British Journal of Pharmacology (1999) 127, 1039–1047
http://www.stockton-press.co.uk/bjp

Influence of the endothelium, nitric oxide and serotonergic receptors on coronary vasomotor responses evoked by ergonovine in conscious dogs

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1 The respective contributions of coronary vascular endothelium, nitric oxide (NO) and serotonergic receptors to the effects of ergonovine on large and small coronary arteries were investigated in conscious dogs.

2 In seven dogs with an endothelium intact, ergonovine (30–1000 μg, i.v.) induced a biphasic response on large coronary artery with an early and transient vasodilatation (up to $+2.9\pm0.5\%$ from 3310 ± 160 μm, $P<0.01$) followed by a sustained vasoconstriction (down to $-4.9\pm0.5\%$, $P<0.001$) which occurred simultaneously with a sustained increase in coronary blood flow (CBF) (up to $+100\pm26\%$ from 28 ± 4 ml min^{-1}, $P<0.001$). After endothelium removal (balloon angioplasty), the ergonovine-induced vasodilatation was abolished and vasoconstriction potentiated ($-6.4\pm0.9\%$ after vs $-4.9\pm0.5\%$ before endothelium removal, $P<0.01$).

3 After blockade of NO synthesis by N^{ω}-nitro-L-arginine (30 mg kg^{-1}) in four other dogs, the early vasodilatation induced by ergonovine was abolished but the delayed vasoconstriction as well as the increase in CBF remained unchanged.

4 Both ketanserin and methiothepin (0.3 mg kg^{-1}) abolished the early vasodilatation and reduced the delayed vasoconstriction induced by ergonovine. Ketanserin decreased and methiothepin abolished the reduction in coronary resistance induced by ergonovine.

5 Thus, the complex interactions between vascular endothelium and serotonergic receptors to ergonovine-induced constriction of large coronary arteries might explain the induction of coronary spasms in patients with endothelial dysfunction.

Keywords: Ergonovine; coronary arteries; endothelium; conscious dogs

Abbreviations: CBF, coronary blood flow; 5-HT, serotonin; L-NNA, N^{ω}-nitro-L-arginine; LV, left ventricle, LV dP/dt, first derivative over time of left ventricular pressure; NO, nitric oxide

Introduction

Although the pathogenesis of coronary spasm is yet unclear, ergonovine remains to date the drug of choice for inducing coronary spasms in patients with resting angina pectoris (Maseri *et al*., 1990; Hamon *et al*., 1996). Numerous experimental studies have reported that the pharmacological mechanisms which underly ergonovine-evoked coronary artery constriction involve activation of serotonergic (5-HT) receptors located on the vascular smooth muscle (Sakanashi *et al*., 1980; Muller-Schweinitzer, 1980; Brazenor & Angus, 1981; Holtz *et al*., 1982; Egashira *et al*., 1992) but several aspects of this mediation have not yet been investigated *in vivo*. Primarily, the involvement of vascular endothelium in the modulation of the coronary constrictor effects of ergonovine, and other related 5-HT$_{1B/1D}$ or 5-HT$_{2A}$ agonists, has been well established *in vitro* but never definitely proven *in vivo* (Brum *et al*., 1984; Cox *et al*., 1989; Shimokawa *et al*., 1989; Egashira *et al*., 1992; Ellwood & Curtis, 1997; Ishida *et al*., 1998; Longmore *et al*., 1997; Villalon *et al*., 1997; Valentin *et al*., 1998), although it might explain the supersensitivity of vascular atherosclerotic segments to ergonovine (Heistad *et al*., 1984; Kawachi *et al*., 1984). Furthermore, the effect of the drug on coronary blood flow has never been assessed in the above mentioned studies, although this is of major interest to understand its mode of action.

The main goal of the present study was thus to investigate the haemodynamic and coronary effects of increasing doses of ergonovine and to assess the respective contributions of the coronary vascular endothelium, of nitric oxide (NO) release and of 5-HT receptors to the effects of ergonovine. For this purpose, we used an experimental model in which the direct dilator and/or constrictor effects of a drug can be investigated *in vivo* at the level of a large epicardial coronary artery in the presence of a functional vascular endothelium and after its subsequent removal by balloon angioplasty (Drieu La Rochelle *et al*., 1992; Berdeaux *et al*., 1994; Ghaleh *et al*., 1995). These experiments were conducted in conscious, chronically instrumented dogs to avoid the deleterious effects of general anaesthesia and acute surgery on coronary dynamics.

Methods

Animal preparation

The animal instrumentation and ensuing experiments were performed in agreement with the official regulations edicted by

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the French Ministry of Agriculture (approval no. 94148) and conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals (DHEW [DHSS] publication no. [NIH] 8523, revised 1985).

Fifteen adult mongrel dogs, weighing 22–26 kg, were anaesthetized with pentobarbitone (30 mg kg^{-1}, i.v.), intubated and ventilated with a respirator. Muscle paralysis was obtained with pancuronium bromide (0.2 mg kg^{-1}, i.v. bolus). Under sterile surgical conditions, a left thoracotomy through the 5th intercostal space was performed and the heart 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 (model VD 5S, Triton Technology, San Diego, CA, U.S.A.) was attached to a Dacron backing and sutured using 5-0 suture (Ethicon Inc., Sommersville, NJ, U.S.A.) to opposing surfaces of the left circumflex coronary artery 2–4 cm from its origin. Care was taken when positioning the transducers to limit dissection of, and damage to, any visible nerves and proper alignment of the crystals was confirmed during surgery by monitoring the ultrasonic signal with an oscilloscope. A Doppler flow probe (10 MHz, Crystal Biotech, Hopkinton, MA, U.S.A.) was implanted distal to the dimension transducers. In seven dogs, a solid state pressure transducer (model P7A, Konigsberg Instruments, Pasadena, CA, U.S.A.) was introduced into the left ventricle through the apical dimple and secured with purse-string sutures. 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 the surgery. Analgesia was obtained with propacetamol (1 g, i.m.).

Measurement of haemodynamic and coronary parameters in conscious dogs

Aortic pressure was measured with a Statham P23 XL pressure gauge transducer (Statham Instruments, Oxnard, CA, U.S.A.). Left ventricular (LV) pressure was measured from the Konigsberg gauge and its first positive derivative over time (i.e., LV dP/dt_{max}) was obtained by electrical differentiation of the LV signal. The external diameter of the left circumflex coronary artery was measured instantaneously and continuously with an ultrasonic transit-time dimension system (System 6, Triton Technology Inc, San Diego, CA, U.S.A.) with a resolution of ± 0.04 mm and mean coronary diameter was measured as previously described (Berdeaux *et al*., 1994). Left circumflex coronary blood flow velocity was measured with a Doppler flowmeter (System 6, Triton Technology Inc, San Diego, CA, U.S.A.). The doppler shift measured with the coronary flow velocity probe was converted to blood flow using the equation:

$$\text{coronary blood flow} = 2.5 \times d^2 \times f,$$

where d is the internal diameter of the coronary artery and f is the doppler shift in kHz. Internal coronary diameter was taken as the internal diameter of the flow probe which becomes adherent to the coronary artery during the healing process, and according to previous postmortem evaluation, wall thickness was considered to be 20% of the external coronary artery diameter. 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 (model ES 2000, Gould Instruments Inc., Cleveland, OH, U.S.A.).

Experimental protocols

All experiments were conducted at least 10–15 days 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. The increase in coronary diameter in response to nitroglycerin (10 μg kg^{-1}, i.v.) reached $6.9 \pm 1.3\%$ in these dogs, demonstrating that vasomotion of the epicardial artery was not impeded by the surgical procedure.

After basal haemodynamic parameters had been obtained, seven dogs received, in random order and on separate days, increasing doses of ergonovine (30–1000 μg, i.v. bolus). To assess the reproducibility of the haemodynamic and coronary effects of ergonovine, one dose of the drug (300 μg) was readministered 7 ± 2 days after its first administration and 9 ± 2 days before endothelium removal in six out of these seven dogs.

At least 1 day after the last administration of ergonovine, endothelium of the epicardial coronary artery was removed at the level of the crystals attachment according to the previously described technique (Berdeaux *et al*., 1994; Ghaleb *et al*., 1995). For this purpose, dogs were lightly reanaesthetized with propofol (200 mg, i.v.) and 0.5% halothane. Under aseptic conditions, an incision was made to expose the right carotid artery. A 8 F 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. To avoid distension of the coronary artery, care was taken to calibrate the balloon catheter both according to the external coronary diameter measured by the ultrasonic transit-time gauge and by estimation of the internal diameter after serial injections of contrast medium. The balloon was inflated with air, and the catheter was gently moved back and forth three times over the entire segment from the proximal circumflex artery to the crystals area. The balloon was then deflated, the catheter withdrawn, and the dog was allowed to fully recover. This procedure caused de-endothelialization on each side of the crystals, leaving the distal circumflex, the left anterior descending and the septal coronary arteries intact, as previously demonstrated by histological and pharmacological studies (Berdeaux *et al*., 1994). Three days after this procedure, the adequacy of endothelium removal and the lack of significant vascular smooth muscle lesion were verified by i.v. bolus injections of the endothelium-dependent vasodilator, acetylcholine (0.3 μg kg^{-1}) and of the endothelium-independent vasodilator, nitroglycerin (1 μg kg^{-1}), respectively. Six out of the seven dogs involved in this protocol fulfilled our criterion for adequacy of endothelium removal, i.e. a vasodilatory response to acetylcholine inhibited by at least 80% and a vasodilatory response to nitroglycerin of at least 75% of the control response, the control value of coronary diameter for each dog being that measured before endothelium removal. Three, 9 and 21 days after endothelium removal, acetylcholine (0.3 μg kg^{-1}), nitroglycerin (1 μg kg^{-1}) and ergonovine (300 μg) were readministered in the same dogs.

In four additional dogs, ergonovine (300 μg) was administered before (control) and after subacute inhibition of NO synthesis by N^{ω}-nitro-L-arginine (L-NNA). Two days after a

control injection of ergonovine (300 μg), the animals underwent acute inhibition of NO-synthase by L-NNA (30 mg kg^{-1} i.v. infusion over 20 min). To avoid the intrinsic haemodynamic effects of L-NNA during the experiment, the injection of ergonovine was performed the following day, after a second administration of L-NNA (20 mg kg^{-1}).

Finally, in order to investigate the relative contribution of 5-HT_{2A} and 5-HT_1 receptors to the effects of ergonovine, the drug (300 μg) was administered in four additional dogs before (control) and, 2 days later, after either ketanserin (a selective 5-HT_{2A} receptor antagonist) or methiothepin (a non selective 5-HT_1 and 5-HT_{2A} receptor antagonist) pre-treatment. Both antagonists were infused over a 10 min period at the dose of 0.3 mg kg^{-1}.

Drugs

Drugs used were pentobarbitone (Sanofi Santé Animale, Libourne, France), pancuronium bromide (Organon Tecknika, Fresnes, France), ergonovine maleate, methiothepin methanesulphonate and acetylcholine hydrochloride (Sigma Chemical Co, St Louis, MO, U.S.A.), nitroglycerin (Besins-Iscovesco, Paris, France), N^{ω}-nitro-L-arginine (Sigma Chimie, la Verpillère, France), ketanserin tartrate (Janssen Research Foundation, Beerse, Belgique), propacetamol (UPSA, Rueil-Malmaison, France), cefazolin (Allard, Paris, France), gentamicin (Dakota, Paris, France), halothane (Belamont, Paris, France) and propofol (Zeneca, Cergy, France). Drugs dosage refer to their salt and were dissolved in isotonic and sterile saline.

Data analysis

All data are expressed as mean values $\pm$ s.e.mean. The hypothesis that the values of a given parameter were different after and before endothelium removal was tested by contrast analysis. The effect of time on a given parameter measured before and 3, 9 and 21 days after endothelium removal was tested by a one way analysis of variance for repeated measures. The hypothesis that (a) the baseline values measured before each dose of ergonovine, and (b) the absolute change of a given parameter after ergonovine administration was dose-dependent, were both tested by a one way analysis of variance for repeated measures. All these statistical analyses were performed on an IBM-compatible personal computer using a BMDP statistical software. A value of $P<0.05$ was considered significant.

Results

Haemodynamic and coronary effects of ergonovine in the normal conscious dog

Figure 1 illustrates the haemodynamic and coronary responses to a bolus injection of ergonovine (300 μg) in one typical dog. Table 1 summarizes the cardiac, systemic and coronary haemodynamic responses to graded doses of ergonovine.

Up to 100 μg, ergonovine did not alter mean aortic pressure and heart rate but increased these parameters and decreased LV dP/dt_{max} starting from 300 μg, these effects being significant at 1000 μg. As shown in Figure 1, ergonovine induced an early and transient epicardial vasodilatation rapidly followed by a sustained vasoconstriction, the latter effect occurring simultaneously with a progressive and sustained increase in coronary blood flow. As shown in Table 1 and illustrated in Figure 2, all these effects were dose-dependent (from 30 – 1000 μg), except the epicardial vasoconstriction which reached a plateau ($-4.9\pm0.5\%$) from 300 μg. The maximal increase in coronary diameter ($+2.9\pm0.5\%$,

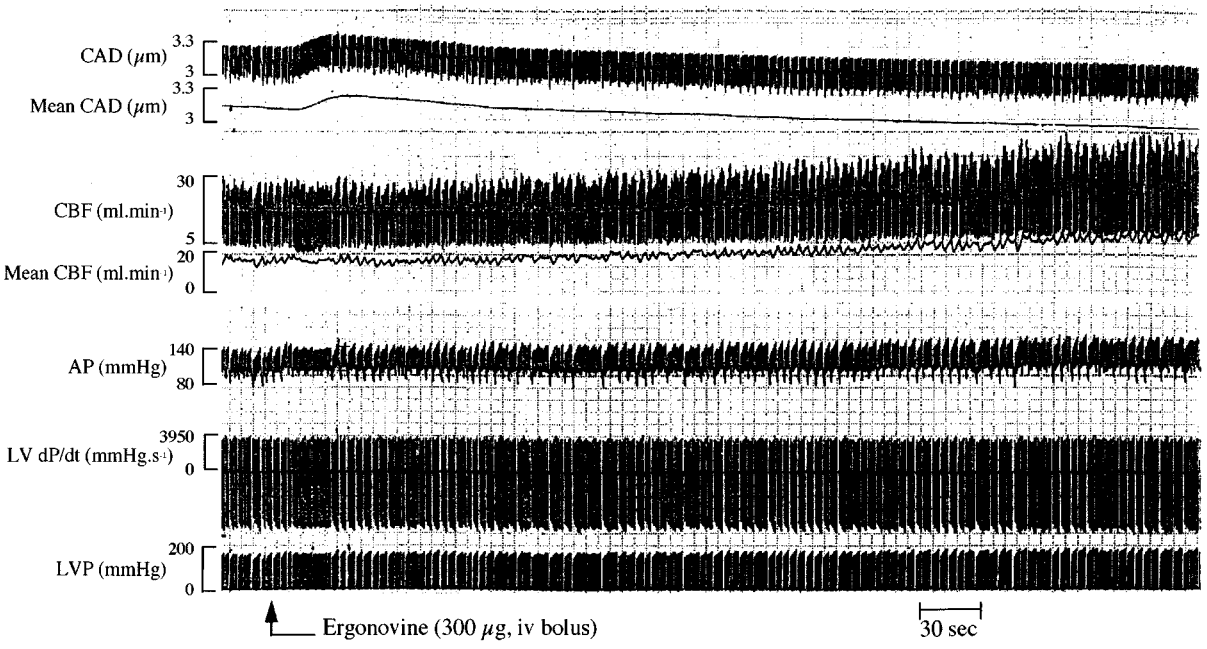

Figure 1 Representative recording of the effects of ergonovine (300 μg, i.v. bolus) in a conscious dog. From top to bottom are presented phasic and mean epicardial coronary artery diameter (CAD), phasic and mean coronary blood flow (CBF), arterial blood pressure (AP), the first derivative of left ventricular pressure (dP/dt) and left ventricular pressure (LVP). The injection of ergonovine is indicated by the arrow. Ergonovine induced a transient increase in coronary artery diameter followed by a sustained vasoconstriction. These effects of ergonovine on epicardial coronary arteries were associated with a significant increase in coronary blood flow but no major changes in systemic haemodynamic parameters were observed.

Table 1 Systemic and coronary haemodynamic effects of ergonovine

	Ergonovine (μg)			
	30	*100*	*300*	*1000*
HR (beats min^{-1})				
Baseline	86±4	90±2	84±4	88±5
Change from baseline	−2±1	+5±1	+7±2[b]	+7±2[a]
MAP (mmHg)				
Baseline	98±4	99±4	97±5	98±4
Change from baseline	+4±1	+1±1	+3±1	+6±1[b]
dP/dt_{max} (mmHg sec^{-1})				
Baseline	3451±209	3305±227	3280±231	3312±159
Change from baseline	−19±34	−187±52	−307±77[a]	−373±64[c]
CAD (μm)				
Baseline	3169±160	3183±162	3141±173	3166±170
Change from baseline (VD)	+18±5[a]	+24±5[a]	+69±18[a]	+93±16[c]
Change from baseline (VC)	−69±12[c]	−104±17[c]	−155±26[c]	−156±25[c]
CBF (ml min^{-1})				
Baseline	28±4	28±4	28±4	28±4
Change from baseline	+4±0[a]	+8±2[a]	+16±4[a]	+28±8[a]
CVR (mmHg min ml^{-1})				
Baseline	4.01±0.60	4.00±0.61	3.92±0.55	3.90±0.60
Change from baseline	−0.26±0.08	−0.72±0.15[b]	−14±0.21[b]	−1.30±0.25[b]

Values are mean±s.e.mean ($n=7$). Significant changes from baseline: [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$. HR: heart rate; MAP: mean arterial pressure; $dPdt_{max}$: maximal elevation of the first derivative of left ventricular pressure; CAD: coronary artery diameter; VD: value taken at the peak of vasodilatation; VC: value taken at the peak of vasoconstriction; CBF: coronary blood flow; CVR: coronary vascular resistance.

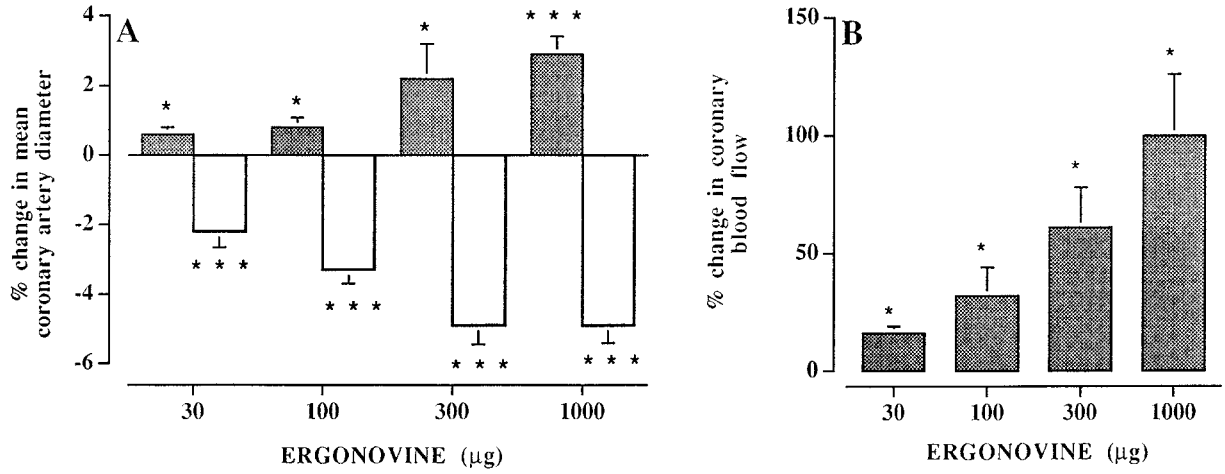

Figure 2 Bargraphs showing the per cent changes in mean coronary artery diameter (A) during the early phase of vasodilatation (shaded bars) and the delayed vasoconstriction phase (open bars), and the per cent changes in mean coronary blood flow (B) at the peak effects of increasing dosees of ergonovine (30–1000 μg, i.v. bolus) in conscious dogs. The coronary effects of ergonovine were dose-dependent but epicardial vasoconstriction plateaued from 300 μg. Significant changes from baseline: *$P<0.05$; ***$P<0.001$ ($n=7$).

$P<0.001$) occurred 43±3 s after the onset of ergonovine injection whereas the maximal decrease in coronary diameter and increase in coronary blood flow (100±26%, $P<0.05$) occurred 8.6±0.8 and 9.0±0.6 min after the drug injection, respectively. Finally, all cardiac, systemic and coronary effects of ergonovine (300 μg) were reproducible during subsequent administrations (data not shown).

Haemodynamic and coronary effects of ergonovine after endothelium removal

As shown in Table 2, endothelium removal *per se* did not significantly affect the baseline values of coronary blood flow and coronary vascular resistance, but increased coronary diameter by 8.6% ($P<0.01$) 3 days after the manoeuvre and this increase in coronary diameter remained significant throughout the measurement period (e.g. 6.1% at day 21, $P<0.05$). Whereas acetylcholine increased coronary diameter before endothelium removal (4.6±0.7%), the response was depressed 3 days after endothelium removal (0.8±0.2%, $P<0.01$) and thereafter almost restored (3.3±0.8 and 3.5±0.5%, 9 and 21 days after endothelium removal, respectively). In contrast, the nitroglycerin-induced increase in coronary diameter was slightly but not significantly altered after endothelium removal (6.9±1 before, 5.2±1, 5.8±1 and 6.1±0.9%, at 3, 9 and 21 days after endothelium removal, respectively). Finally, the increase in coronary blood flow and the fall in coronary vascular resistance evoked by acetylcholine and nitroglycerin were not affected by endothelium removal (Table 2).

As shown in Figure 3, the ergonovine (300 μg)-evoked increase in coronary diameter before endothelium removal was

Table 2 Coronary effects of nitroglycerin (1 μg kg^{-1}) and acetylcholine (0.3 μg kg^{-1}) before and at different days after endothelium removal

	Before endothelium removal	*Days after endothelium removal*		
		3	*9*	*21*
Nitroglycerin				
CAD (μm)				
Baseline	2856±161	3101±195[b]	3058±186[a]	3030±168[a]
Change from baseline	+198±25	+163±34	+176±35	+183±38
CBF (ml min^{-1})				
Baseline	28±4	32±6	30±6	30±6
Change from baseline	+8±2	+12±4	+12±2	+10±2
CVR (mmHg min ml^{-1})				
Baseline	4.02±0.77	3.31±0.53	4.08±0.95	3.76±0.78
Change from baseline	−1.08±0.21	−0.96±0.20	−1.46±0.38	−1.08±0.18
Acetylcholine				
CAD (μm)				
Baseline	2848±155	3118±190[b]	3068±187[a]	3040±168[a]
Change from baseline	+132±26	+24±7[b]	+103±24	+107±22
CBF (ml min^{-1})				
Baseline	30±4	34±6	32±6	30±6
Change from baseline	+32±8	+36±8	+32±8	+30±8
CVR (mmHg min ml^{-1})				
Baseline	3.74±0.74	3.30±0.53	3.84±0.90	3.68±0.63
Change from baseline	−2.13±0.36	−1.99±0.25	−2.30±0.50	−2.22±0.27

Values are mean±s.e.mean ($n=6$). Significant changes vs corresponding value before endothelium removal: [a]$P<0.05$, [b]$P<0.01$. All changes from corresponding baseline values were statistically significant ($P<0.05$). CAD: coronary artery diameter; CBF: coronary blood flow; CVR: coronary vascular resistance.

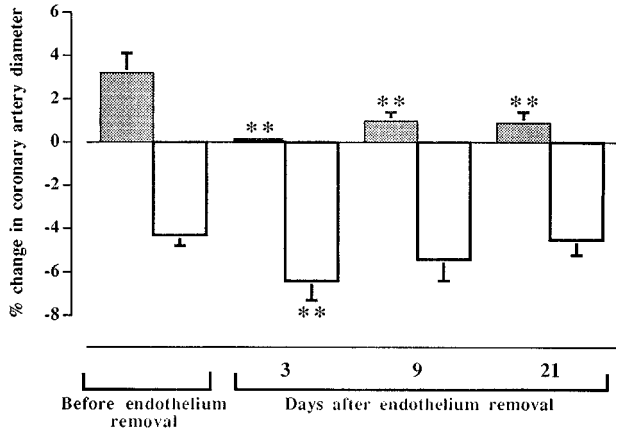

Figure 3 Bargraphs showing the per cent changes in mean coronary artery diameter during the early phase of vasodilatatioan (shaded bars) and the delayed vasoconstriction phase (open bars) after administration of ergonovine (300 μg, i.v. bolus) before and at different days after endothelium removal. Significant changes from corresponding value before endothelium removal: $^{**}P<0.01$, ($n=6$).

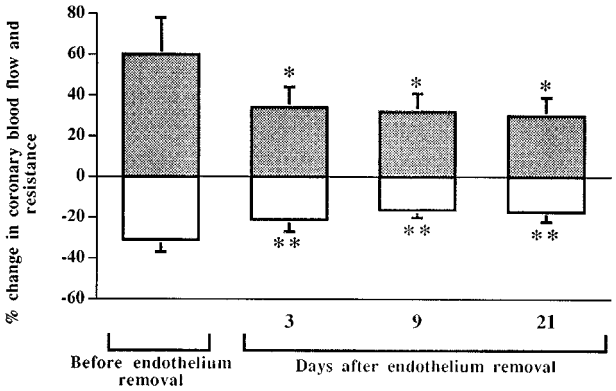

Figure 4 Bargraphs showing the per cent changes in coronary blood flow (shaded bars) and coronary vascular resistance (open bars) at the peak effects of ergonovine (300 μg, i.v. bolus) before and at different days after endothelium removal. Significant changes from corresponding value before endothelium removal: $^{*}P<0.05$; $^{**}P<0.01$, ($n=6$).

abolished 3 days after the manoeuvre and this vasodilatory response remained significantly reduced as compared to the corresponding control response thereafter (1±0.4 and 0.9±0.5%, at 9 and 21 days after endothelium removal, respectively, $P<0.01$). Conversely, ergonovine-induced decrease in coronary diameter was more marked 3 days after endothelium removal than before (−6.4±0.9 vs −4.9±0.5%, $P<0.01$). Thereafter, the ergonovine-induced decrease in coronary diameter returned to its control value. As shown in Figure 4, the ergonovine-induced increase in coronary blood flow and decrease in coronary vascular resistance were significantly reduced 3, 9 and 21 days after endothelium removal. In contrast, the increases in coronary blood flow evoked by acetylcholine and nitroglycerin remained unaffected throughout the procedure (Table 2).

Influence of NO-synthesis inhibition on haemodynamic and coronary effects of ergonovine

As shown in Table 3, inhibition of NO-synthesis by L-NNA decreased heart rate (−48±2%, $P<0.001$) and coronary diameter (−10.6±2%, $P<0.01$) but coronary blood flow, coronary vascular resistance and mean arterial pressure remained unchanged as compared to their corresponding control values. The early vasodilatory response evoked by ergonovine at the level of large epicardial coronary arteries was

abolished by L-NNA but the delayed vasoconstrictor component of the response as well as the simultaneous increase in coronary blood flow and decrease in coronary vascular resistance induced by ergonovine remained unchanged after L-NNA.

Influence of ketanserin and methiothepin on haemodynamic and coronary effects of ergonovine

As shown in Table 4, ketanserin and methiothepin *per se* did not alter systemic haemodynamics and coronary blood flow but increased the epicardial coronary diameter (ketanserin: $1.5\pm0.6\%$ from 3215 ± 227 μm, $P<0.05$; methiothepin: $3.6\pm0.7\%$ from 3215 ± 201 μm, $P<0.01$). As shown in Table 4, both ketanserin and methiothepin abolished the early and transient epicardial vasodilatation induced by ergonovine and reduced by 43 and 75%, respectively, the delayed and sustained vasoconstriction induced by ergonovine on epicardial coronary arteriers. Finally, ketanserin decreased by 35% and methiothepin abolished the reduction in coronary vascular resistance induced by ergonovine.

Table 3 Effects of ergonovine (300 μg) before and after L-NNA induced NOS inhibition

	Before L-NNA induced NOS inhibtion	*After L-NNA induced NOS inhibiton*
HR (beats min^{-1})		
Baseline	100 ± 4	53 ± 3^{d}
Change from baseline	$+6\pm3$	$+1\pm3$
MAP (mmHg)		
Baseline	98 ± 5	90 ± 4
Change from baseline	4 ± 3	$+5\pm2$
CAD (μm)		
Baseline	3143 ± 134	2815 ± 179^{c}
Change from baseline (VD)	$+118\pm35^{a}$	0 ± 0^{b}
Change from baseline (VC)	-170 ± 31^{a}	-165 ± 32^{a}
CBF (ml min^{-1})		
Baseline	32 ± 4	32 ± 4
Change from baseline	$+10\pm2^{a}$	$+14\pm6^{a}$
CVR (mmHg min ml^{-1})		
Baseline	3.35 ± 0.48	3.12 ± 0.60
Change from baseline	-0.60 ± 0.12^{a}	-0.58 ± 0.12^{a}

Values are mean$\pm$s.e.mean; ($n=4$). Significant change from baseline: $^{a}P<0.05$. Significant change vs corresponding value before L-NNA induced NOS inhibition: $^{b}P<0.05$; $^{c}P<0.01$; $^{d}P<0.001$. HR: heart rate; MAP: mean arterial pressure; CAD: coronary artery diameter; VD: value taken at the peak of vasodilatation; VC: value taken at the peak of vasoconstriction; CBF: coronary blood flow; CVR: coronary vascular resistance; NOS: nitric oxide synthase.

Discussion

The present study demonstrates that, in chronically instrumented conscious dogs, ergonovine induces a biphasic effect on epicardial coronary arteries: an early but transient endothelium- and NO-dependent coronary vasodilatation followed by a sustained vasoconstriction, both responses being sensitive to ketanserin and methiothepin. This vasoconstrictor effect of ergonovine at the level of large epicardial coronary arteries is increased 3 days after endothelium removal, partially inhibited by ketanserin and almost abolished by methiothepin. Ergonovine also induces a sustained vasodilatation of small coronary arteries, an effect which is also partially inhibited by ketanserin and abolished by methiothepin but is independent of local NO synthesis.

Effects of ergonovine on large epicardial coronary arteries

The present study confirms that in conscious dogs with an intact coronary endothelium, ergonovine, like serotonin, induces a dose-related biphasic response on large epicardial coronary arteries characterized by an initial brief increase in coronary artery diameter followed by a delayed and sustained vasoconstriction (Holtz *et al*., 1982; Chu & Cobb, 1987; Egashira *et al*., 1992). As previously reported with serotonin in anaesthetized dogs (Brum *et al*., 1984), we observed that the initial dilatory effect exhibited by ergonovine was endothelium-dependent and mediated through NO-release since this effect disappeared after both endothelium removal and NO synthesis inhibition by L-NNA. However, the inhibition of this endothelium-dependent dilatation by ketanserin is rather surprising inasmuch as it was previously reported that

Table 4 Effects of ketanserin and methiothepin on systemic and coronary haemodynamic effects of ergonovine

	Ergonovine 300 µg (control)	*Ketanserin* (0.3 mg kg^{-1})	*Ergonovine after ketanserin*	*Methiothepin* (0.3 mg kg^{-1})	*Ergonovine after methiothepin*
HR (beats min^{-1})					
Baseline	97 ± 7	97 ± 14	111 ± 17	100 ± 17	114 ± 16
Change from baseline	0 ± 0	14 ± 9	-3 ± 6	14 ± 5	-3 ± 5
MAP (mmHg)					
Baseline	84 ± 3	86 ± 3	82 ± 9	82 ± 4	78 ± 5
Change from baseline	2 ± 1	-4 ± 9	4 ± 6	-4 ± 3	-1 ± 2
CAD (μm)					
Baseline	3196 ± 180	3215 ± 227	3285 ± 238	3215 ± 201	3333 ± 224
Change from baseline (VD)	103 ± 10^{b}	50 ± 19^{a}	8 ± 12^{d}	118 ± 29^{b}	0 ± 0^{d}
Change from baseline (VC)	-182 ± 29^{b}	–	$-103\pm16^{a,c}$	–	$-45\pm21^{a,d}$
CBF (ml min^{-1})					
Baseline	26 ± 3	29 ± 3	30 ± 6	25 ± 5	27 ± 8
Change from baseline	12 ± 3^{a}	1 ± 7	6 ± 4	2 ± 3	2 ± 3
CVR (mmHg ml min^{-1})					
Baseline	4.95 ± 0.60	5.56 ± 0.70	5.65 ± 0.80	6.69 ± 0.95	6.20 ± 0.90
Change from baseline	-1.31 ± 0.24^{b}	$+0.72\pm0.60^{a}$	-0.97 ± 0.35^{a}	-0.49 ± 0.20^{b}	$-0.03\pm0.10^{b,d}$

Values are mean$\pm$s.e.mean ($n=4$). Significant change from baseline: $^{a}P<0.05$; $^{b}P<0.01$. Significant change vs corresponding control value: $^{c}P<0.05$; $^{d}P<0.01$. HR: heart rate; MAP: mean arterial pressure; CAD: coronary artery diameter; VD: value taken at the peak of vasodilatation; VC: value taken at the peak of vasoconstriction; CBF: coronary blood flow; CVR: coronary vascular resistance.

endothelial 5-HT receptors are of 5-$HT_{1B/1D}$ (Schoeffter & Hoyer, 1990; Valentin *et al*., 1996) and 5-HT_{2B} (Glusa & Roos, 1996) subtypes and that ketanserin exhibits relatively low affinity for these receptors ($pK_i \leqslant 5$).

Following this initial vasodilatation, ergonovine induced a prolonged and dose-dependent epicardial coronary vasoconstriction. Under control conditions this effect appears to be partially opposed by the endothelium through a flow-dependent mechanism since coronary blood flow increases simultaneously. Moreover, we observed in this study that 3 days after mechanical removal of the endothelium, the magnitude of the vasoconstrictor action of ergonovine was significantly greater than that observed when the endothelium was intact. Similar findings were previously reported *in vitro* following application of several 5-$HT_{1B/1D}$ agonists on isolated rabbit saphenous veins (Valentin *et al*., 1996) and canine coronary arteries (Valentin *et al*., 1998). The ergonovine-induced epicardial coronary vasoconstriction then returned to its control values 9 days after endothelium removal, i.e. when restoration of a functional endothelium has occurred, as previously demonstrated in this animal model (Hayashi *et al*., 1988; Cox *et al*., 1989; Drieu La Rochelle *et al*., 1992; Berdeaux *et al*., 1994). Although Hayashi *et al*. (1988) reported a non-significant increase in ergonovine-induced vasoconstriction 10 days after endothelium removal, it must be noticed that the absolute changes in large coronary diameter reported in their study were similar to those observed in the present one. In hypercholesterolaemic dogs, Kawachi *et al*. (1984) observed an enhancement of epicardial coronary constriction by ergonovine up to 3 months following endothelium removal, a phenomenon which might be explained by the long-lasting dysfunction of the regenerated endothelium induced by hypercholesterolaemia. Finally, Egashira *et al*. (1992) reported that ergonovine-induced epicardial coronary constriction was potentiated only 1 month after endothelium removal in conscious dogs. This apparent discrepancy with our data might be due to the fact that in the study by Egashira *et al*. (1992), dogs underwent endothelium removal 1 month before surgery and that ergonovine was administered only a few days after instrumentation, a manoeuvre which is known to induce histological and functional lesions of the coronary arteries that were still present when ergonovine was administered. Thus, despite some minor discrepancies between the different studies, all of them and the present one tend to demonstrate a protective role of the coronary vascular endothelium against ergonovine-induced constriction of large coronary arteries and this observation is highly relevant when it is transposed to pathological situations.

Also in agreement with previous studies conducted *in vitro* (Muller-Schweinitzer, 1980; Sakanashi & Yonemura, 1980; Brazenor & Angus, 1981) and *in vivo* (Holtz *et al*., 1982; Egashira *et al*., 1992), we observed a significant decrease in ergonovine-induced epicardial coronary constriction after 5-HT receptors blockade. Although the aim of this study was not to investigate the nature of 5-HT receptor subtype(s) mediating this vasoconstrictor response to ergonovine, our study clearly demonstrates that 5-HT_1 and 5-HT_{2A} receptors are both involved in the vasoconstrictor effect of ergonovine on large coronary arteries. Regarding 5-HT_1 receptors, one may reasonably hypothesize that these receptors are, at least, of the 5-$HT_{1B/1D}$ subtype since sumatriptan has been shown to also evoke constriction of epicardial coronary arteries in dogs (Gupta *et al*., 1995) and in man (Mac Intyre *et al*., 1992). Recent *in vitro* studies have also shown that numerous 5-HT_{1B} agonists induce a sustained, dose- and time-dependent coronary constriction similar to that exhibited by ergonovine (VanDenbrink *et al*., 1998; Valentin *et al*., 1998; Parsons *et al*., 1998). At the level of large coronary arteries these receptors are clearly, and exclusively, of the 5-$HT_{1B/1D}$ subtype as demonstrated by the available pharmacological (Kaumann *et al*., 1993; 1994; Longmore *et al*., 1998) and local mRNA expression studies (Hamel *et al*., 1993; Sgard *et al*., 1996). However, the lack of highly selective 5-HT_1 receptor subtypes antagonists does not allow definitive conclusion as to which of these subtypes is involved in the vasoconstrictor effect of ergonovine at the level of large epicardial vessels. Regarding 5-HT_{2A} receptors, the present study confirms the *in vitro* investigation of Ellwood & Curtis (1997) demonstrating their presence at the level of epicardial coronary arteries. However, as ketanserin failed to prevent spontaneous (De Caterina *et al*., 1984) or ergonovine-induced coronary spasms (Freedman *et al*., 1984) in patients with variant angina, it is likely that these receptors are not primarily involved in the pathophysiology of coronary spasm in man.

Effects of ergonovine on small coronary arteries

To date, very few studies have investigated the effects of ergonovine at the level of small coronary arteries. An increase in coronary vascular resistance was reported in *ex-vivo* beating hearts of baboons (Chilian *et al*., 1990), whereas coronary blood flow was not affected by an intracoronary injection of ergonovine in anaesthetized dogs (Sudhir *et al*., 1993; Tsunoda *et al*., 1994). The present study clearly demonstrates that ergonovine dilates in a dose-dependent manner the small coronary arteries in conscious dogs. This effect is neither related to an indirect increase in myocardial oxygen demand nor the release of NO, since cardiac and systemic haemodynamic parameters were only minimally, if at all, affected by ergonovine and since this effect persisted following L-NNA administration. In contrast, this vasodilatory response was abolished by methiothepin, demonstrating that 5-HT_1 receptors mediate both ergonovine-induced constriction of large and dilatation of small coronary arteries. The fact that the vasodilatory response of small coronary arteries to ergonovine was also reduced by ketanserin is, however, more surprising since activation of 5-HT_{2A} receptors usually cause contraction and not relaxation of vascular smooth muscle. Such a pattern of responses to ergonovine on small coronary arteries is quite similar to that previously reported with serotonin in conscious dogs (Chu & Cobb, 1987; Woodman, 1990). In anaesthetized dogs with previous blockade of 5-HT_2 and 5-HT_3 receptors, Cambridge *et al*. (1995) reported a dose-related, methiothepin-sensitive vasodilatation of small coronary arteries with serotonin and selected tryptamine analogues. In agreement with the results of the present study, these authors also demonstrated that inhibition of NO synthesis had little or no effect upon the vasodilatation of small coronary arteries induced by 5-carboxamidotryptamine, suggesting that the response was mediated through a non-endothelial 5-HT receptor (Cambridge *et al*., 1991). However, as above mentioned, the present study cannot determine the exact nature of the 5-HT receptor subtype involved in ergonovine-induced dilatation of small coronary arteries but on the basis of the sensitivity of this response to ketanserin it may be hypothesized that ergonovine activates the presynaptic 5-HT_{1D} heteroceptors located on cardiac sympathetic nerve endings as previously reported on the human atrium (Molderings *et al*., 1996). Indeed, ketanserin exhibits higher affinity for 5-HT_{1D} ($pK_i = 7.1$) than for 5-HT_{1B} ($pK_i < 5.0$) receptors (Kaumann *et al*., 1994) and at the dose of 0.3 mg kg^{-1} used in the present

study, ketanserin may have blocked both 5-HT_{2A} and 5-HT_{1D} receptors. Activation of these 5-HT_{1D} presynaptic heteroceptors by ergonovine might limit the release of noradrenaline at the level of small coronary arteries, and hence contribute to the drug-induced dilatation of the small coronary arteries.

Surprisingly, we observed a reduction of the ergonovine-induced dilatation of small coronary arteries after removal of the endothelium at the level of large epicardial arteries. This finding is rather paradoxical since our technique for mechanical removal of the endothelium only concerns a limited segment of the proximal large circumflex coronary artery. This significant reduction of the ergonovine-induced relaxation of small coronary vessels cannot be accounted for by a time-related tachyphylaxis phenomenon since the ergonovine-induced increase in coronary blood flow and decrease in coronary vascular resistance, before and throughout the 3 weeks following endothelium removal, were highly reproducible. By the same time, the increase in coronary blood flow and the fall in coronary vascular resistance evoked by acetylcholine and nitroglycerin were not affected at all by endothelium removal in the same animals, thus excluding any artifact related to the balloon angioplasty technique. We have presently no explanation for this unexpected observation but the interactions of ergonovine with the numerous platelets derived vasoconstrictor substances released after balloon angioplasty (e.g., thromboxane A_2, ADP, serotonin, etc) need to be further investigated (Golinio *et al*., 1989; Willerson *et al*., 1991).

The present study highlights the role of vascular endothelium in modulating the coronary effects of ergonovine and clearly shows that the vasoconstrictor action of the drug is reinforced in the absence of endothelium. We also evidenced the involvement of 5-HT_{1B} and 5-HT_{2A} receptors in ergonovine-induced constriction of large coronary arteries. The similarity between ergonovine and serotonin coronary effects, and the high sensibility and specificity of ergonovine at detecting coronary spasm are strong arguments in favour of the involvement of serotonin itself and/or its receptors in the pathophysiology of variant angina (McFadden *et al*., 1991; Hoyer *et al*., 1994). Further studies, however, are necessary to investigate the role of the different 5-$HT_{1B/1D}$ receptor subtypes in the pathophysiology of coronary spasm and consequently to propose new therapeutic approaches of this disease with selective antagonists (Pauwels, 1997).

Finally, the present study also suggests that even a limited angioplasty procedure on large coronary arteries can lead to an abnormal response of the coronary arteriolar bed to ergonovine and that local basal sympathetic tone or 5-HT receptors could be involved in this phenomenon. Such an alteration has been described in humans after angioplasty since myocardial perfusion remains abnormal for weeks after this procedure (El-Tamini *et al*., 1991).

The authors are greatly indebted to Alain Bizé for his excellent technical assistance. We also thank Drs Ivan Carel (Faculté de Médecine Paris Sud) and Christophe Drieu La Rochelle (Preclinical Pharmacology Unit, Biotrial, Rennes, France) for fruitful discussions. D Karila-Cohen was supported by a Fellowship Grant from the Fondation de la Recherche Médicale.

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(Received January 18, 1999
Revised March 29, 1999
Accepted April 6, 1999)