechanism for the design of new cardiovascular drugs. K⁺ channel openers increase K⁺ conductance towards the extracellular space, promote cellular hyperpolarization and elevate smooth muscle activation threshold. This mechanism is principally involved in the reduction in blood pressure produced by K⁺ channel openers already observed in experimental models (Buckingham *et al.*, 1986; Buckingham, 1988; Cook & Hof, 1988; Cook *et al.*, 1988; Caveno *et al.*, 1989; Richer *et al.*, 1989) and in man (Vanden Burg *et al.*, 1986).

Vascular resistance is under the active control of sympathetic nerve fibres and vascular vasomotor tone has been shown to be principally dependent upon activation of α -adrenoceptors. Various vasodilator drugs such as angiotensin converting enzyme inhibitors, calcium antagonists, ketanserin, etc. have previously been reported to interact differentially with the α -adrenergic sympathetic nervous system. All these drugs exert sympathoinhibitory effects vs α_1 - (ketanserin, Scalbert *et al.*, 1989) or α_2 - (calcium antagonists, Van Zwieten *et al.*, 1986) or α_1 - and α_2 - (converting enzyme inhibitors, Richer *et al.*, 1984) adrenoceptor-mediated systemic and regional vascular responses. These sympathoinhibitory effects may contribute to their vasodilator and antihypertensive properties.

Regarding K⁺ channel openers, *in vitro* experiments have shown that cromakalim inhibits contractile responses to nor-adrenaline in rat aorta (Weir & Weston, 1986) and portal vein (Hamilton *et al.*, 1986) and in rabbit mesenteric artery (Clapham & Wilson, 1986). Moreover, *in vivo* cromakalim inhibits pressor responses to electrical stimulation of the spinal sympathetic outflow in rats (Buckingham, 1988; Shropshire *et al.*, 1989). In this context, the present study was conducted in order (1) to assess whether the previously described

systemic sympathoinhibitory effect of cromakalim also develops at the regional vascular level, (2) if so, to determine whether it affects the different vascular beds homogeneously, (3) to investigate some of the characteristics of this sympathoinhibitory effect, and (4) to assess if the latter is a specific property of cromakalim or if it is also shared by other K⁺ channel openers, e.g. SR 44866, a newly developed drug of this group (Richer *et al.*, 1989; Maruyama *et al.*, 1989).

Methods

Animal preparation

Male SHRs (Iffa Credo Laboratories, L'Arbresle, France) (body weight: 370 ± 3 g, systolic blood pressure: 231 ± 2 mmHg) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p., $0.1 \text{ ml } 100 \text{ g}^{-1}$). The animals were pithed and placed immediately under artificial ventilation (Harvard respirator, model 680). A femoral and a jugular vein were cannulated respectively for agonist (cirazoline, UK-14,304) bolus injections and for K⁺ channel openers or solvent infusions (Harvard perfusor, model 907 A). A carotid artery was cannulated for blood pressure measurement with a transducer (Statham P50) connected to an appropriate preamplifier (Gould Instruments, model 13-4615-10). Heart rate (HR) was derived from the arterial pressure pulse (Gould Instruments Biotach amplifier, model 13-4615-66). Systolic (SAP) and diastolic (DAP) blood pressures as well as HR were continuously recorded (Gould Polygraph, model 6610-06). The animals were bivagotomized and given i.v. atropine (1 mg kg^{-1}) and gallamine (20 mg kg^{-1}). Blood velocity Doppler probes were placed around the upper abdominal aorta, the left renal artery, the superior mesenteric artery and the lower abdominal aorta and were connected to a pulsed Doppler flowmeter (Directional pulsed Doppler, model 545C, University of Iowa, Iowa City, U.S.A.). The velocity values obtained have been demonstrated to be directly and linearly proportional to the corresponding blood flows (BF), respectively cardiac output (CO), renal (RBF), mesenteric (MBF) and hindlimb (HBF)

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blood flows (Hartley & Cole, 1974; Richer *et al.*, 1987). After a 15 min-stabilization period the basal values of all parameters investigated were recorded.

Experimental protocols

The animals were randomly assigned to one of the three following treatment groups: controls, cromakalim and SR 44866. After the end of the stabilization period, an infusion ($0.09 \text{ ml kg}^{-1} \text{ min}^{-1}$) of solvent (controls), of cromakalim ($1 \mu\text{g kg}^{-1} \text{ min}^{-1}$) or of SR 44866 ($0.25 \mu\text{g kg}^{-1} \text{ min}^{-1}$) was started and maintained for 15 min. After stopping the solvent or K^+ channel opener infusion, the values of all parameters investigated were again determined.

Experiment A: systemic and regional vascular reactivity to postsynaptic α_1 -adrenoceptor stimulation Systemic and regional vascular responses to intravenous injections of cirazoline (0.3, 1, 3, $10 \mu\text{g kg}^{-1}$) were assessed in 10–15 animals from each group immediately after the end of the K^+ channel opener or solvent infusion.

Experiment B: systemic and regional vascular reactivity to post-synaptic α_2 -adrenoceptor stimulation Systemic and regional vascular responses to intravenous injections of UK-14,304 (3, 10, 30, 100, $300 \mu\text{g kg}^{-1}$) were assessed in 10–15 animals from each group immediately after the end of the K^+ channel opener or solvent infusion.

Experiments C and D: systemic and regional vascular reactivity to the electrical stimulation of the whole spinal cord Systemic and regional vascular responses were assessed during electrical stimulation of the spinal cord for 20 s at frequencies of 0.25, 0.5, 1, 2 and 4 Hz with a rectangular pulse of 1 ms at maximal voltage (60 V) with a ST Stimulator (Janssen Scientific Instruments, model 198): either immediately after the end of the K^+ channel opener or solvent infusion in 10–15 animals from each group (Experiment C); or after restoration of blood pressure to its pre K^+ channel opener infusion level (Experiment D). This restoration was achieved after cromakalim or SR 44866 infusion, either by a prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) infusion ($0.008 \text{ ml kg}^{-1} \text{ min}^{-1}$, $30 \mu\text{g kg}^{-1} \text{ min}^{-1}$), or by a vasopressin infusion ($0.09 \text{ ml kg}^{-1} \text{ min}^{-1}$, $4 \mu\text{g kg}^{-1} \text{ min}^{-1}$) ($n = 8$ –10).

Drugs

Drugs used were atropine sulphate (Sigma Chemical Co., St-Louis, MO, U.S.A.), cirazoline hydrochloride (Synthelabo, Paris, France), cromakalim (Sanofi Recherche, Montpellier, France), gallamine triethiodide (Rhône-Poulenc Santé, Vitry-sur-Seine, France), pentobarbitone sodium (Abbott, Saint Remy sur Arve, France), prostaglandin $\text{F}_{2\alpha}$ (Upjohn Kalamazoo, MI, U.S.A.), SR 44866 or 6-cyano-2,2-dimethyl-4-[2-oxo-1,2-dihydro-1-pyridyl]-2H-1-benzopyran (Sanofi Recherche, Montpellier, France), UK-14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) tartrate, (Pfizer, Orsay, France) and vasopressin (Sigma Chemical Co., St-Louis, MO, U.S.A.). Doses are expressed in terms of the indicated free base or salt.

Solutions of cromakalim (0.1 mg ml^{-1}) and SR 44866 (0.1 mg ml^{-1}) were prepared in the following solvent: distilled water-PEG 200 (93.75:6.25). These solutions were subsequently diluted with saline (1:10). Control experiments were performed with the active drugs' solvent diluted with saline (1:10).

Calculations and statistical analysis of data

Mean arterial pressure (MAP) was calculated from SAP and DAP as follows:

$$\text{MAP} = \text{DAP} + \frac{\text{SAP} - \text{DAP}}{3}$$

Total peripheral (TPVR) and regional vascular resistances renal (RVR), mesenteric (MVR), hindlimb (HVR) values were calculated as the ratios of MAP to the value (in kHz) of the corresponding Doppler signals (CO, RBF, MBF and HBF, respectively) and expressed in arbitrary units (AU). Reported values are means $\pm$ s.e.mean.

Systemic and regional haemodynamic effects of cromakalim, SR 44866 and solvent Mean baseline values for each investigated parameter (SAP, DAP, MAP, HR, RBF, MBF, HBF, CO, RVR, MVR, HVR, TPVR) were calculated for combined A, B and C experiments in the three treatment groups (controls, cromakalim and SR 44866) and compared by analysis of variance.

For each investigated parameter, percentage variations induced by solvent, cromakalim or SR 44866 were calculated for combined A, B and C experiments in the three treatment groups. Statistical analysis was performed within the control group by Student's paired *t* test or between the three treatment groups by analysis of variance.

Interactions of cromakalim, SR 44866 and solvent with the sympathetic system In experiments A, B, C and D, absolute changes in blood pressure, heart rate, systemic and regional blood flows and vascular resistances induced by each dose of agonist (Experiments A and B) or by each stimulation frequency (Experiments C and D) were determined from the corresponding values observed just after the end of the solvent or K^+ channel opener infusion (Experiments A, B and C) or just after restoration of blood pressure to its pre- K^+ channel opener level by either $\text{PGF}_{2\alpha}$ or vasopressin infusions (Experiment D). Their means were then calculated and compared by analysis of variance.

Results

Table 1 shows the mean basal values of each investigated parameter for combined A, B and C experiments in the three treatment groups. Analysis of variance indicates that for none of these parameters was there a significant difference between the three calculated basal values.

Systemic and regional haemodynamic effects of cromakalim, SR 44866 and solvent infusions in pithed SHR

Table 2 shows the mean percentage changes in SAP, DAP, MAP, HR, RBF, MBF, HBF, CO, RVR, MVR, HVR and TPVR induced by cromakalim, SR 44866 and solvent infusions in combined A, B and C experiments. It appears that the solvent slightly but significantly reduced blood pressure, RBF, MBF, HBF and CO and increased TPVR, MVR and HVR. Cromakalim and SR 44866 also decreased blood pressure significantly and to the same extent, this effect being significantly greater than that of the solvent, but did not affect HR. When compared with the control group, RBF and MBF were significantly lowered by both drugs, CO was significantly decreased by cromakalim but not by SR 44866 and HBF was not significantly affected by either drug. Finally, TPVR and especially HVR were significantly lowered by both drugs whereas RVR and HVR were not significantly modified.

Systemic and regional haemodynamic effects of cirazoline in the pithed SHR and their modifications by cromakalim and SR 44866 (Experiment A)

Figure 1 shows that neither cromakalim nor SR 44866 induced any significant change in cirazoline pressor or vasoconstrictor dose-responses.

Table 1 Baseline values for systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure, heart rate (HR), cardiac output (CO), total peripheral resistance (TPVR), renal (RBF, RVR), mesenteric (MBF, MVR), hindlimb (HBF, HVR) blood flows (BF) and vascular resistances (VR) for combined A, B and C experiments in the three treatment groups (solvent, cromakalim and SR 44866) of pithed SHR

Group	n	Blood pressure (mmHg)			HR (beats min ⁻¹)	RBF	Blood flows (kHz)			CO	Vascular resistances (AU)			
		SAP	DAP	MAP			MBF	HBF	RVR		MVR	HVR	TPVR	
Solvent	38	70.9	42.5	52.0	284.3	2.35	3.34	2.13	3.11	25.2	17.5	28.1	18.0	
SR 44866	45	± 1.3	± 1.3	± 1.3	± 5.7	± 0.14	± 0.16	± 0.13	± 0.15	± 1.6	± 1.7	± 2.1	± 0.8	
		71.3	44.8	53.6	278.8	2.36	3.50	2.11	2.82	27.6	18.5	28.4	21.7	
Cromakalim	45	± 1.4	± 1.1	± 1.2	± 5.3	± 0.15	± 0.20	± 0.14	± 0.16	± 2.1	± 1.4	± 2.1	± 1.6	
		71.0	43.8	52.9	289.0	2.45	3.16	2.14	2.79	24.5	19.8	27.8	21.2	
Anova F ² ₁₂₇		± 1.5	± 1.4	± 1.3	± 6.2	± 0.14	± 0.18	± 0.12	± 0.14	± 1.5	± 1.5	± 1.5	± 1.2	
		0.053	0.744	0.390	0.829	0.061	0.739	0.071	1.309	0.867	0.521	0.024	2.310	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Values are means ± s.e.mean. NS: not significant.
AU: arbitrary units.

Systemic and regional haemodynamic effects of UK-14,304 in the pithed SHR and their modifications by cromakalim and SR 44866 (Experiment B)

The two K⁺ channel openers induced minor changes in the UK-14,304 pressor or vasoconstrictor responses. Figure 2 shows that only UK-14,304-induced increases in MVR and HVR were significantly reduced by both drugs.

Systemic and regional haemodynamic effects of electrical stimulation of the spinal cord in the pithed SHR and their modifications by cromakalim and SR 44866 (Experiment C)

Figure 3 shows that the hypertensive responses to electrical stimulation were significantly depressed by both K⁺ channel openers at all frequencies and to the same extent. The cardiac output increases elicited by electrical stimulation were also

reduced by both drugs and significantly so at low stimulation frequencies. In contrast, the positive chronotropic and the systemic vasoconstrictor responses (TPVR increases) elicited were not significantly modified by either drug. Similarly, regional blood flow increases were not modified but regional vasoconstrictor responses in the mesentery, in the kidney and in the hindlimb were significantly decreased by both drugs (Figure 4). This decrease was not homogeneously distributed in the three investigated vascular beds, the drugs preferentially affecting the mesentery (cromakalim: -86%, SR 44866: -91%), followed by the kidney (cromakalim: -67%, SR 44866: -42%) and finally the hindlimb bed (cromakalim: -44%, SR 44866: -37%).

Table 3 shows MAP and HVR absolute values before (basal values) starting K⁺ channel openers infusions, after the end of these infusions and after restoration of MAP to its

Figure 1 Absolute variations (s.e.mean shown by vertical bars) of mean arterial pressure (MAP, mmHg) and peripheral (TPVR), renal (RVR), mesenteric (MVR) and hindlimb (HVR) vascular resistances (arbitrary units) induced by increasing doses of cirazoline in pithed SHR pretreated either with cromakalim (□) or with SR 44866 (●) or with the solvent (○).

Figure 2 Absolute variations (s.e.mean shown by vertical bars) of mean arterial pressure (MAP, mmHg) and peripheral (TPVR), renal (RVR), mesenteric (MVR) and hindlimb (HVR) vascular resistances (arbitrary units) induced by increasing doses of UK-14,304 in pithed SHR pretreated either with cromakalim (□) or with SR 44866 (●) or with the solvent (○).

* Value significantly different from corresponding value in the solvent pretreated group: *P* at least <0.05.

Table 2 Percentage variations of systolic (SAP), diastolic (DAP), mean (MAP) arterial blood pressure, heart rate (HR), cardiac output (CO), total peripheral resistance (TPVR), renal (RBF, RVR), mesenteric (MBF, MVR) and hindlimb (HBF, HVR) blood flows (BF) and vascular resistances (VR) induced by cromakalim, SR 44866 or solvent infusion for combined A, B and C experiments in pithed SHR

Treatment	n	SAP	DAP	MAP	HR	Blood flows (BF)			Vascular resistances (VR)				
						RBF	MBF	HBF	CO	RVR	MVR	HVR	TPVR
Solvent	38	-5.4*** ± 1.0	-3.9* ± 1.6	-4.7*** ± 1.2	0.7 ± 0.7	-6.9*** ± 1.9	-10.8*** ± 2.1	-10.9*** ± 2.0	-12.8*** ± 1.6	3.9 ± 2.3	8.6*** ± 2.4	9.3** ± 3.0	10.4*** ± 2.1
SR 44866	45	-15.0 ^c ± 0.9	-20.1 ^c ± 1.3	-17.9 ^c ± 1.0	0.5 ± 0.8	-14.7* ± 2.0	-22.9 ^c ± 1.9	-9.9 ± 2.5	-14.9 ± 1.4	-1.6 ± 2.3	9.0 ± 2.7	-6.0 ^c ± 2.9	-2.5 ^c ± 1.7
Cromakalim	45	-17.5 ± 1.0	-25.0 ^{c,d} ± 1.5	-21.7 ^{c,d} ± 1.2	-0.7 ± 0.8	-19.0 ^c ± 2.2	-25.6 ^c ± 1.9	-12.9 ± 2.5	-19.6 ^{b,d} ± 1.4	0.6 ± 3.5	7.1 ± 3.1	-7.5 ^c ± 2.7	-1.8 ^c ± 1.8
Anova F _{1,27}		40.666 S 1%	53.877 S 1%	57.303 S 1%	0.850 NS	9.118 S 1%	15.181 S 1%	0.400 NS	5.780 S 1%	0.909 NS	0.138 NS	10.199 S 1%	14.431 S 1%

Values are means ± s.e.mean. S: significant, NS: not significant.

Significant % variation (Student's paired *t* test: * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001).

% variation significantly different from that observed in solvent group (ANOVA): ^a *P* < 0.05; ^b *P* < 0.01; ^c *P* < 0.001.

% variation significantly different from that observed in SR 44866 group (ANOVA): ^d *P* < 0.05.

Figure 3 Absolute variations (s.e.mean shown by vertical bars) of mean arterial pressure (MAP, mmHg), heart rate (HR, beats min⁻¹), cardiac output (CO, kH₂O) and total peripheral vascular resistance (TPVR, arbitrary units) induced by increasing stimulation frequencies of the spinal cord in pithed SHR pretreated either with cromakalim (□) or with SR 44866 (●) or with the solvent (○).

* Value significantly different from corresponding value in the solvent pretreated group: *P* at least <0.05.

basal value by either PGF_{2α} or vasopressin infusion, i.e. just before starting electrical stimulation of the spinal cord sympathetic outflow. Table 3 shows that, whatever the treatment group, the vasoconstrictor drug infusion restored blood pressure to within the control range, indicating that the respective doses of PGF_{2α} and vasopressin were properly adjusted. It

Figure 4 Absolute variations (s.e.mean shown by vertical bars) of renal (RBF, RVR), mesenteric (MBF, MVR) and hindlimb (HBF, HVR) blood flows (BF, kH₂O) and vascular resistances (VR, arbitrary units) induced by increasing stimulation frequencies of the spinal cord in pithed SHR pretreated either with cromakalim (□) or with SR 44866 (●) or with the solvent (○).

* Value significantly different from corresponding value in the solvent pretreated group: *P* at least <0.05.

Figure 5 Absolute variations (s.e.mean shown by vertical bars) of mean arterial pressure (MAP, mmHg, a and c) and hindlimb vascular resistance (HVR, arbitrary units, b and d) induced by increasing stimulation frequencies of the spinal cord in pithed SHR pretreated: (a and b) with cromakalim (■), with cromakalim after prostaglandin F_{2α} (PGF_{2α}, ▲), with cromakalim after vasopressin (△) or with the solvent (○), and (c and d) with SR 44866 (●), with SR 44866 after PGF_{2α} (□), with cromakalim after vasopressin (▽) or with the solvent (○).

* Value significantly different from corresponding value in solvent group: *P* at least <0.05.

† Value significantly different from corresponding value in K⁺ channel opener group: *P* at least <0.05.

also appears that these doses of PGF_{2α} and vasopressin raised hindlimb (Table 3), renal and mesenteric vascular resistances (data not shown) to values higher than those initially observed before K⁺ channel opener infusion.

Figure 5 shows that the sympathoinhibitory effects of cromakalim and SR 44866 were abolished after restoration of MAP to its initial value by vasopressin infusion, the pressor and vasoconstrictor responses evoked by electrical stimulation of the spinal cord being even potentiated.

In contrast, Figure 5 shows that the sympathoinhibitory effects of cromakalim and SR 44866 vs the pressor and vasoconstrictor responses evoked by electrical stimulation of the spinal cord were not modified when restoration of MAP to its initial level was achieved by PGF_{2α} infusion, the magnitude of the pressor and vasoconstrictor responses observed after cromakalim or SR 44866 infusion being decreased to the same extent as in the absence of PGF_{2α}.

Discussion

Few data are available at present regarding the interactions between K⁺ channel openers and the sympathetic nervous system (see Introduction). The main purpose of this study was thus to investigate more thoroughly this problem at the systemic but also at the regional vascular level by use of two different compounds from this group of drugs.

In as much as most of the studies investigating the interactions between antihypertensive drugs of various types and the sympathetic nervous system have been performed previously in SHR (Antonaccio & Kerwin, 1981; Richer *et al.*, 1984; 1985; Buckingham, 1988; Scalbert *et al.*, 1989), this strain was also used in our experiments in order to allow comparisons. In addition, the respective doses of cromakalim and SR 44866 and the protocol design were chosen according to previous results which showed that in normotensive anaesthetized rats, SR 44866 was about four times more potent than cromakalim in lowering blood pressure and that the

Table 3 Absolute values ($\pm$ s.e.mean) of mean arterial pressure (MAP) and hindlimb vascular resistance (HVR) before (basal values), immediately after the end of K^+ channel opener infusion and after restoration of MAP to its pre- K^+ channel opener infusion level either by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or vasopressin infusions

Treatment	Cromakalim		SR 44866	
	MAP	HVR	MAP	HVR
$PGF_{2\alpha}$				
Basal values	50.9 $\pm$ 1.3	30.3 $\pm$ 1.8	49.8 $\pm$ 2.0	30.0 $\pm$ 2.9
After K^+ channel opener	40.2 $\pm$ 1.3 ^c	27.2 $\pm$ 2.0 ^b	39.4 $\pm$ 1.7 ^a	28.3 $\pm$ 3.5 ^a
After K^+ channel opener + $PGF_{2\alpha}$	48.5 $\pm$ 1.6	37.9 $\pm$ 2.7 ^a	51.7 $\pm$ 2.2	32.1 $\pm$ 2.9
Vasopressin				
Basal values	39.7 $\pm$ 1.3	33.7 $\pm$ 4.7	42.4 $\pm$ 2.2	40.1 $\pm$ 5.3
After K^+ channel opener	30.0 $\pm$ 1.6 ^b	27.8 $\pm$ 4.8 ^b	34.5 $\pm$ 3.0 ^a	30.2 $\pm$ 1.7 ^a
After K^+ channel opener + vasopressin	44.9 $\pm$ 3.3	44.3 $\pm$ 9.5	41.1 $\pm$ 2.6	36.7 $\pm$ 2.9

Value significantly different from corresponding basal value (Student's paired *t* test): ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$.

duration of the systemic and regional haemodynamic effects of both drugs exceeded 30 min (Richer *et al.*, 1989).

Our present results confirm in pithed SHR the potency ratio of the two drugs investigated but also show that this experimental model is more sensitive to the K^+ channel openers blood pressure lowering effect than the intact normotensive rat. Thus the doses of cromakalim and SR 44866 required to produce an approximately 20 percent decrease in blood pressure were found to be $1 \mu\text{g kg}^{-1} \text{min}^{-1}$ and $0.25 \mu\text{g kg}^{-1} \text{min}^{-1}$ respectively in the pithed SHR, the corresponding values in the intact normotensive anaesthetized rat being $2.8 \mu\text{g kg}^{-1} \text{min}^{-1}$ and $0.7 \mu\text{g kg}^{-1} \text{min}^{-1}$ respectively (Richer *et al.*, 1989). It is also noteworthy that in the pithed SHR, HR was, as expected, not affected whereas in conscious animals, with a fully operational autonomic nervous system, tachycardia accompanies the blood pressure lowering effect (Buckingham *et al.*, 1986). Finally, in the pithed SHR, the regional vasodilator profiles of the two drugs were similar, confirming their preferential vasodilator effect in skeletal muscle beds (Cook & Hof, 1988; Cavero *et al.*, 1989).

At equihypotensive doses, cromakalim and SR 44866 exhibited a similar pattern of interactions with cirazoline, UK-14,304 and spinal cord stimulation-induced systemic pressor and regional vasoconstrictor effects. This finding indicates that this pattern is not specific to cromakalim but is shared by other K^+ channel openers, suggesting that it is related to the common effects of these drugs on cell membrane K^+ permeability.

The inability of both drugs to oppose pressor and vascular responses to cirazoline, a selective α_1 -postsynaptic adrenoceptor agonist, indicates that they are devoid of any sympathoinhibitory effect versus the systemic and regional vascular responses to postsynaptic α_1 -adrenoceptor stimulation.

Systemic and renal vasoconstrictor responses to UK-14,304, a selective postsynaptic α_2 -adrenoceptor agonist were not significantly modified by either drug but mesenteric and hindlimb vasoconstrictor responses were significantly reduced and to the same extent by cromakalim and SR 44866. These results demonstrate that these two K^+ channel openers develop only weak sympathoinhibitory effects versus the systemic and regional vascular responses to postsynaptic α_2 -adrenoceptor stimulation. Calcium antagonists have previously been shown to reduce the pressor and regional vasoconstrictor effects of selective α_2 -agonists but to decrease only slightly those of selective α_1 -agonists (Van Zwieten *et al.*, 1986). Our data indicate that K^+ channel openers which exhibit no α_1 - and only a weak α_2 -sympathoinhibitory effect interact very similarly to calcium antagonists with the sympathetic nervous system. The mechanism of this interaction could be, as it is for calcium antagonists, a reduction of the penetration of Ca^{2+} into the cell, related to the K^+ channel openers-induced hyperpolarization of the cell membrane, rather than the mobilization of intracellular Ca^{2+} .

In anaesthetized pithed SHRs, cromakalim and SR 44866 both exhibited sympathoinhibitory effects against the systemic pressor and regional vasoconstrictor responses induced by spinal cord stimulation. This finding confirms the observation reported by Buckingham (1988) with cromakalim at the systemic level and extends it not only to the three investigated regional vascular beds but also to another K^+ channel opener, namely SR 44866. Furthermore, our data show that the sympathoinhibitory effects of the two K^+ channel openers do not equally affect the three investigated vascular beds, the decreasing rank order being: mesentery > kidney > hindlimb. The mechanism and significance of this heterogeneity in the K^+ channel openers sympathoinhibitory effects are not yet clear and require further investigation. Previously, converting enzyme inhibitors have also been shown to exert non-homogeneous sympathoinhibitory effects, affecting preferentially the renal bed (Richer *et al.*, 1984). On the other hand, nifedipine and ketanserin have been shown to decrease sympathetic mediated responses homogeneously in the different vascular beds investigated (Richer *et al.*, 1985; Scalbert *et al.*, 1989).

In contrast to the vascular responses, the tachycardia induced by electrical stimulation remained unaffected by the two drugs. Despite this unchanged tachycardic response, the increases in cardiac output elicited by electrical stimulation of the spinal cord were significantly reduced by both drugs. This suggests that the inhibitory effects of K^+ channel openers on the pressor responses to sympathetic nerve stimulation result not only from their action on vascular smooth muscle but also from the reduction in cardiac output they induce. This reduction could possibly be linked either to a decreased venous return or to the intrinsic negative inotropic effect of the drugs (Gotanda *et al.*, 1988).

Responses to endogenous catecholamines released by spinal cord stimulation are due mainly to the activation of postsynaptic α_1 -adrenoceptors (Langer *et al.*, 1981). In as much as the latter were not affected by the two K^+ channel openers used in our experiments, the mechanism of the sympathoinhibitory effects exhibited by the two drugs versus the vascular responses to spinal cord stimulation appears to be pre-rather than postjunctional in its location. However, the precise site where sympathoinhibition develops and the specific involvement of K^+ channels in its genesis remain to be demonstrated. Our experiments also clearly show that K^+ channel openers exert their sympathoinhibitory effects only at the vascular level but not at the sinus node since the tachycardic responses to spinal cord stimulation remained unaffected. A similar phenomenon has previously been reported with converting enzyme inhibitors (Antonaccio & Kerwin, 1981).

It also appears from our experiments that the sympathoinhibitory effects of the two K^+ channel openers versus the responses to electrical stimulation of the spinal cord are not

dependent upon the blood pressure level, as previously postulated (Buckingham, 1988). And indeed, if it is true that the sympathoinhibitory effect of cromakalim (Buckingham, 1988) and also of SR 44866 disappears when blood pressure is restored to pre-drug infusion levels by vasopressin, this is not the case when PGF_{2α} is used as a vasoconstrictor agent. The reason for this discrepancy is not clear but might simply be due to an interaction between the vasoconstrictor agent itself and the sympathetic nervous system. In fact, vasopressin has recently been shown to potentiate markedly vasoconstrictor responses to electrical stimulation and to noradrenaline in rabbits (Patel & Schmid, 1988), a phenomenon that could have blunted the sympathoinhibitory effects of K⁺ channel openers in our and in Buckingham's (1988) experiments.

Finally, the additional fall in both blood pressure and vascular resistance produced by cromakalim and SR 44866 in pithed SHR demonstrates that these two K⁺ channel openers do not require an intact autonomic nervous system in order to

exert their hypotensive action. Thus, the sympathoinhibitory effects of cromakalim and SR 44866 appear not to be involved in the blood pressure decrease. However, the possibility cannot be excluded that they might partly contribute *in vivo* to attenuate some sympathetic vascular reflexes which could limit the vasodilator and/or antihypertensive effects of K⁺ channel openers.

In conclusion, it appears that the two K⁺ channel openers, cromakalim and SR 44866, exert sympathoinhibitory effects in SHRs. These effects, which selectively affect some vascular beds (mesentery > kidney > hindlimb), are not dependent upon the arterial pressure level and their site of action is most probably prejunctionally located.

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