British Journal of Pharmacology (1995) 114, 496–502

Comparisons of the effects of nicorandil, pinacidil, nicardipine and nitroglycerin on coronary vessels in the conscious dog: role of the endothelium

Bijan Ghaleh, *Jean-Luc Dubois-Randé, *Luc Hittinger, [1]Jean-François Giudicelli & *Alain Berdeaux

[1]Département de Pharmacologie, Faculté de Médecine Paris-Sud, 63 rue Gabriel Péri, 94276 - Le Kremlin Bicêtre Cédex, France and *Service de Cardiologie et Institut National de la Santé et de la Recherche Médicale, U400, Hôpital Henri Mondor, Créteil, France

1 The vasodilator properties of nicorandil on large and small coronary arteries were compared to those of nicardipine, pinacidil, nitroglycerin and acetylcholine in six conscious dogs.

2 Intravenous bolus injections of acetylcholine (0.1 $\mu g\,kg^{-1}$), nitroglycerin (0.3–3 $\mu g\,kg^{-1}$), pinacidil (10–100 $\mu g\,kg^{-1}$), nicardipine (3–30 $\mu g\,kg^{-1}$) and nicorandil (10–100 $\mu g\,kg^{-1}$) dose-dependently increased circumflex coronary artery diameter and decreased coronary vascular resistance, indicating vasodilator effects on both conduit and resistance coronary arteries.

3 Three days after removal of the endothelium of the circumflex coronary artery (balloon angioplasty), pinacidil- and nicardipine-induced dilatation of large coronary arteries was greatly reduced (both −76%, $P<0.01$) whereas that produced by nitroglycerin and nicorandil was decreased only slightly and to a similar extent for both drugs (−19%, $P<0.01$ and −28%, $P<0.05$, respectively).

4 Thus in conscious dogs, nicardipine- and pinacidil-induced dilatation of large coronary arteries is endothelium-dependent. In contrast, the vasodilator effects of nitroglycerin and nicorandil on conduit vessels are endothelium-independent.

5 Finally, our results demonstrate that nicorandil dilates the large coronary arteries through its nitrate-like action and that the ATP-potassium channel opening properties of the drug are not involved in this effect in the conscious dog.

Keywords: Nicorandil; potassium channel opener; calcium antagonist; nitroglycerin; coronary arteries; endothelium; conscious dogs

Introduction

It is now well established from both experimental and clinical studies that the endothelium modulates the responses of large and small coronary arteries to vasodilator and vasoconstrictor agents (Young & Vatner, 1986). However, among coronary vasodilators, only nitrate-like derivatives and NO donors clearly exert their vascular relaxant effect, at least at the level of large coronary arteries, through an endothelium-independent mechanism whether this effect has been investigated *in vitro* and *in vivo* (Drieu La Rochelle *et al.*, 1993). In contrast, for non-nitrate coronary vasodilators, there are discrepancies in the literature about the endothelium-dependency or -independency of their coronary effects, especially between the *in vivo* and *in vitro* data. The major factor responsible for these discrepancies may be that *in vivo* an endothelium-dependent dilatation of large coronary arteries develops secondary to the primary arteriolar dilatation and to the subsequent increase in coronary blood flow, a phenomenon that is not observed *in vitro* (Holtz *et al.*, 1983; Hintze & Vatner, 1984; Drieu La Rochelle *et al.*, 1992b). Although the flow-dependent component of the coronary vasodilator effect of a drug is suppressed when coronary flow is kept constant by means of an occluding device, a direct endothelium-dependent component, if present, does persist. The only way to solve the problem is to perform experiments in vessels without endothelium. Recently, we developed an experimental model in which the direct dilator effect of a drug can be investigated at the level of an epicardial coronary artery in the presence of a functional vascular endothelium and after its subsequent removal by balloon angioplasty (Drieu La Rochelle *et al.*, 1992a; Berdeaux *et al.*, 1994). This model was developed in conscious, chronically instrumented dogs to avoid the deleterious effects of general anaesthesia and acute surgery on coronary dynamics.

Among coronary vasodilators, nicorandil, an anti-anginal drug in currrent use, is of special interest because this drug has at least two mechanisms of action. On the one hand, because of its chemical structure, nicorandil can increase guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in vascular smooth muscle cells (Meisheri *et al.*, 1991) through a nitrate-like effect (Sakai *et al.*, 1989). On the other hand, nicorandil can enhance membrane potassium conductance by opening ATP-sensitive potassium (K_{ATP}) channels (Weir & Weston, 1986; Meisheri *et al.*, 1991). However, even though nicorandil has these two mechanisms of action *in vitro*, controversies still exist about how the drug acts *in vivo*, especially regarding the role of the vascular endothelium in the coronary effects of the drug.

Thus, the goal of the present study was to investigate this problem by comparing the coronary vasodilator profile of nicorandil in the presence and in the absence of a functional endothelium at the level of a large epicardial coronary artery in six conscious dogs. The effects of nicorandil were compared in the same six animals to those of other coronary vasodilator drugs acting through different pharmacological mechanisms, i.e., nitroglycerin, pinacidil, nicardipine and acetylcholine.

Methods

Animal preparation

Six adult mongrel dogs weighing 20 to 31 kg, were anaesthetized with sodium pentobarbitone (30 $mg\,kg^{-1}$, i.v.),

[1] Author for correspondence.

intubated and ventilated with a respirator. Under sterile surgical conditions, a left thoracotomy through the fifth intercostal space was performed, and the heart was suspended in a pericardial cradle. Catheters were implanted in the descending thoracic aorta and in the pulmonary artery. A pair of ultrasonic dimension transducers, 5 MHz piezoelectric crystals (VD 5S, Triton Technology, San Diego, CA, U.S.A.), was attached to a Dacron backing and sutured using ethicon 5-0 suture (Ethicon, Inc., Sommersville, NJ, U.S.A.) to opposing surfaces of the left circumflex coronary artery 2 to 4 cm from its origin. Care was taken when positioning the transducers to limit dissection of, and damage to, any visible nerves; proper alignment of the crystals was confirmed during surgery by monitoring the ultrasonic signal with an oscilloscope. In addition, a 10 MHz Doppler flow probe (Crystal Biotech, Hopkinton, MA, U.S.A.) and, downstream of this, a hydraulic occluder (Jones Instruments, Silver Springs, MD, U.S.A.), were implanted distal to the dimension transducers. The pericardium was left partially closed and all wires and catheters were passed subcutaneously to the back of the dog and brought through the skin between the scapulae. The pneumothorax was evacuated through a chest tube inserted in the 6th intercostal space. Cefazolin (1 g) and gentamicin (80 mg) were administered 30 min before incision and at the end of surgery. The animal instrumentation and the ensuing experiments were performed in accordance with the official regulations of the French Ministry of Agriculture.

Measurement of haemodynamic parameters in conscious dogs

Aortic pressure was measured with a Statham P23ID pressure transducer (Statham Instruments, Oxnard, CA, U.S.A.). Left circumflex coronary artery external diameter was measured instantaneously by means of an ultrasonic transit-time dimension gauge with a resolution of ± 0.04 mm (Triton Technology Inc., System 6 model 200, San Diego, CA, U.S.A.). Left circumflex coronary blood flow was measured with a Doppler flowmeter (Triton Technology Inc., System 6 model 200, San Diego, CA, U.S.A.). Left circumflex coronary vascular resistance, which reflects coronary arteriolar tone, was calculated as the ratio of mean arterial pressure to mean coronary blood flow. Data were recorded continuously on a multichannel electrostatic recorder (ES 2000, Gould Instruments Inc., Cleveland, OH, U.S.A.).

Experimental protocol

All experiments were conducted at least 2–3 weeks after the initial surgery, when the dogs were healthy, apyretic and had been trained to lie quietly on their right side on the experimental table. After recording baseline haemodynamic parameters in the conscious state, the dogs received in randomized order and on separate days increasing doses of acetylcholine ($0.1-1\ \mu g\ kg^{-1}$), nitroglycerin ($0.3-3\ \mu g\ kg^{-1}$), nicardipine ($3-30\ \mu g\ kg^{-1}$), pinacidil ($10-100\ \mu g\ kg^{-1}$) and nicorandil ($30-300\ \mu g\ kg^{-1}$) as i.v. bolus injections through the pulmonary artery catheter. In addition, recordings were made of vasodilatation of the circumflex coronary artery during reactive hyperaemia evoked by the release of a 20 s coronary occlusion (Hintze & Vatner, 1984; Hayashi *et al.*, 1990).

As previously described (Drieu La Rochelle *et al.*, 1992a), three days after the last drug administration the dogs were lightly re-anaesthetized with propofol (200 mg, i.v.) and 0.5% halothane. Under aseptic conditions, an incision was made to expose the right carotid artery. An 8 French left coronary guiding catheter (Schneider Climo, Lyon, France) was inserted through the right carotid artery and positioned in the left coronary ostium under fluoroscopic guidance. A balloon angioplasty catheter (Thruflex, Medtronic, Fourmies, France) was inserted through the guiding catheter into the left circumflex coronary artery into the area of the piezoelectric crystals. The balloon was inflated with air and the catheter was moved backwards and forwards 3 times over the entire region between the proximal circumflex coronary artery and the area of the crystals. This procedure causes removal of the endothelium on each side of the crystals as previously demonstrated by histological and pharmacological studies (Berdeaux *et al.*, 1994), leaving the distal circumflex, the left anterior descending and the septal arteries intact. The balloon was then deflated and the catheter withdrawn. Two to three days after endothelium removal, i.e., before any significant endothelial regeneration occurred in this preparation (Hayashi *et al.*, 1988; Berdeaux *et al.*, 1994), the following experiments were performed. Dilatations of left circumflex coronary artery in response to nitroglycerin ($1\ \mu g\ kg^{-1}$), acetylcholine ($0.3\ \mu g\ kg^{-1}$), reactive hyperaemia (20 s), nicorandil ($100\ \mu g\ kg^{-1}$), nicardipine ($30\ \mu g\ kg^{-1}$) and pinacidil ($30\ \mu g\ kg^{-1}$) were assessed again. Because of the long-lasting effects of these drugs, only one dose of each was administered in the described order. The doses of nicardipine, pinacidil and nicorandil were selected as inducing similar non-maximal large coronary artery dilatations. Each drug was administered only when the effects of the previous one had completely disappeared, i.e., when all parameters had returned to their corresponding control values.

Drugs

The drugs used were nicardipine (Laboratoires Sandoz, Rueil-Malmaison, France), pinacidil (Laboratoires Léo, Montigny-le-Bretonneux, France), nitroglycerin (Laboratoires Besins-Iscovesco, Paris, France), acetylcholine hydrochloride (Sigma Chimie, la Verpillère, France) and nicorandil (Laboratoires Bellon, Neuilly sur Seine, France).

Data analysis

Data shown are mean values $\pm$ s.e. All haemodynamic parameters except coronary artery diameter were measured at baseline and at the time of the peak increase in coronary blood flow. Coronary artery diameter was measured before and at the time of its peak increase to a given stimulus. Results are expressed either as absolute or percentage changes from baseline values. Sequential changes of mean values among periods were evaluated by a two-way analysis of variance for repeated measures. When overall differences were detected, individual comparisons were made with a paired t test with Bonferroni's correction. Comparisons between the effects of a treatment before *vs.* after removal of the endothelium were performed on the absolute variations of the investigated parameter using a paired t test. Because of a slight but significant depression of the response to nitroglycerin after removal of the endothelium with a balloon (Hayashi *et al.*, 1988; Drieu La Rochelle *et al.*, 1992a), we calculated an index of large coronary artery dilatation as the ratio of the absolute change in mean coronary artery diameter induced by a given drug to its corresponding absolute change induced by nitroglycerin ($1\ \mu g\ kg^{-1}$). The effect of the procedure for removing the endothelium on the responses of the left circumflex coronary artery to a given drug was then assessed by comparing the ratios calculated before and after endothelium removal by using a paired t test.

These statistical analyses were performed on a PC compatible using BMDP statistical software (BMDP, Los Angeles, U.S.A.). A P value <0.05 was considered as significant.

Results

Coronary vascular responses with intact endothelium

As shown in Table 1 there was no significant difference between the baseline values of the different investigated

Table 1 Values of coronary and systemic haemodynamic parameters at baseline and at the time of peak increase in mean coronary blood flow (CBF) or mean coronary artery diameter (CAD) after acetylcholine, nitroglycerin, pinacidil, nicardipine and nicorandil administrations in the conscious dog with endothelium intact (E+) and after removal of endothelium (E−)

Drug		Heart rate		Mean arterial pressure		Mean coronary blood flow		Mean coronary artery diameter		Mean coronary vascular resistance	
		Baseline	Peak CBF	Baseline	Peak CBF	Baseline	Peak CBF	Baseline	Peak CAD	Baseline	Peak CBF
(μg kg^{-1})		(beats min^{-1})		(mmHg)		(cm s^{-1})		(μm)		(mmHg s cm^{-1})	
Acetylcholine											
0.1	E+	80 ± 5	95 ± 4**	88 ± 3	76 ± 2**	11.2 ± 2.4	19.2 ± 4.2**	2967 ± 248	3048 ± 243**	10.4 ± 3.0	5.0 ± 1.2**
0.3	E+	81 ± 6	104 ± 4**	87 ± 3	76 ± 2**	11.2 ± 2.4	22.7 ± 5.9**	2971 ± 246	3083 ± 249**	10.3 ± 3.0	4.5 ± 1.2**
1.0	E+	80 ± 5	109 ± 6**	86 ± 2	70 ± 2**	11.0 ± 2.4	27.3 ± 6.8**	2968 ± 246	3118 ± 246**	10.3 ± 2.8	3.5 ± 0.9**
0.3	E−	77 ± 5	99 ± 5**	96 ± 3^b	79 ± 2**^{b}	9.8 ± 1.9	22.7 ± 5.3**	3258 ± 286^b	3279 ± 287**^{b}	12.6 ± 3.4	4.4 ± 0.9**
Nitroglycerin											
0.3	E+	75 ± 6	80 ± 7*	88 ± 2	86 ± 2	11.3 ± 2.4	12.5 ± 2.5	2972 ± 246	3145 ± 244**	10.2 ± 2.9	9.4 ± 3.0
1.0	E+	76 ± 7	90 ± 7**	88 ± 2	84 ± 3**	11.3 ± 2.4	15.0 ± 2.4**	2978 ± 245	3179 ± 244**	10.3 ± 2.8	7.0 ± 2.0**
3.0	E+	75 ± 6	103 ± 4**	89 ± 3	80 ± 2**	11.3 ± 2.4	20.2 ± 3.5**	2973 ± 245	3205 ± 246**	10.5 ± 3.0	5.2 ± 1.6**
1.0	E−	79 ± 5	86 ± 6**	95 ± 2^a	89 ± 1**	10.7 ± 2.1	12.5 ± 1.9**^{b}	3243 ± 285^b	3403 ± 287**^{b}	11.7 ± 3.1	8.1 ± 1.3*
Pinacidil											
10	E+	85 ± 3	94 ± 5*	88 ± 3	84 ± 4**	11.0 ± 2.3	16.7 ± 3.5*	2948 ± 255	3013 ± 254**	10.5 ± 2.7	7.2 ± 2.3**
30	E+	84 ± 4	116 ± 9**	89 ± 4	81 ± 4**	11.0 ± 2.2	33.3 ± 6.8**	2949 ± 256	3149 ± 244**	10.3 ± 2.6	3.2 ± 0.9**
100	E+	84 ± 3	139 ± 9**	90 ± 4	74 ± 3**	11.7 ± 2.0	45.8 ± 7.3**	2952 ± 252	3225 ± 232**	9.8 ± 2.8	2.0 ± 0.5**
30	E−	85 ± 5	113 ± 5**	100 ± 6	95 ± 6**	10.8 ± 1.9	24.7 ± 3.3**^{b}	3301 ± 295^b	3345 ± 296*^{b}	11.1 ± 2.4	4.2 ± 0.6**
Nicardipine											
3	E+	82 ± 3	91 ± 5**	91 ± 6	86 ± 3**	11.7 ± 2.2	13.8 ± 2.1	2971 ± 248	3005 ± 250*	9.9 ± 2.9	7.2 ± 1.5**
10	E+	83 ± 4	113 ± 6**	91 ± 5	84 ± 3**	12.3 ± 2.0	19.3 ± 2.8**	2977 ± 249	3077 ± 246**	9.0 ± 2.2	5.0 ± 1.0**
30	E+	82 ± 3	135 ± 4**	96 ± 7	82 ± 4**	11.7 ± 2.3	25.8 ± 5.0**	2968 ± 258	3128 ± 254**	10.8 ± 3.2	4.0 ± 1.0**
30	E−	78 ± 5	129 ± 9**	97 ± 6	83 ± 4**	10.3 ± 1.8	22.5 ± 4.6**	3284 ± 298^b	3323 ± 302*^{b}	11.2 ± 2.5	4.7 ± 1.0**
Nicorandil											
30	E+	75 ± 5	84 ± 3*	89 ± 3	87 ± 4	11.0 ± 2.1	15.2 ± 2.7	2962 ± 245	3058 ± 241**	10.5 ± 3.0	6.9 ± 1.6**
100	E+	72 ± 5	105 ± 7**	94 ± 2	83 ± 4**	11.2 ± 2.3	32.3 ± 5.9**	2982 ± 245	3196 ± 236**	10.9 ± 2.9	3.7 ± 1.4**
300	E+	76 ± 4	133 ± 4**	92 ± 2	80 ± 1**	11.3 ± 2.6	47.7 ± 10.2**	2966 ± 249	3254 ± 240**	10.7 ± 2.9	2.2 ± 0.6**
100	E−	73 ± 5	109 ± 10**	97 ± 3^a	88 ± 3**	9.7 ± 2.0	30.5 ± 7.8**	3268 ± 289^b	3418 ± 291**^{b}	12.9 ± 3.1	4.3 ± 1.3**

Significant ($^aP<0.05$; $^bP<0.01$) vs. corresponding value before removal of endothelium. Significant ($*P<0.05$; $**P<0.01$) vs. baseline.
Values are mean ± s.e.mean.

parameters, measured or calculated before administration of any dose of any of the coronary vasodilators.

All drugs induced dose-dependent decreases in mean arterial pressure and coronary vascular resistance and dose-dependent increases in heart rate, coronary blood flow and coronary artery diameter, except the lowest doses of nitroglycerin (0.3 μg kg^{-1}) and nicorandil (30 μg kg^{-1}) for which the decrease in mean arterial pressure was not significant at the time of the peak increase in coronary blood flow. Furthermore, it must be noted that the tachycardia induced by nicardipine, pinacidil and nicorandil was more marked and of longer duration than that induced by acetylcholine and nitroglycerin, regardless of the dose administered (data not shown).

As shown in Table 1 and Figure 1, all vasodilators induced a dose-dependent decrease in coronary vascular resistance and dose-dependent increases in coronary blood flow and coronary artery diameter, except for nitroglycerin which at the lowest dose (0.3 μg kg^{-1}) selectively increased coronary artery diameter but had no effect on coronary vascular resistance. As shown in Table 2 and illustrated in Figures 2 and 3, the peak decrease in coronary vascular resistance induced by all vasodilator drugs always occurred earlier than the corresponding peak increase in coronary artery diameter. The effects on coronary vascular resistance observed after administration of nitroglycerin and acetylcholine were transient as coronary vascular resistance had returned to its baseline value when the maximal increase in coronary artery diameter occurred. In contrast, the decrease in coronary vascular resistance induced by nicardipine, pinacidil and nicorandil was still present when the peak effects of these drugs on coronary artery diameter occurred.

Coronary vascular responses after endothelium removal

As shown in Table 1, removal of the endothelium *per se* did not significantly affect the baseline values of mean coronary blood flow, mean coronary vascular resistance or heart rate. In contrast, coronary artery diameter was significantly increased by approximately 10%. Baseline values of mean arterial pressure before administration of acetylcholine, nitroglycerin and nicorandil were also slightly increased after endothelium removal. Because the occluder malfunctioned in one dog, responses during reactive hyperaemia were examined in only five dogs. Before endothelium removal, release of a 20 s coronary artery occlusion was associated with a 327 ± 35% increase in coronary blood flow and a delayed increase in coronary artery diameter of 178 ± 25 μm from a control value of 3064 ± 285 μm. After removal of the endothelium, and despite a similar increase in coronary blood flow (361 ± 28%), the increase in coronary artery diameter (14 ± 5 μm from a control value of 3397 ± 348 μm) was reduced by 91% ($P<0.01$) compared with the corresponding value before endothelium removal.

As shown in Figure 4, the increases in circumflex coronary artery diameter evoked by acetylcholine (− 82%, $P<0.001$), nicardipine (− 76%, $P<0.01$) and pinacidil (− 76%, $P<0.01$) were strongly reduced after endothelium removal. In contrast, nitroglycerin and nicorandil were still able to strongly dilate the coronary artery segment without endothelium although the response was significantly reduced relative to that evoked with intact endothelium (− 19%, $P<0.01$ and − 28%, $P<0.05$, respectively). In order to take into account this reduction in nitroglycerin responsiveness after endothelium removal which reflects a partial decrease in the

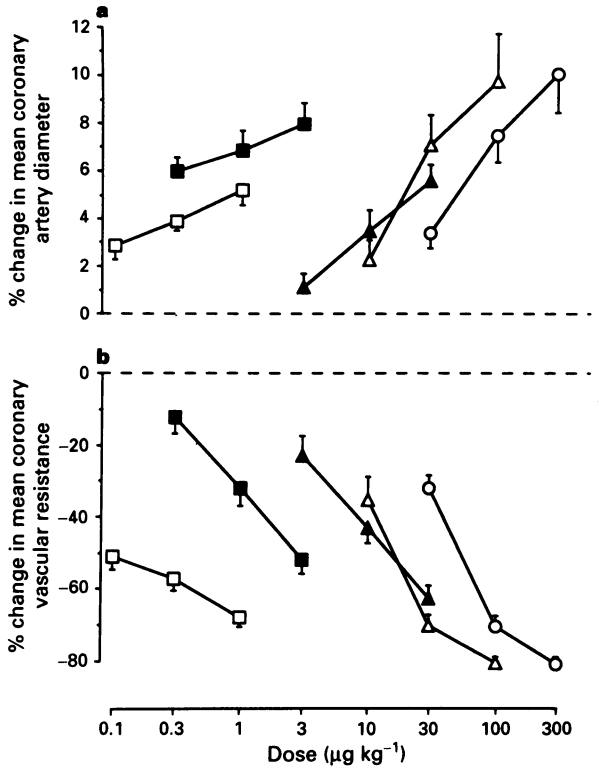

Figure 1 Dose-response curves for the maximal increase in mean coronary artery diameter (a, upper panel) and for the maximal decrease in mean coronary vascular resistance (b, lower panel) induced by increasing doses of acetylcholine (□), nitroglycerin (■), nicardipine (▲), pinacidil (△) and nicorandil (○) in conscious dogs with intact endothelium. Results are expressed as percent changes from the corresponding baseline values.

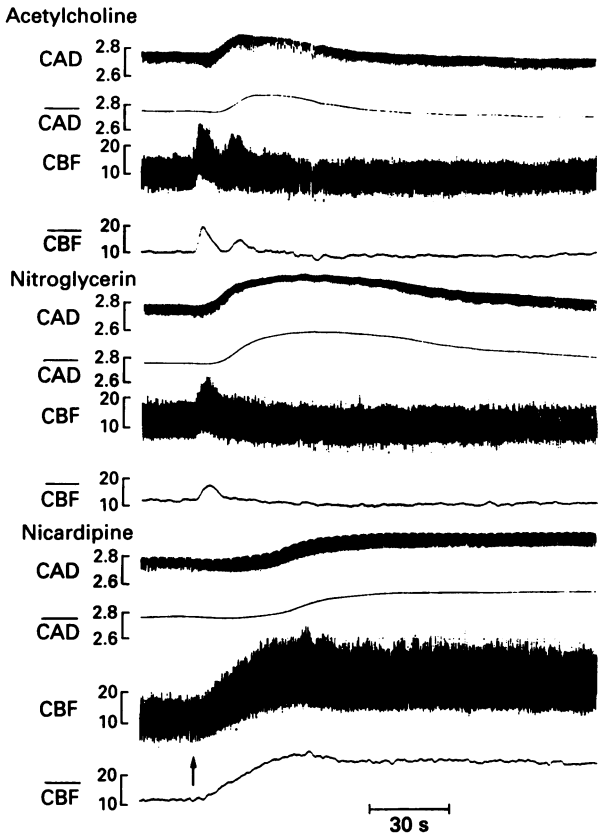

Figure 2 Representative tracings of the effects of the i.v. administration of acetylcholine (0.3 μg kg^{-1}), nitroglycerin (1 μg kg^{-1}) and nicardipine (30 μg kg^{-1}) on phasic and mean coronary artery diameter (CAD and $\overline{CAD}$, mm, respectively) and on phasic and mean coronary blood flow (CBF and $\overline{CBF}$, cm s^{-1}, respectively) in a conscious dog. The drugs were administered as a bolus at the time indicated by the arrow at the bottom of the figure.

reactivity of coronary smooth muscle cells (Drieu La Rochelle *et al.*, 1992a; Berdeaux *et al.*, 1994), we calculated the ratio of the absolute change in coronary artery diameter induced by each vasodilator drug to the corresponding absolute change induced by nitroglycerin (see Methods). Thus, as compared to nitroglycerin, large epicardial coronary artery responses to acetylcholine, nicardipine and pinacidil were still significantly reduced after endothelium removal (Table 3). In contrast, lack of endothelium did not affect the dilator response of the large epicardial coronary artery to nicorandil ($P = 0.29$). Furthermore, both nitroglycerin (1 μg kg^{-1}) and nicorandil (100 μg kg^{-1}) induced similar and non-significantly different increases in coronary artery diameter before (214 ± 29 μm and 201 ± 21 μm, respectively) and after endothelium removal (161 ± 17 μm and 150 ± 25 μm, respectively).

Discussion

The present study conducted in conscious dogs (a) compares the effects on large and small coronary arteries of nicorandil to those of acetylcholine, nitroglycerin, nicardipine and pinacidil, and (b) investigates the role of coronary endothelium in the vasodilator effects of these drugs at the level of the large epicardial coronary arteries.

It appears from our data that all drugs, at the doses investigated, induced a dose-dependent decrease in mean arterial pressure and an increase in heart rate. These results are in agreement with those of Cox *et al.* (1983) with acetylcholine and of Zhang *et al.* (1993) with nitroglycerin who showed that these drugs induce a transient tachycardia and a fall in mean arterial pressure. The positive chronotropic effects observed following administration of both potassium

Table 2 Values of the times (s) to peak decrease in coronary vascular resistance and increase in coronary artery diameter following administration of acetylcholine, nitroglycerin, pinacidil, nicardipine or nicorandil in the conscious dog with intact coronary endothelium

Drug (μg kg^{-1})	*Time (s) to peak* *Coronary vascular resistance*	*Coronary artery diameter*
Acetylcholine		
0.1	12 ± 1	30 ± 1
0.3	13 ± 1	32 ± 1
1	14 ± 1	40 ± 3
Nitroglycerin		
0.3	11 ± 2	48 ± 2
1	11 ± 2	54 ± 4
3	12 ± 2	65 ± 4
Pinacidil		
10	43 ± 2	73 ± 5
30	45 ± 4	108 ± 9
100	90 ± 15	267 ± 35
Nicardipine		
3	26 ± 4	53 ± 8
10	39 ± 3	83 ± 5
30	44 ± 6	175 ± 17
Nicorandil		
30	29 ± 2	64 ± 8
100	32 ± 3	117 ± 9
300	56 ± 8	265 ± 22

Values are mean ± s.e.mean.

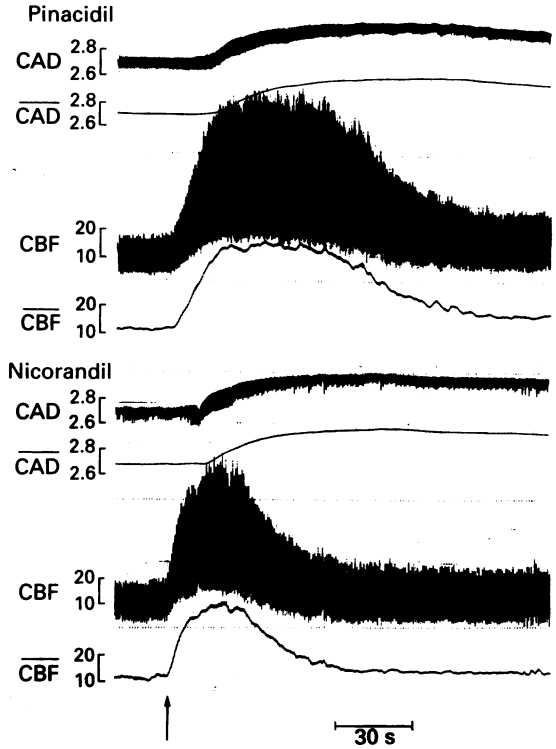

Figure 3 Representative tracings of the effects of the i.v. administration of pinacidil (30 $\mu g\,kg^{-1}$) and nicorandil (100 $\mu g\,kg^{-1}$) on phasic and mean coronary artery diameter (CAD and $\overline{CAD}$, mm, respectively) and on phasic and mean coronary blood flow (CBF and $\overline{CBF}$, $cm\,s^{-1}$, respectively) in a conscious dog. The drugs were administered as a bolus at the time indicated by the arrow at the bottom of the figure.

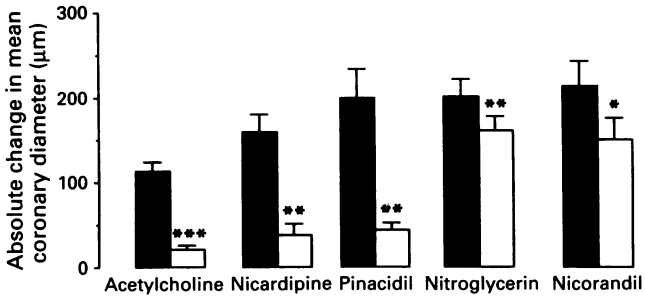

Figure 4 Peak increases in mean coronary artery diameter after acetylcholine (0.3 $\mu g\,kg^{-1}$), nicardipine (30 $\mu g\,kg^{-1}$), pinacidil (30 $\mu g\,kg^{-1}$), nitroglycerin (1 $\mu g\,kg^{-1}$) and nicorandil (100 $\mu g\,kg^{-1}$) administrations in conscious dogs before (solid bars) and after (open bars) endothelium removal. *, **, ***: significant ($P<0.05$; $P<0.01$; $P<0.001$) *vs.* before endothelium removal.

Table 3 Values of the ratios of absolute change in mean coronary artery diameter induced by reactive hyperaemia or a drug to the corresponding change induced by nitroglycerin (see Methods) calculated before and after coronary endothelium removal following reactive hyperaemia and administration of acetylcholine, pinacidil, nicardipine and nicorandil in the conscious dog

	With endothelium	*Without endothelium*
Reactive hyperaemia	0.82 ± 0.02	0.09 ± 0.04[a]
Acetylcholine 0.3 $\mu g\,kg^{-1}$	0.58 ± 0.07	0.14 ± 0.04[b]
Pinacidil 30 $\mu g\,kg^{-1}$	0.97 ± 0.09	0.27 ± 0.04[b]
Nicardipine 30 $\mu g\,kg^{-1}$	0.80 ± 0.09	0.25 ± 0.05[a]
Nicorandil 100 $\mu g\,kg^{-1}$	1.06 ± 0.09	0.92 ± 0.09

Value are mean ± s.e.mean.
Significant ([a]$P<0.01$; [b]$P<0.001$) vs. before removal of endothelium.

channel openers and calcium antagonists (McLeay *et al.*, 1983; Kawashima & Liang, 1985; Buckingham *et al.*, 1986) and observed here with nicardipine and pinacidil are reflexly mediated through unloading of the baroreceptors. Nicorandil also decreased blood pressure and increased heart rate. This tachycardia also most probably results from a compensatory increase in sympathetic tone elicited by hypotension since it is not observed in patients with congestive heart failure (Galié *et al.*, 1990), in whom the baroreceptor reflex is desensitized.

Our study confirms that nicorandil and the other investigated vasodilators dose-dependently dilate the large, as well as the small, coronary arteries (Vatner *et al.*, 1980; Hintze & Vatner, 1983; Cox *et al.*, 1983; Preuss *et al.*, 1985; Giudicelli *et al.*, 1990). However, although all these drugs act on the coronary vasculature, there are clearly some differences in the kinetics of their coronary effects. Thus, the peak decrease in coronary vascular resistance induced by nicardipine, pinacidil and nicorandil occurred later than that induced by acetylcholine and nitroglycerin. At the level of small coronary arteries, the effects on coronary blood flow following administration of both acetylcholine and nitroglycerin were transient and were no longer present at the time of peak increase in coronary artery diameter. In contrast, nicardipine, as well as both pinacidil and nicardipine, elicited a substantial and prolonged increase in coronary blood flow which was still present at the time of the peak increase in coronary artery diameter. It is not likely that the prolonged effect of nicorandil on small coronary arteries was due to a slow release of NO. Shen & Vatner (1993) have demonstrated that both the time to peak coronary vasodilatation as well as the duration of vasodilatation induced by lemakalim, a K_{ATP} channel opener, are significantly longer than those induced by nifedipine, acetylcholine and nitroglycerin. By using glibenclamide, Miwa *et al.* (1993) showed that the K_{ATP} channel opening properties of nicorandil are of major importance in the small endocardial vessels. Furthermore, glibenclamide has been reported to block the changes in coronary blood flow and coronary vascular resistance induced by nicorandil in anaesthetized dogs (Ogawa *et al.*, 1992). Thus, it is extremely likely that the dilatation of the small coronary arteries which nicorandil induced in our study was related to the drug's K_{ATP} channel opening properties.

Because an increase in coronary blood flow can trigger dilatation of large coronary arteries through an endothelium-dependent mechanism (Holtz *et al.*, 1983), we compared the responses of the large coronary arteries to the five investigated coronary dilators before and after endothelium removal by using a balloon angioplasty technique. As we showed previously (Berdeaux *et al.*, 1994), this procedure results in a local endothelium removal in the circumflex coronary artery at the site of attachment of the crystals. This is supported by the fact that coronary dilatation induced by reactive hyperaemia (flow-dependent dilatation) and acetylcholine (endothelium-dependent dilatation) were almost abolished, confirming the absence of functional coronary endothelium, in agreement with previous studies performed under similar experimental conditions (Inoue *et al.*, 1988; Hayashi *et al.*, 1988; Weidinger *et al.*, 1990). Our post-endothelium removal experiments were performed 2–3 days after angioplasty, when no functional regeneration of the endothelium has occurred; recovery of normal responses to removal of vascular occlusion, acetylcholine (Hayashi *et al.*, 1988) and treadmill exercise (Berdeaux *et al.*, 1994) requires about 9 days after endothelium removal. The observation that endothelium removal induced a significant increase in basal coronary artery diameter can be accounted for, as previously described, by a passive distension of the blood vessel during balloon inflation, rather than by the lack of endothelium *per se* (Berdeaux *et al.*, 1994).

Our experiments clearly showed that endothelium removal strongly reduced the dilatation of large coronary arteries induced by pinacidil, confirming our previous data which

indicated that potassium channel openers dilate large coronary arteries mainly through an endothelium-dependent mechanism (Drieu La Rochelle *et al.*, 1992a). More precisely, their mechanism of action seems to be mainly flow-dependent as increases in circumflex coronary diameter were no longer observed when coronary blood flow was maintained constant using a cuff occluder (Giudicelli *et al.*, 1990). Similarly, the nicardipine-induced increase in coronary artery diameter was almost completely abolished after endothelium removal. This result is in agreement with previous studies performed at constant coronary blood flow which showed that the dilatations of large coronary arteries induced by other calcium entry blockers such as nifedipine, verapamil and diltiazem are partly flow-dependent (Holtz *et al.*, 1983; Drieu La Rochelle *et al.*, 1992b). Thus it seems reasonable to conclude that the potassium channel opener, pinacidil, and the calcium antagonist, nicardipine, preferentially dilate coronary arterioles and secondarily dilate large coronary arteries through an indirect endothelium-dependent mechanism, which is most likely flow-dependent.

In contrast, the dilatation of large coronary arteries induced by nitroglycerin and nicorandil was only slightly decreased after endothelium removal, an effect previously described with nitroglycerin (Hayashi *et al.*, 1988; Drieu La Rochelle *et al.*, 1992a; Berdeaux *et al.*, 1994). As the response to organic nitrates is a widely accepted index of smooth muscle integrity after *in vivo* endothelium removal (Chu & Cobb, 1987; Hayashi *et al.*, 1988; Inoue *et al.*, 1988), the slight reduction in the nitroglycerin-induced responses probably reflects a small angioplasty-induced alteration of smooth muscle cells. Taking into account this alteration by calculation of a dilatation index (see Methods), our data show that the nicorandil-induced vasodilatation of large coronary arteries is endothelium-independent (Table 3). As potassium channel openers have been shown to dilate these vessels through an endothelium-dependent mechanism (see above), it seems likely that the nicorandil-induced dilatation of the large epicardial coronary arteries occurs through the drug's nitrate-like properties and that its K_{ATP} channel opening activity is not involved in this effect. This result is in agreement with a previous study reporting that the spasmolytic effect of nicorandil (Imagawa *et al.*, 1992) is not modified by administration of the K_{ATP} channel antagonist, glibenclamide. Miwa *et al.* (1993) have also reported that nicorandil-induced relaxation of epi-myocardial coronary arteries is not affected by glibenclamide, but is inhibited by oxyhaemoglobin.

In conclusion, the present study demonstrates that nicorandil dilates the large coronary arteries in conscious dogs through an endothelium-independent mechanism, by a nitrate-like action. It also shows that the drug's action on K_{ATP} channels is predominantly at the level of the small coronary arteries. Thus it seems that nicorandil contrasts with pinacidil and nicardipine which dilate large coronary arteries through an indirect endothelium-dependent mechanism.

The authors are greatly indebted to Alain Bizé for his excellent technical assistance. This work was supported by grant 930403 from Institut National de la Santé et de la Recherche Médicale.

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(Received June 13, 1994
Revised August 30, 1994
Accepted September 23, 1994)