

# Effects of A<sub>1</sub> adenosine receptor blockade by bamiphylline on ischaemic preconditioning during coronary angioplasty

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**Objective** The role of A<sub>1</sub> adenosine receptors in preconditioning in humans is unknown. To establish whether bamiphylline, a selective antagonist of A<sub>1</sub> adenosine receptors, abolishes ischaemic preconditioning in man, 36 consecutive patients undergoing single-vessel coronary angioplasty were randomized to receive intravenous infusion of bamiphylline (5 mg · kg<sup>-1</sup>) or placebo (0.9% NaCl) immediately prior to the procedure.

**Design** The mean values (± 1 SD) of ST segment shifts on the surface and intracoronary electrocardiograms were measured at the end of the first and second balloon inflations, both 2 min long. The severity of cardiac pain was obtained at the same time using a visual analogue scale.

**Results** In bamiphylline-treated patients, the mean ST segment shift and the severity of cardiac pain during the second inflation were similar to those during the first

inflation (14 ± 15 vs 16 ± 16 mm, ns and 31 ± 28 vs 31 ± 29, ns, respectively). Conversely, in placebo-treated patients both the mean ST segment shift and the severity of cardiac pain during the second inflation were significantly less than those during the first inflation (10 ± 6 vs 17 ± 7 mm, *P* < 0.001 and 25 ± 21 vs 39 ± 31 mm, *P* < 0.01, respectively). Thus, bamiphylline abolishes ischaemic preconditioning observed in man during repeated coronary balloon inflations.

**Conclusion** These results suggest that, in this setting, ischaemic preconditioning is mediated, at least in part, by A<sub>1</sub> adenosine receptors.

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**Key Words:** A<sub>1</sub> adenosine receptors, bamiphylline, coronary angioplasty, ischaemic preconditioning.

## Introduction

The ability of brief periods of ischaemia to limit cell death following a subsequent sustained episode of ischaemia has been called preconditioning<sup>[1]</sup>. The phenomenon of ischaemic preconditioning has now been demonstrated in several animal species, including dogs<sup>[1–3]</sup>, pigs<sup>[4]</sup>, rabbits<sup>[5]</sup>, and rats<sup>[6]</sup>. Deutsch *et al.*<sup>[7]</sup>, Cribier *et al.*<sup>[8]</sup>, and we<sup>[9]</sup> have shown that, during coronary angioplasty, the severity of myocardial ischaemia during the second balloon inflation is less than that

during the first inflation, thus suggesting that ischaemic preconditioning may also occur in humans. Moreover, Yellon *et al.*<sup>[10]</sup> have recently confirmed ischaemic preconditioning in the human heart in the setting of coronary artery bypass surgery.

We have recently demonstrated that, in man, the ischaemic preconditioning observed during coronary angioplasty following repeated balloon inflations is abolished by pretreatment with glibenclamide, thus suggesting that the activation of ATP-sensitive K<sup>+</sup> channels plays an important role<sup>[11]</sup>. Several studies have shown, however, that preconditioning results from a complex series of events, involving not only ATP-sensitive K<sup>+</sup> channels<sup>[3,12–14]</sup> but also adenosine receptors<sup>[5,15–23]</sup>, G-proteins<sup>[24]</sup> and α<sub>1</sub>-adrenergic receptors<sup>[25]</sup>. In particular, numerous recent experimental studies have shown that activation of A<sub>1</sub> adenosine receptors mimics and their blockade abolishes ischaemic preconditioning<sup>[15,15–20]</sup>.

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Thus, A<sub>1</sub> adenosine receptors might also play an important role in preconditioning in humans, as recently shown in isolated human muscle<sup>[26]</sup>.

To establish in man the role played by A<sub>1</sub> adenosine receptors in preconditioning, we assessed the effect of bamiphylline, the most selective antagonist of A<sub>1</sub> receptors available for clinical use in Europe<sup>[27]</sup>, in patients undergoing repeated coronary occlusions in the setting of elective angioplasty of an isolated coronary stenosis.

## Methods

### Study patients

We studied 36 consecutive patients undergoing uncomplicated elective coronary angioplasty with: (1) history of chronic stable angina pectoris lasting  $\geq 3$  months; (2) isolated obstructive lesion (internal diameter reduction  $>70\%$  by visual assessment) in the proximal two-thirds of a major epicardial coronary artery; (3) no history of previous myocardial infarction; (4) no angiographic evidence of coronary collateral vessels (grade 0, according to Rentrop's classification)<sup>[28]</sup>. No patient had evidence of left ventricular hypertrophy on the echocardiogram or conduction defects on the electrocardiogram (ECG) that could have interfered with the interpretation of ST segment changes. All patients had normal hepatic and renal function, and fasting blood glucose levels. All patients gave written informed consent for participation in the study, which was approved by the Institutional Ethics Committee in April, 1993.

### Study protocol

In this double-blind study, patients were randomly allocated to two groups. One group consisted of 18 patients (16 men, 2 women; mean  $[\pm \text{SD}]$  age  $55 \pm 10$  years, range 34–71) who received an intravenous infusion of bamiphylline ( $5 \text{ mg} \cdot \text{kg}^{-1}$  in 20 min) (bamiphyllini hydrochloridum 300 mg/5 ml dissolved in 20 ml of 0.9% NaCl; Christiaens SA, Brussels, Belgium) after introduction of the femoral sheath, immediately prior to coronary angioplasty. The other group consisted of 18 patients (16 men, 2 women; mean  $[\pm \text{SD}]$  age  $52 \pm 9$  years, range 38–69) who received an intravenous infusion of placebo (20 ml of 0.9% NaCl in 20 min) after introduction of the femoral sheath, immediately prior to coronary angioplasty. As the half-life of intravenous bamiphylline is of approximately  $2 \text{ h}$ <sup>[29]</sup>, effective plasma levels of the drug were obtained during both balloon inflations. All patients were on oral aspirin (100 mg o.d.), diltiazem (60 mg t.i.d.) and isosorbide dinitrate (40 mg b.i.d.) for  $\geq 48 \text{ h}$  before coronary angioplasty. All patients received the morning dose of treatment prior to coronary angioplasty, which was performed within the following 4 h. No patient received sublingual

or intravenous nitrates in the last 24 h prior to the study or throughout the study. Patients were not premedicated with diazepam or other sedatives.

Coronary angioplasty of the stenosed artery was performed as previously described<sup>[11]</sup>. Briefly, after placement of the guiding catheter and performance of baseline angiography, the guide wire was placed across the lesion in the distal segment of the stenosed artery. The balloon catheter was then placed within the stenosis and the balloon was inflated for 2 min. After balloon deflation and withdrawal proximal to the lesion, with the guide wire still across the lesion, a recovery period of  $\geq 5 \text{ min}$  was allowed to re-establish baseline haemodynamic and ECG conditions. A second balloon inflation for 2 min was then performed. In each individual patient balloon pressure during the first and second inflation was identical. After the first two inflations, coronary angioplasty was completed on the basis of the specific needs of individual patients.

### Assessment of myocardial ischaemia

Standard surface 12-lead and intracoronary ECGs derived from the coronary guide wire were continuously monitored and simultaneously recorded (Mingograph 7, Siemens) at a paper speed  $25 \text{ mm} \cdot \text{s}^{-1}$  throughout the study. To avoid electrode interference with fluoroscopic imaging during the angioplasty procedure, radiotranslucent precordial electrodes were used. The ECGs were analysed by a cardiologist who had no knowledge of the study protocol. At baseline (with just the guide wire across the lesion) and at the end of the first two inflations, ST segment shift was measured 80 ms after the J point. The severity of myocardial ischaemia was expressed as: (1) the summation of the absolute values of the ST segment elevation and ST segment depression from baseline, on surface ECG, from all 12 leads; (2) the absolute values of ST segment elevation or ST segment depression from baseline on intracoronary ECG; (3) the summation of the absolute values of ST segment elevation and ST segment depression on both the surface and the intracoronary ECGs. ST segment shifts were expressed in millimetres ( $1 \text{ mm} = 0.1 \text{ mV}$ ).

### Assessment of cardiac pain

At the beginning of each coronary angioplasty procedure, patients were informed that they might develop chest pain. At the end of the first two balloon inflations, the intensity of cardiac pain was assessed by using a visual analogue scale<sup>[30]</sup>. Patients were asked to put a mark on a 100-mm scale marked from no symptoms to severe symptoms. The scale was measured from 0 to the subject's mark in millimetres.

### Statistical analysis

Two-factor analysis of variance (ANOVA) with repeated measures on one factor was used to compare

**Table 1** Clinical, anatomical and haemodynamic features

	Bamiphylline (n=18)	Placebo (n=18)
Age (years)	55 ± 10	52 ± 9
Male/female	16/2	16/2
Vessel disease (%)		
LAD	50	44
LCx	22	28
RCA	28	28
Heart rate, beats per minute		
Baseline	79 ± 15	79 ± 15
End of infusion	80 ± 11	79 ± 15
Inflation 1	79 ± 11	77 ± 14
Inflation 2	78 ± 11	76 ± 14
Mean aortic pressure (mmHg)		
Baseline	95 ± 12	96 ± 11
End of infusion	94 ± 10	96 ± 10
Inflation 1	96 ± 11	97 ± 12
Inflation 2	96 ± 12	96 ± 11

LAD=left anterior descending coronary artery; LCx=left circumflex coronary artery; RCA=right coronary artery.

haemodynamic and ECG data during balloon inflations in the two groups of patients. When significant differences were detected, pairwise comparisons were made using the Scheffé F-test. Comparisons of the remaining continuous or discrete variables between the two groups were performed using an unpaired Student's t-test or a  $\chi^2$  test, respectively. Visual-analogue scales were analysed using the Wilcoxon signed rank test or the Mann-Whitney U test as appropriate. Data are expressed as mean  $\pm$  1 SD; *P* values <0.05 were considered significant.

## Results

Clinical, anatomical and haemodynamic features in the two groups of patients are summarized in Table 1. During intravenous infusion of bamiphylline prior to coronary angioplasty, no patient experienced symptoms. In both bamiphylline- and placebo-treated patients, the values of heart rate and mean aortic pressure were similar at baseline, at the end of bamiphylline or placebo infusion and at the end of the first and the second inflations (Table 1).

### Coronary angioplasty

Coronary angioplasty was successfully performed in all 36 patients (residual stenosis <50%). The mean balloon pressure was similar in bamiphylline- and placebo-treated patients ( $4.4 \pm 1.2$  vs  $5.1 \pm 1.3$  atm, ns, respectively). The recovery period between the two balloon inflations was similar in bamiphylline- and placebo-treated patients ( $8.1 \pm 1$  vs  $8 \pm 2$  min, ns, respectively).

## Myocardial ischaemia

The ST segment shift values at baseline and at the end of the first two inflations, as changes from baseline, are reported in Table 2. In bamiphylline-treated patients, the mean ST segment shift during the second balloon inflation was similar to that during the first inflation on the surface ECG ( $7 \pm 9$  vs  $8 \pm 10$  mm, ns), the intracoronary ECG ( $7 \pm 8$  vs  $8 \pm 8$  mm, ns) and the surface plus intracoronary ECGs ( $14 \pm 15$  vs  $16 \pm 16$  mm, ns). Conversely, in placebo-treated patients, the mean ST segment shift during the second balloon inflation was significantly less than that during the first inflation on the surface ECG ( $5 \pm 4$  vs  $8 \pm 5$  mm, *P*<0.001), the intracoronary ECG ( $4 \pm 3$  vs  $9 \pm 5$  mm, *P*<0.001) and the surface plus intracoronary ECGs ( $10 \pm 6$  vs  $17 \pm 7$  mm, *P*<0.001) (Fig. 1). It is noteworthy that there was no significant difference between the two groups of patients in the degree of ST segment shift at baseline and at the end of the first inflation on either surface or intracoronary ECG (Table 2).

## Cardiac pain

In bamiphylline-treated patients, the severity of cardiac pain during the second inflation was similar to that during the first inflation ( $31 \pm 28$  vs  $31 \pm 29$  mm, ns). Conversely, in placebo-treated patients, the severity of cardiac pain during the second inflation was less than that during the first inflation ( $25 \pm 21$  vs  $39 \pm 31$  mm, *P*<0.01) (Fig. 2). Of note, in bamiphylline-treated patients cardiac pain severity at the end of the first inflation was less than that in placebo-treated patients, although the difference did not achieve statistical significance ( $31 \pm 29$  vs  $39 \pm 31$  mm, *P*=0.35) (Table 2).

## Discussion

This study shows that the adaptation to ischaemia during coronary angioplasty may be prevented by pre-treatment with bamiphylline, the most selective antagonist of A<sub>1</sub> adenosine receptors available for clinical use in Europe<sup>[27]</sup>. In fact, we found that, in bamiphylline-treated patients, the mean ST segment shift and the severity of cardiac pain at the end of the second balloon inflation were similar to those at the end of the first inflation, while in placebo-treated patients they were significantly less. Our findings, therefore, indicate that, in humans, A<sub>1</sub> adenosine receptors play an important role in ischaemic preconditioning, in agreement with the results of previous studies in animal models<sup>[5,15-20]</sup> and in isolated human muscle<sup>[26]</sup>.

### Pharmacology of bamiphylline

Bamiphylline, a 7,8-bisubstituted of aminophylline, has been used successfully to treat bronchial asthma and

**Table 2** ST-segment shift values and cardiac pain severity

	Bamiphylline (n=18)			Placebo (n=18)	
	Baseline	$\Delta$		Baseline	$\Delta$
ST segment shift on S-ECG (mm)					
Inflation 1	2 ± 2	8 ± 10	*	2 ± 2	8 ± 5
Inflation 2	2 ± 1	7 ± 9		2 ± 1	5 ± 4‡
ST segment shift on IC-ECG (mm)					
Inflation 1	2 ± 2	8 ± 8	*	2 ± 2	9 ± 5
Inflation 2	2 ± 2	7 ± 8		2 ± 2	4 ± 3‡
ST segment shift on S- plus IC-ECG (mm)					
Inflation 1	4 ± 3	16 ± 16	†	4 ± 3	17 ± 7
Inflation 2	4 ± 2	14 ± 15		4 ± 3	10 ± 6‡
Pain severity (mm)					
Inflation 1	—	31 ± 29	*	—	39 ± 31
Inflation 2	—	31 ± 28		—	25 ± 21§

\* $P < 0.05$ ; † $P < 0.01$ , bamiphylline vs placebo (changes from inflation 1 to inflation 2). ‡ $P < 0.001$  vs inflation 1 value; § $P < 0.01$  vs inflation 1 value.

IC-ECG = intracoronary electrocardiogram; S-ECG = surface 12-lead electrocardiogram;  $\Delta$  = changes from baseline values.

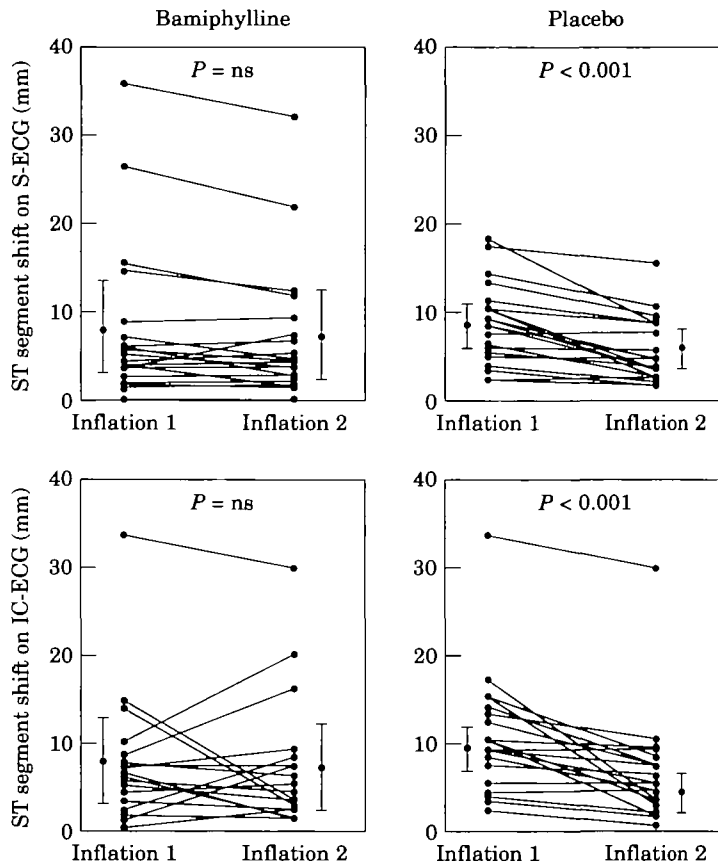
lung anaphylaxis in young children and infants<sup>[31]</sup> and chronic obstructive pulmonary disease in adults with an efficacy comparable to that of aminophylline but with substantially fewer side-effects<sup>[32,33]</sup>. The therapeutic threshold of bamiphylline is almost 50 times lower than that of aminophylline ( $0.2 \mu\text{g} \cdot \text{ml}^{-1}$  vs  $10 \mu\text{g} \cdot \text{ml}^{-1}$ ), whereas the tolerance range is almost 100 times wider<sup>[34,35]</sup>. In crude synaptic membranes prepared from rat brain, bamiphylline displaces radioligands from  $A_1$  adenosine receptors with a potency similar to that of 8-phenyl-theophylline, whereas it shows a much lower potency on  $A_2$  adenosine receptors. This results in a high degree of  $A_1$  receptor selectivity indicated by an  $A_2/A_1$  ratio of 596<sup>[27]</sup>. A critical issue in our study was the choice of an appropriate dose of bamiphylline. At the dose used in the present study the mean plasma concentration of bamiphylline (about  $0.5 \times 10^{-5}$  M) displaces 80% of  $^3\text{H}$ -Diethyl-8-phenyl-xanthine (an antagonist of  $A_1$  adenosine receptors), 50% of  $^3\text{H}$ -Cyclo-hexyl-adenosine (an agonist of  $A_1$  adenosine receptors), but only 5% of  $^3\text{H}$ -5'-N-ethyl-carboxamino-adenosine (an agonist of  $A_2$  adenosine receptors)<sup>[27]</sup>. Thus, at the dose used in this study, bamiphylline results in a rather selective blockade of  $A_1$  adenosine receptors. Accordingly, we have recently shown that, at this dose, bamiphylline does not suppress adenosine-induced cutaneous vasodilation<sup>[36]</sup>, or adenosine-induced coronary vasodilation<sup>[37]</sup>, which are both mediated by vascular  $A_2$  receptors<sup>[38]</sup>. Thus, it is very unlikely that, in our patients, bamiphylline prevented ischaemic preconditioning by limiting an  $A_2$  receptor-mediated increase of coronary blood flow. Finally, we cannot exclude that ischaemic preconditioning was prevented through blockade of  $A_3$  receptors, which appear to mediate preconditioning in rabbits<sup>[21,22]</sup>; however, the affinity of bamiphylline for  $A_3$  receptors has not yet been investigated.

As  $A_1$  adenosine receptor blockade by bamiphylline prevents the algogenic effects of adenosine<sup>[36,37]</sup>, it is somewhat intriguing that, although in our patients treated with bamiphylline pain severity during the first inflation was less than that in patients treated with placebo, this difference did not achieve statistical significance in the presence of similar electrocardiographic changes in the two groups of patients. However, we have previously shown that during coronary angioplasty the anginal pain is also determined by the mechanical stretching of the arterial wall<sup>[9]</sup>, thus probably accounting for the partial failure of bamiphylline to reduce the anginal pain in this study.

We have also previously shown that bamiphylline improves exercise-induced myocardial ischaemia<sup>[39]</sup>, while in this study the severity of ischaemia during the first balloon inflation was similar following placebo or bamiphylline. This difference is probably related to the different pathophysiology of ischaemia caused by an increase of myocardial oxygen consumption as opposed to that caused by coronary occlusion.

### *Role of $A_1$ adenosine receptors in ischaemic preconditioning in man*

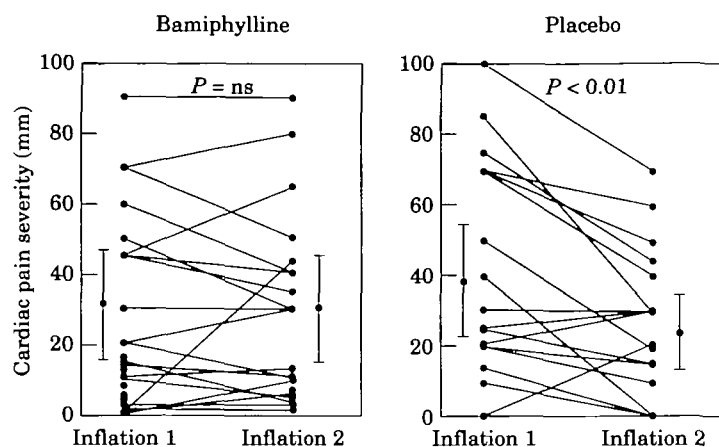
$A_1$  adenosine receptors are present in perivascular sympathetic nerves and in cardiomyocytes<sup>[40,41]</sup>. The blockade by bamiphylline of  $A_1$  adenosine receptors located in the perivascular nerves causes an increase in catecholamine release<sup>[42,43]</sup> and might influence the severity of myocardial ischaemia during balloon occlusion in two different ways. First, an increase of catecholamine release may increase myocardial oxygen consumption, thus worsening the severity of myocardial ischaemia during coronary occlusion. However, if this were the



**Figure 1** Plots of individual values of ST segment shifts on the surface ECG (S-ECG) and the intracoronary ECG (IC-ECG) at the end of the first and second balloon inflations in the two groups of patients. In bamiphylline-treated patients, ST segment changes at the end of the second balloon inflation were similar to those at the end of the first inflation. Conversely, in placebo-treated patients, ST segment changes at the end of the second balloon inflation were significantly less than those at the end of the first inflation. Of note, two bamiphylline-treated patients had zero ST segment shift value on S-ECG during both inflations.

case we should have obtained greater electrocardiographic changes and more severe pain in bamiphylline-treated patients also at the end of the first inflation. Instead, the magnitude of ischaemic electrocardiographic changes, the severity of pain and the systemic haemodynamic parameters at the end of the first inflation were similar in bamiphylline- and in placebo-treated patients. Second, an increase of catecholamine release may enhance preconditioning, as Banerjee *et al.*<sup>[25]</sup> demonstrated in isolated rat hearts that  $\alpha_1$ -adrenergic receptors stimulation can mimic preconditioning. However, if this were the case,  $A_1$  adenosine receptor blockade by bamiphylline should have resulted in cardioprotection rather than prevention of preconditioning. Thus, it would appear that the prevention of preconditioning by bamiphylline observed in our study, during repeated brief periods of coronary occlusion, is not mediated by the blockade of  $A_1$  adenosine receptors located on perivascular sympathetic nerves, but, more

likely, by  $A_1$  or, perhaps,  $A_3$  receptors<sup>[21,22]</sup> located on the surface of myocardial fibres. Indeed, experimental studies have shown that  $A_1$  adenosine receptors appear to mediate ischaemic preconditioning by directly enhancing the opening of the ATP-sensitive  $K^+$  channels<sup>[12,19]</sup>. In rat ventricular myocytes, Kirsch *et al.*<sup>[44]</sup> showed, indeed, that  $A_1$  adenosine receptors are coupled to ATP-sensitive  $K^+$  channels. Moreover, in dogs, glibenclamide, an ATP-sensitive  $K^+$  channel blocker, not only prevents ischaemic preconditioning, but also  $A_1$  adenosine receptor-mediated cardioprotection<sup>[12,45]</sup> and attenuates adenosine-induced bradycardia<sup>[46]</sup>. In agreement with the results of experimental observations<sup>[3,13,14]</sup>, we have demonstrated in a previous study that glibenclamide completely abolishes the ischaemic preconditioning observed in humans during brief repeated coronary occlusions<sup>[11]</sup>. Thus, in man, both bamiphylline and glibenclamide appear to prevent ischaemic preconditioning. How  $A_1$  adenosine receptors



**Figure 2** Plots of individual values of cardiac pain severity at the end of the first and second balloon inflations in the two groups of patients. In bamiphylline-treated patients, cardiac pain severity during the second balloon inflation was similar to that during the first inflation. Conversely, in placebo-treated patients, cardiac pain severity during the second balloon inflation was significantly less than that during the first inflation. Of note, two bamiphylline-treated patients had no pain during either inflation.

and ATP-sensitive  $K^+$  channels interact in humans in determining preconditioning cannot be deduced from the results of our studies.

### Study limitations

We based the assessment of myocardial ischaemia on the electrocardiographic changes which do not represent direct evidence of ischaemia and on the anginal pain severity which is rather subjective. However, the surface 12-lead and the intracoronary ECGs represent well accepted methods for the evaluation of myocardial ischaemia during coronary angioplasty<sup>[7-9,47-49]</sup>. Moreover, we compared ST segment changes in the same patient where other variables being constant, the most important parameter determining the degree of ST segment shift appears to be the severity of myocardial ischaemia, as previously shown in experimental studies<sup>[50,51]</sup>. Regarding the assessment of the anginal pain, the visual analogue scale is a well accepted method for the evaluation of pain perception<sup>[30]</sup>, which we utilized in several previous studies<sup>[9,36,37,52]</sup>. Finally, it is possible that the adaptation to ischaemia observed in this human model is mediated by progressive collateral recruitment<sup>[8]</sup>, which we did not assess in our study. However, if this were the case, it would be difficult to explain the ability of bamiphylline to prevent the adaptation to ischaemia as, at the dose used in this study, bamiphylline does not appear to antagonise  $A_2$  receptors, which mediate the vascular effects of adenosine<sup>[37]</sup>. Of note, the results of our study appear to confirm the observation that ischaemic preconditioning in isolated human muscle, a model where vascular effects can be ruled out, is mediated by  $A_1$  adenosine receptors<sup>[26]</sup>.

### Conclusions and clinical implications

In patients with stable angina pectoris undergoing elective coronary angioplasty, ischaemic preconditioning occurring during brief repeated coronary occlusions is prevented by bamiphylline, a selective antagonist of  $A_1$  adenosine receptors. These findings indicate that, in humans,  $A_1$  adenosine receptors may play an important role in ischaemic preconditioning. Our study suggests that adenosine antagonists should be used with caution in those patients with ischaemic heart disease in whom ischaemic preconditioning is likely to play an important cardioprotective role, i.e. those with unstable angina or undergoing coronary artery bypass surgery.

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