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Differential anti-ischaemic effects of muscarinic receptor blockade in patients with obstructive coronary artery disease

Impaired vs normal left ventricular function

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Aims In patients with coronary artery disease acetylcholine (a muscarinic agonist) causes vasoconstriction. The effect of atropine (a muscarinic antagonist) on coronary vasotone in patients with normal or impaired left ventricular function is unknown.

Methods and Results Twenty-four patients who required atropine infusion (to supplement heart rate response) during atrial pacing (pacing was conducted to assess ischaemia as part of an experimental protocol) were studied; 17 patients had normal and seven impaired left ventricular function (ejection fraction $\leq 0{\cdot}40$). Two control groups were selected from a large database (from patients in whom atrial pacing was carried out but to whom atropine was not administered) to match the normal (n=20) and dysfunction (n=10) groups. In the normal left ventricular function group atropine increased rate pressure product by $12 \pm 4\%$, as compared to those without atropine ($P<0{\cdot}05$). Left ventricular end diastolic pressure increased less in the atropine group ($+40 \pm 8\%$ vs $+78 \pm 6\%$; $P<0{\cdot}05$). Arterial norepinephrine increased similarly in both groups, but coronary flow (as assessed by using a thermodiluting method in the coronary sinus) increased $23 \pm 4\%$ more in the atropine group ($P<0{\cdot}05$). Further, there were lower levels of myocardial lactate production and ST-segment depression in the atropine group [lactate extraction $+13 \pm 6\%$ (atropine) vs $-19 \pm 4\%$ (controls), ST-segment depression $1{\cdot}3 \pm 0{\cdot}6$ (atropine) vs $1{\cdot}8 \pm 0{\cdot}2$ mm (control), both $P<0{\cdot}05$ between groups]. In contrast, in the dysfunction group the overall effect of atropine was less pronounced.

Conclusion In patients with normal left ventricular function atropine improves coronary flow and reduces myocardial lactate production and ST-segment depression during atrial pacing, suggesting a reduction in myocardial ischaemia.

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Key Words: Coronary disease, endothelial function ischaemia, muscarinic antagonists.

Introduction

Experimental and clinical studies have shown that the coronary flow is affected by autonomic tone[1–3], and both sympathetic and vagal fibres have been identified in the coronary vasculature[4–5]. In normal coronary arteries, intracoronary injection of acetylcholine elicitates vasodilatation[6,7] through the release of endothelial derived relaxing factor (nitric oxide) by the endothelium. Acetylcholine stimulates the muscarinic receptors, which are part of the parasympathetic nervous system, and muscarine receptors (subtype M_3) can be seen in the coronary arteries[8,9]. The release of endothelial derived relaxing factor has been linked to muscarine receptor activation[6–9]. In patients with coronary artery disease, however, acetylcholine causes vasoconstriction but not vasodilatation. This method can be used to discriminate between normal and diseased endothelium[10,11].

The atrial pacing stress test is a reliable model for inducing myocardial ischaemia in patients with coronary

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artery disease[12,13]. In patients who develop atrioventricular block, however, this model is somewhat limited, since induction (and consequently) exclusion of ischaemia is dependent on the maximally achieved heart rate. Atropine, which is a non-selective muscarinic (parasympathetic) antagonist[14], may be used in these patients to increase maximal heart rate. Whether atropine by itself effects the coronary vasculature and myocardial ischaemia is unknown. In addition, it is unclear whether abnormalities in baseline autonomic tone, as is the case in patients with impaired left ventricular function, affects the influence of atropine.

In the present study we examined the effects of atropine in patients with coronary artery disease, who either had normal or impaired left ventricular function. We therefore studied lactate extraction, intracardiac pressures, ST-segment changes on the electrocardiogram, and plasma neurohormones during fast atrial pacing.

Methods

Design of the study

All patients in the present study had signs of ischaemia ($\geq$1 mm ST-segment depression) during exercise. Patients had undergone coronary arteriography for clinical indications. They had at least a single >70% stenosis. Following the initial coronary arteriography, atrial pacing stress testing was performed to evaluate myocardial ischaemia and perfusion as part of an experimental protocol. We studied 24 patients who required additional atropine infusion (to supplement heart rate response) during incremental atrial pacing, of whom 17 had a normal left ventricular ejection fraction (>0·40, determined by angioventriculography) and seven had an impaired left ventricular function (left ventricular ejection fraction $\leq$0·40). Two age-, sex-, ejection fraction- and severity of coronary artery disease- control groups were selected from a large database (from patients in whom atrial pacing was performed but atropine was not administered) to match the normal (n=20) and left ventricular dysfunction (n=10) groups. The investigation conforms with the principles outlined in the Declaration of Helsinki. The protocol was approved by the local Institutional Ethical Review Committee and all patients gave their written informed consent.

Patients

All patients underwent coronary angiography because of a history of angina pectoris and signs of ischaemia during stress testing. Patients in the present study were eligible if they had $\geq$1 stenosis of $\geq$70% in at least one left-sided epicardial coronary artery, i.e. the left anterior descending, or the left circumflex artery. All cardiovascular drugs were withdrawn for >5 half-lives prior to the study, except for short-acting nitroglycerine which was permitted until 5 h before the study.

Exclusion criteria were unstable angina pectoris, hypertension (systolic blood pressure $\geq$180 mmHg and/or a diastolic blood pressure $\geq$100 mmHg), advanced heart failure (New York Heart Association functional class III and IV), recent myocardial infarction (<3 months), conduction disturbances, valvular heart disease, insulin-dependent diabetes mellitus, significant renal dysfunction, (serum creatinine $\geq$150 μmol . l^{-1} and chronic obstructive pulmonary disease.

Catheterization procedure

Left and right-sided cardiac catheterization and coronary angiography were performed through the femoral artery and vein, as previously described in detail[12,13]. In short, a triple lumen 7 Fr Swan Ganz catheter was advanced to the pulmonary artery. In addition a 7 Fr coronary sinus thermodilution and pacing catheter was positioned in the mid portion of the coronary sinus. This catheter was inserted to measure coronary flow and collect blood, and for atrial pacing. Finally a 7 Fr Sentron pigtail micromanometer catheter was introduced into the left ventricle.

Study protocol

After positioning the catheters, a stabilization period was allowed so that there was a minimum interval of $\geq$40 min between preceding coronary angiography and the study. After the stabilization period, control measurements were carried out. Subsequently, the atrial pacing stress test was performed with increments in heart rate of 10 beats . 2 min^{-1} until angina pectoris developed, >2 mm ST-segment depression occurred or a heart rate of 170 beats . min^{-1} was reached. If atrioventricular dissociation (Wenckebach) occurred before this time, atropine (0·5 mg intravenously) was administered at a pacing rate of 120 beats . min^{-1}. All haemodynamic and electrocardiography measurements were repeated at maximal pacing rate.

Haemodynamic measurements

After calibration, all pressures, the first derivative of left ventricular systolic pressure, cardiac output, coronary flow and the electrocardiogram were recorded on paper, using a standard catheterization laboratory system[12]. In addition, haemodynamic parameters were determined on-line by a catheterization laboratory computer system[12], as an index of myocardial oxygen demand, rate pressure product was calculated. Coronary sinus blood flow was determined during a continuous 30 s infusion of 30 ml saline at room temperature[15]. We also calculated the rate pressure product/coronary sinus blood

Table 1 Patients' characteristics

	Normal LV (controls)	Normal LV (atropine)	LV dysfunction (controls)	LV dysfunction (atropine)
Sex (male/female)	20/0	17/0	9/1	6/1
Age (years)	60 ± 2	60 ± 3	59 ± 2	58 ± 4
LV ejection fraction	0·54 ± 0·02	0·56 ± 0·04	0·36 ± 0·02	0·38 ± 0·02
Previous MI	8 (40%)	8 (47%)	9 (90%)	6 (86%)
Coronary stenosis >70%:				
1-vessel	8 (40%)	7 (41%)	2 (20%)	1 (14%)
2-vessel	8(40%)	7 (41%)	3 (30%)	2 (29%)
3-vessel	4 (20%)	3 (18%)	5 (50%)	4 (57%)

mean ± SD (%); LV=left ventricular; MI=myocardial infarction.

flow ratio as an index of the balance of oxygen demand/supply. Calculations were made from mean flow curves, using standard formulae[16]. The electrocardiogram was monitored continuously and ST-segments were determined at a paper speed of 100 mm . s^{-1} in five successive beats, 60 ms after the J point of the QRS complex.

Neurohumoral and metabolic sampling

Simultaneous sampling of arterial and coronary sinus blood, in chilled tubes, was carried out for the measurements of (nor)epinephrine, angiotensin II, oxygen and lactate, as previously described[12,13]. Myocardial oxygen consumption (MVO_2) and cardiac balance of neurohormones were determined as follows: (arterial concentration–coronary venous concentration) × coronary sinus blood flow. Myocardial lactate metabolism was calculated as (arterial lactate concentration–coronary venous lactate concentration)/arterial concentration × 100%; a positive value denotes lactate uptake and a negative value denotes net lactate production.

Statistical analysis

Data are presented as mean value ± standard deviation. The changes in values between measurements during pacing and baseline were calculated. A two-way analysis of variance (ANOVA) multiple comparison with Bonferroni correction was performed. A two-tailed P value <0·05 was considered significant.

Results

Of the 24 patients who received atropine seven had left ventricular dysfunction (ejection fraction ≤0·40; mean0·38 ± 0·02) and 17 patients normal left ventricular function (ejection fraction >0·40; mean 0·56 ± 0·04) (Table 1). These 24 were matched with 30 control patients who did not receive atropine, of whom 20 had a normal left ventricular function (ejection fraction 0·54 ± 0·04) and 10 left ventricular dysfunction (ejection fraction 0·36 ± 0·02). During pacing all patients experienced myocardial ischaemia as defined by ≥1 mm ST-segment depression. Reasons for discontinuing pacing were angina pectoris in 74% of the patients, >2 mm ST-segment depression in 20% and target maximal heart rate in the remaining patients. Reasons for stopping were not significantly different between groups. Atropine was administered at a mean pacing frequency of 120 beats . min^{-1}.

Effects of atropine in patients with normal left ventricular function

Baseline haemodynamics were comparable in the atropine and control groups. In the control group, maximal heart rate during pacing was 145 ± 4 beats . min^{-1}, and rate pressure product increased by 90 ± 10%; MVO_2 increased 55 ± 6% (Table 2). At maximal pacing coronary sinus blood flow was 177 ± 18 ml . min^{-1}. In the atropine group, maximal heart rate during pacing was 155 ± 8 beats . min^{-1} (+7 ± 2% vs control patients) and rate–pressure product was 12 ± 4% higher; MVO_2 increased 64 ± 11% (all P<0·05). In addition, coronary sinus blood flow increased 23 ± 10% further in the atropine group, as compared to the control group (P<0·05) (Fig. 1). Although the ratio rate pressure product: coronary sinus blood flow was slightly lower in the atropine group than in the control group (1·3 ± 0·4 vs 1·4 ± 0·6, respectively) this difference was not statistically significant. During pacing left ventricular end-diastolic pressure increased in all groups, but significantly more in the controls than in the atropine group (78 ± 6% vs 40 ± 8%; P<0·05) (Fig. 2).

Arterial norepinephrine increased during pacing in all groups [+361 ± 88 (atropine) vs +354 ± 46 (control) pg . ml^{-1}; P=ns]. Arterial epinephrine increased slightly more in the control group than in the atropine group (+122 ± 22 vs +75 ± 18 pg . ml^{-1}, respectively, P<0·05 control vs atropine. In contrast, angiotensin II increased only in the atropine group (+2·6 ± 2·4 pg . ml^{-1}) but decreased (−0·4 ± 0·8 pg . ml^{-1}) in the control group (P<0·05 atropine vs

Table 2 Oxygen demandlsupply

		Baseline	Maximal pacing
RPP × 1000 (mmHg . min^{-1})	Normal LV function — control	10 ± 0·6	20 ± 0·8
	Normal LV function — atropine	11 ± 1·2	23 ± 2·0#
	LV dysfunction — control	12 ± 0·8	21 ± 1·2
	LV dysfunction — atropine	10 ± 1·6	20 ± 1·4
CVR (mm Hg . ml^{-1} . min^{-1})	Normal LV function — control	1·05 ± 0·1	0·75 ± 0·1
	Normal LV function — atropine	0·83 ± 0·1	0·57 ± 0·1#
	LV dysfunction — control	0·80 ± 0·1	0·58 ± 0·1
	LV dysfunction — atropine	1·06 ± 0·3	0·66 ± 0·2
MVO_2 (ml . min^{-1})	Normal LV function — control	14·2 ± 1·4	22·0 ± 2·4
	Normal LV function — atropine	16·5 ± 4·0	27·1 ± 7·4△
	LV dysfunction — control	20·8 ± 4·0	31·9 ± 6·6
	LV dysfunction — atropine	12·8 ± 4·0	23·0 ± 8·8△

#P<0·05 control vs atropine, for normal LV or LV dysfunction; △P<0·05=percentage of change during pacing in control group vs atropine group, for normal LV or LV dysfunction. CVR=coronary vascular resistance; LV=left ventricular; MVO_2=myocardial oxygen consumption; RPP=rate pressure product.

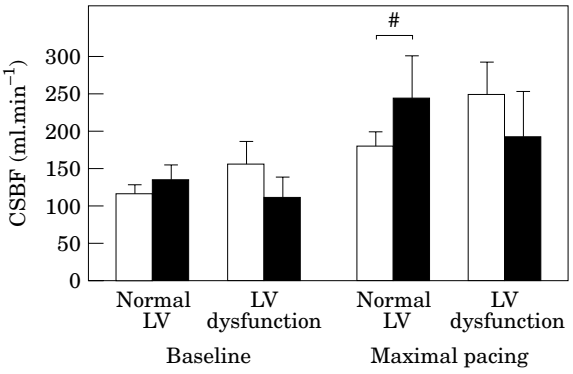

Figure 1 **□=control patients; ■=patients who received atropine. Coronary sinus blood flow (CSBF) at baseline and maximal pacing. #P<0·05 control vs atropine, for the normal left ventricular group.**

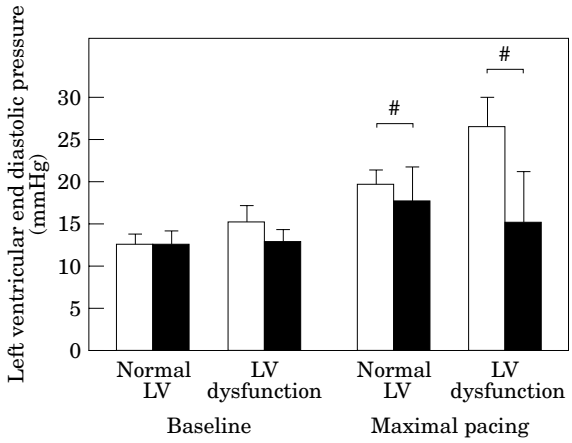

Figure 2 **□=control patients; ■=patients who received atropine. Left ventricular end diastolic pressure (mmHg) at baseline and maximal pacing. #P<0·05 control vs atropine, for the normal left ventricular group and the left ventricular dysfunction group.**

controls). The cardiac balance of norepinephrine decreased only in the atropine group (Fig. 3). The cardiac balance of other neurohormones was not significantly affected by atropine.

Myocardial lactate production was lower in the atropine group [lactate extraction +13 ± 12% (atropine) vs −19 ± 8% (control)] (P<0·05) (Fig. 4(a)). ST-segment depression was 1·8 ± 0·2 mm in the control group and 1·3 ± 0·6 mm in the atropine group (P<0·05 among groups) (Fig. 4(b)).

Effects of atropine in patients with left ventricular dysfunction

Baseline haemodynamics were comparable in the atropine and control groups. In the control group, peak heart rate during pacing was 146 ± 6 beats . min^{-1} and rate pressure product and MVO_2 increased 75 ± 10% and 53 ± 10% respectively. At maximal pacing coronary sinus blood flow was 245 ± 44 ml . min^{-1}. In the atropine group, both peak heart rate and rate pressure product were comparable to the control group, but MVO_2 increased further (80 ± 23%; P<0·05 vs controls). In addition, coronary sinus blood flow increased to a similar extent in all groups (Fig. 1). The ratio of rate pressure product: coronary sinus blood flow was similar in all groups (1·1 ± 0·4). During pacing, left ventricular end-diastolic pressure increased significantly more in the controls than in the atropine group (+94 ± 10% vs +14 ± 4%; P<0·05) (Fig. 2).

Arterial norepinephrine increased during pacing in all groups [+432 ± 92 pg . ml^{-1} (atropine) vs +501 ± 98 pg . ml^{-1}; P=ns] but arterial epinephrine increased further in the control group (+138 ± 28 pg . ml^{-1}) than

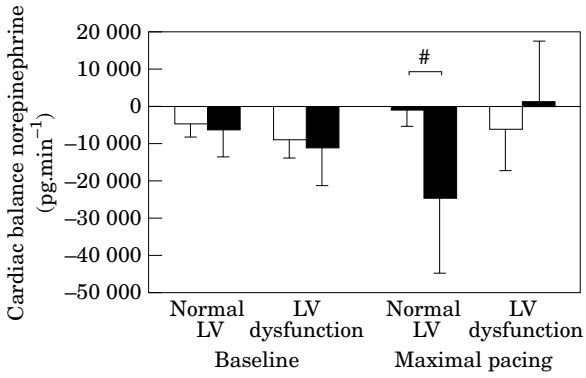

Figure 3 **□=control patients; ■=patients who received atropine. Cardiac balance of norepinephrine ([arterial minus coronary venous norepinephrine concentration] × coronary sinus blood flow) at baseline and maximal pacing. #$P<0{\cdot}05$ control vs atropine, for normal left ventricular group.**

in the atropine group ($+77 \pm 20$ pg . ml^{-1}) ($P<0{\cdot}05$) between groups. Arterial angiotensin II and cardiac balance of neurohormones was not affected by atropine.

Myocardial lactate metabolism was comparable in all groups [lactate extraction $-18 \pm 18\%$ (atropine) vs $-19 \pm 14\%$ (controls); P=ns] (Fig. 4(a)). ST-segment depression was $1{\cdot}6 \pm 0{\cdot}4$ mm in the atropine group and $1{\cdot}5 \pm 0{\cdot}4$ mm in the control group (P=ns between groups) (Fig. 4(b)).

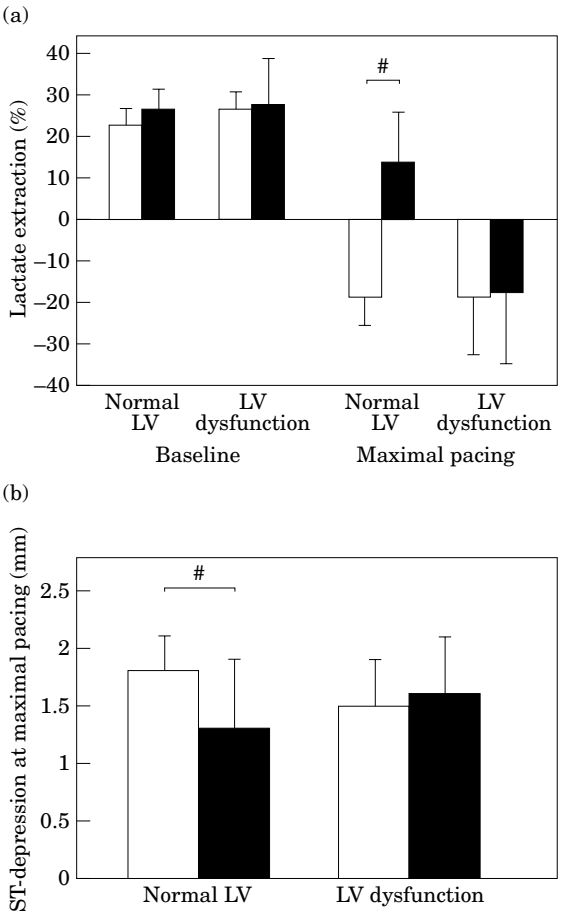

Figure 4 **□=control patients; ■=patients who received atropine. (a) Lactate extraction (when negative: lactate production by the heart; when positive there is lactate uptake) at baseline and immediately after maximal pacing; (b) ST-segment depression (mm) at maximal pacing. #$P<0{\cdot}05$ control vs atropine, for normal left ventricular group.**

Discussion

Acetylcholine (the universal muscarinic agonist) is widely used in cardiovascular disease to discriminate between healthy and diseased endothelium. The same applies to atropine (the universal muscarinic antagonist) which, in addition, is used as an adjunct in the setting of dobutamine stress echocardiography. Atropine may also exert endothelium-mediated effects, but this has not been studied to our knowledge. Further, because atropine has parasympatholytic effects, the baseline parasympathetic tone (which is reduced in left ventricular dysfunction/heart failure) might influence the effects of atropine.

In the present study, blockade of muscarinic receptors by atropine results in pronounced anti-ischaemic effects in patients with coronary artery disease and normal left ventricular function, while the effect in those with left ventricular dysfunction was mostly absent. This result was obtained despite similar systemic sympathetic activation (as measured by arterial catecholamines) and more pronounced oxygen demand during atrial pacing test.

The primary determinants of coronary blood flow are coronary perfusion pressure, myocardial oxygen demand, extravascular forces (wall stress) and neurohumoral activation[17]. The results of the present study are primarily attributable to a neural reflex mediating cholinergic (parasympathetically controlled) vasoconstriction. Marraccini *et al.* showed that atropine, given intravenously to patients with a positive exercise test, increased rate pressure product, but did not aggravate myocardial ischaemia, probably caused by an increase in coronary blood flow[18]. In the present study, the effects of atropine on coronary sinus blood flow were less pronounced in patients with left ventricular dysfunction, which may be related to the decreased parasympathetic tone[19,20] or the increased left ventricular wall stress[21] in patients with left ventricular dysfunction/heart failure.

The observation of Furchgott *et al.*[22] regarding the key role of the endothelium in the regulation of coronary vascular tone has emphasized the importance of tone disturbances in de-endotheliated coronary atherosclerotic vessel segments. Evidence exists that the endothelium is the source of the endothelium-derived relaxing factor which counterbalances direct constrictor stimuli of the muscular wall of the vessel[23]. Several agents, such as acetylcholine, platelet products,

neuropeptides, hormones and physicochemical stimuli, have at the same time been shown to exert an endothelium-mediated vasodilating effect and a direct constrictor effect on vascular smooth muscle[23,24]. Experimental data, which shows that adventitial application of acetylcholine on coronary arteries can produce endothelium-mediated vasodilation[25], may be the key factor in a possible pathophysiological interpretation of the impairment of local acetylcholine leading to coronary dilation in coronary artery disease[26]. On this basis, increased coronary tone during exercise might be related to the persistence of direct parasympathetic coronary vasoconstriction[27] that is not counterbalanced by adequate production of endothelium-derived relaxing factor and this may lead to myocardial perfusion defects[28]. When adding the muscarinic antagonist atropine, the coronary vasoconstriction may be reversed, as the results of the present study suggest, by decreasing the parasympathetic coronary vasoconstriction[27].

The more pronounced decrease in left ventricular end-diastolic pressure by atropine in the left ventricular dysfunction group in the present study, as compared to the normal left ventricular function group, might be caused by the fact that ischaemia in left ventricular dysfunction already plays an important role and therefore is more affected by atropine administration. The positive inotropic effects of atropine[14] may explain this change, showing viability of myocardium in these left ventricular dysfunction patients, which was accompanied by more oxygen consumption (MVO_2). This effect of atropine is well known when this drug is used as an adjunct to dobutamine in stress echocardiography to identify viable myocardium[29].

In a previous study, it was reported that ischaemia significantly changed net cardiac norepinephrine release, as present at baseline, to net uptake in patients with the most myocardial ischaemia, suggesting an alteration in norepinephrine kinetics in the ischaemic myocardium[30]. In the present study, patients with a normal left ventricular function, receiving atropine, released the most cardiac norepinephrine. This is in accordance with the finding that these patients did not produce lactate, and thus were not ischaemic, during the atrial pacing stress test. Another explanation for the present results is the known presynaptic inhibiting effect on the release of norepinephrine by the parasympathetic system[31]. This effect is blocked by atropine, which indirectly facilitates the presynaptic release of norepinephrine. In the present model the vasoconstricting actions of the increased release of norepinephrine ($alpha_1$-mediated), is probably counterbalanced by the blocking effects of M_3 receptors on smooth muscle cells in the vessel wall, resulting in coronary vasodilatation and less ischaemia.

In conclusion, in patients with normal left ventricular function atropine improves coronary flow and reduces myocardial lactate production and ST-segment depression during atrial pacing, suggesting a reduction in myocardial ischaemia. However, atropine cannot be proposed as a drug for the treatment of effort ischaemia because of its concomitant effect on myocardial oxygen demand. Non-selective muscarinic blockers also would not be suitable because of their inhibitory effects on vagal tone to the pacemaker and conducting system tissues, given that reduced vagal tone is associated with increased sudden death mortality[19]. The search for antimuscarinic agents with selective affects coronary vessels (M_3), but not on sinus node automaticity (M_2), might be interesting from the perspective of defining an alternative physiological approach to the treatment of effort ischaemia.

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