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Dobutamine administration exacerbates postischaemic myocardial dysfunction in isolated rat hearts: an effect reversed by thyroxine pretreatment

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Abstract

The present study has investigated the effects of dobutamine on postischaemic dysfunction in the setting of global ischaemia and reperfusion in a model of isolated heart preparation. Isolated rat hearts were subjected to 20 min of zero-flow global ischaemia followed by 45 min of reperfusion. Dobutamine administration (10 μg/kg/min) during the reperfusion period resulted in deterioration of functional recovery, which was abolished by propranolol administration. Long-term thyroxine pretreatment (12.5 μg 100 g^{-1} body weight, b.i.d., s.c., for 2 weeks) reversed the detrimental effect of dobutamine and increased postischaemic recovery of function. We conclude that the combination of thyroxine pretreatment and dobutamine administration could potentially be a new therapeutic strategy to improve postischaemic dysfunction particularly in clinical settings such as cardiopulmonary bypass and/or myocardial infarction.

Keywords: Inotrope; Ischaemia; Dobutamine; Thyroxine; Heart, rat

1. Introduction

Positive inotropic agents are currently considered as the most effective treatment for supporting haemodynamics in various clinical conditions, particularly in the setting of myocardial infarction and in aortocoronary bypass surgery. However, most of those agents and particularly the β-adrenoceptor agonists exert their action at the expense of a disproportionate increase in the myocardial oxygen requirements, a phenomenon known as the oxygen-wasting effect (Suga et al., 1983; Vanoverschelde et al., 1993). This effect is of clinical relevance in conditions in which there is a critical balance between the oxygen supply and demand. In fact, imposing an imbalance on the ischaemic myocardium might further jeopardize the metabolic integrity of myocardial cells and facilitate irreversible damage. In addition, this adverse effect might also be exacerbated by alterations in calcium handling that are found to occur in postischaemic myocardium in which the activity of sarcoplasmic reticulum Ca^{2+}-ATPase is reported to be reduced (Meissner and Morgan, 1995). Therefore, one could raise the question as to whether inotropes with β-adrenoceptor agonist action can actually improve myocardial function in an already ischaemic heart.

Dobutamine is one of the most potent inotropes, which are clinically available and widely used for the haemodynamic support in various clinical conditions. Dobutamine is a cardioselective β-adrenoceptor agonist that increases cardiac output and myocardial blood flow with little change in vascular resistance and heart rate. Its inotropic effect has been reported to be associated with low energy efficiency of the heart and increased myocardial adenosine release suggesting that treatment with this agent can reduce the oxygen supply vs. demand ratio (Vanoverschelde et al., 1993; Ko et al., 1993). These properties might

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be undesirable for the haemodynamic support of the ischaemic myocardium and probably limit the use of dobutamine as a suitable agent in providing the optimum therapeutic strategy for the failing ischaemic myocardium. However, one could hypothesize that preischaemic cardioprotective interventions that modify the reperfusion state following sustained ischaemia could potentially improve the dobutamine effect. This hypothesis has not been addressed in the current literature. Therefore, our study was designed to investigate the effects of dobutamine on postischaemic myocardium and consequently whether cardioprotective interventions such as thyroxine (Pantos et al., 2000, 2002a) pretreatment can modify this effect.

2. Methods

2.1. Thyroxine, dobutamine and propranolol administration

L-Thyroxine (T4) (Sigma, St. Louis, MO, USA) was dissolved in 99% ethanol by adding a small volume (20 μl) of 25% NaOH, and diluted 33 times by adding 0.9% NaCl to obtain a stock solution of 1 mg ml^{-1}. Before each injection, a fresh solution was made in 0.9% NaCl to a concentration of 50 μg T4 ml^{-1}. Thyroxine 12.5 μg 100 g^{-1} body weight was given subcutaneously at 08.00 and 20.00 h daily for 14 days. This treatment results in a long-term, moderate hyperthyroidism (Grofte et al., 1997; Pantos et al., 1999, 2002b). These animals were designated as THYR.

Dobutamine (dobutamine hydrochloride, Eli Lilly Pharmaceuticals, USA) was diluted in Krebs–Henseleit buffer and administered at an equivalent dose of 10 μg/kg/min.

Propranolol (propranolol hydrochloride, Sigma) was diluted in Krebs–Henseleit buffer in order to achieve a concentration of 10^{-7} M.

2.2. Isolated heart preparation

A nonejecting isolated rat heart preparation was perfused according to the Langendorff technique. In this model, coronary flow was maintained constant. An intraventricular balloon allowed measurement of contractility under isovolumic conditions. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 8 mm Hg in all groups and was held constant thereafter throughout the experiment. Since the balloon was not compressible, left ventricular contraction was isovolumic. As intraventricular volume was maintained at a constant value, diastolic fiber length, which represented preload, did not change. Thus, the left ventricular peak systolic pressure and the left ventricular developed pressure (LVDP), defined as the difference between left ventricular peak systolic pressure and left ventricular end-diastolic pressure, represented a contractility index obtained under isometric conditions. Rats were anaesthetized with ketamine hydrochloric acid and heparin 1000 IU/kg was given intravenously before thoracotomy. The hearts were rapidly excised, placed in ice-cold Krebs–Henseleit buffer (composition in mmol/l: sodium chloride 118, potassium chloride 4.7, potassium phosphate monobasic 1.2, magnesium sulfate 1.2, calcium chloride 1.4, sodium bicarbonate 25 and glucose 11) and mounted on the aortic cannula of the Langendorff perfusion system. Perfusion with oxygenated (95% O_2/5% CO_2) Krebs–Henseleit buffer was established within 60 s after thoracotomy. The perfusion apparatus was heated to ensure a temperature of 37 °C throughout the experiments. Hearts were paced at 320 bpm with a Harvard pacemaker. The pacemaker was turned off during ischaemia. The water-filled balloon connected to a pressure transducer and coupled to a Gould RS 3400 recorder was advanced into the left ventricle through an incision in the left atrium (Pantos et al., 1996, 2002a,b, 2003 in press).

2.3. Experimental protocol

All the experimental protocols performed in this study are shown in Fig. 1.

2.4. Dobutamine administration in normal hearts without ischaemia

Normal hearts were subjected to 53 min of stabilization, which was followed by dobutamine administration for 45 min (DOB-BASE).

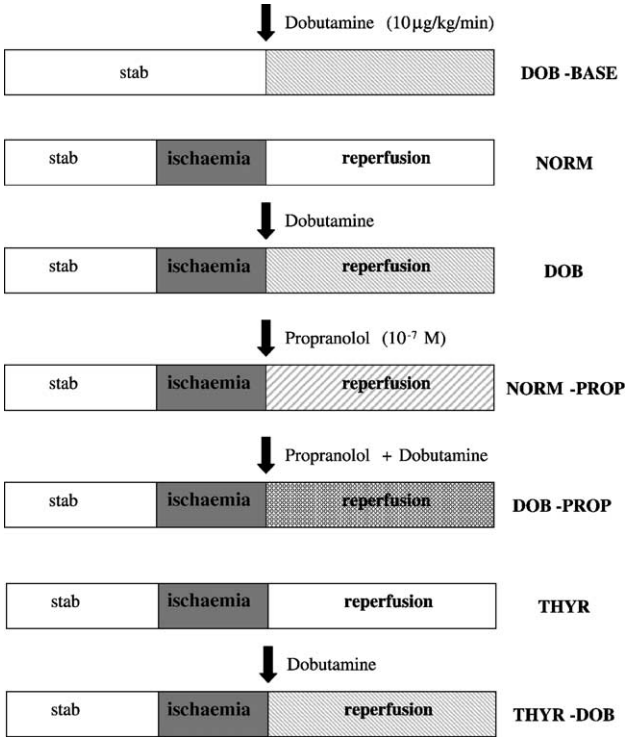


Fig. 1. Experimental protocols. Stab = stabilization period.

Table 1
Left ventricular developed pressure (LVDP) at baseline and 45 min of reperfusion, recovery of left ventricular developed pressure (LVDP%) and left ventricular end-diastolic pressure (LVEDP) at 45 min of reperfusion

Group	LVDP baseline	LVDP 45 min reperfusion	LVDP (%)	LVEDP 45 min reperfusion
NORM ($n=6$)	97.0 (7.1)	45.2 (6.5)	45.4% (4.3)	65.5 (8.7)
DOB ($n=8$)	96.8 (4.2)	18.1 (2.5)*	19.1% (2.8)*	96.8 (6.4)*
NORM-PROP ($n=6$)	102.6 (5.3)	38.0 (5.2)**	37.9% (5.6)**	76.0 (10.7)**
DOB-PROP ($n=6$)	104.0 (5.2)	40.0 (3.5)**	38.4% (2.7)**	69.6 (3.5)**
THYR ($n=10$)	149.8 (6.7)*	88.4 (7.7)*	59.4% (4.7)*	50.0 (5.1)
THYR-DOB ($n=12$)	142.0 (5.5)*	103.6 (7.2)	72.1% (2.9)***	36.5 (5.8)**

The values are means (S.E.M.). NORM = normal hearts, DOB = dobutamine administration at reperfusion, PROP = propranolol administration at reperfusion, THYR = thyroxine-treated hearts.
* $P<0.05$ vs. NORM.
** $P<0.05$ vs. DOB.
*** $P<0.05$ vs. THYR.

2.5. Dobutamine administration in normal hearts after ischaemia

Normal hearts underwent an initial 33 min stabilization period. They were then subjected to 20 min of global, zero-flow ischaemia followed by 45 min of reperfusion.
(a) Dobutamine was not infused at reperfusion (NORM), $n=6$.
(b) Dobutamine was infused throughout the reperfusion period (DOB), $n=8$.

2.6. Dobutamine and propranolol administration in normal hearts after ischaemia

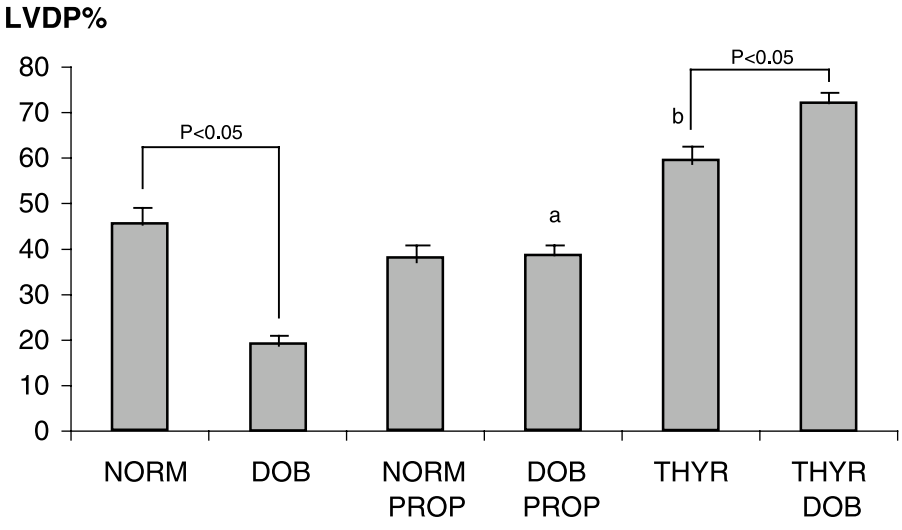
Isolated rat hearts underwent an initial 33 min stabilization period. They were then subjected to 20 min of ischaemia and 45 min of reperfusion.
(a) Propranolol was only infused at reperfusion (NORM-PROP), $n=6$.
(b) Propranolol and dobutamine were infused throughout the reperfusion period (DOB-PROP), $n=6$.

2.7. Dobutamine administration in thyroxine-treated hearts after ischaemia

Isolated rat hearts underwent an initial 33 min stabilization period. They were then subjected to 20 min of global, zero-flow ischaemia followed by 45 min reperfusion.
(a) Dobutamine was not infused at reperfusion (THYR), $n=10$.
(b) Dobutamine was infused throughout the reperfusion (THYR-DOB), $n=12$.

2.8. Measurement of mechanical function

Left ventricular systolic function was assessed by recording LVDP, which was measured at the end of the stabilization period and at 45 min of reperfusion. Recovery of LVDP was expressed as percentage of the initial value (LVDP%). Diastolic function was assessed by monitoring isovolumic left ventricular end-diastolic pressure (LVEDP) as a measure of diastolic chamber distensibility. Left ventricular end-diastolic pressure was measured at 45 min of reperfusion. In normal hearts without ischaemia, LVDP was measured at the end of the stabilization period before dobutamine



[a] P<0.05 vs DOB, [b] P<0.05 vs NORM

Fig. 2. Recovery of left ventricular developed pressure (LVDP%) in NORM, DOB, NORM-PROP, DOB-PROP, THYR and THYR-PROP groups.

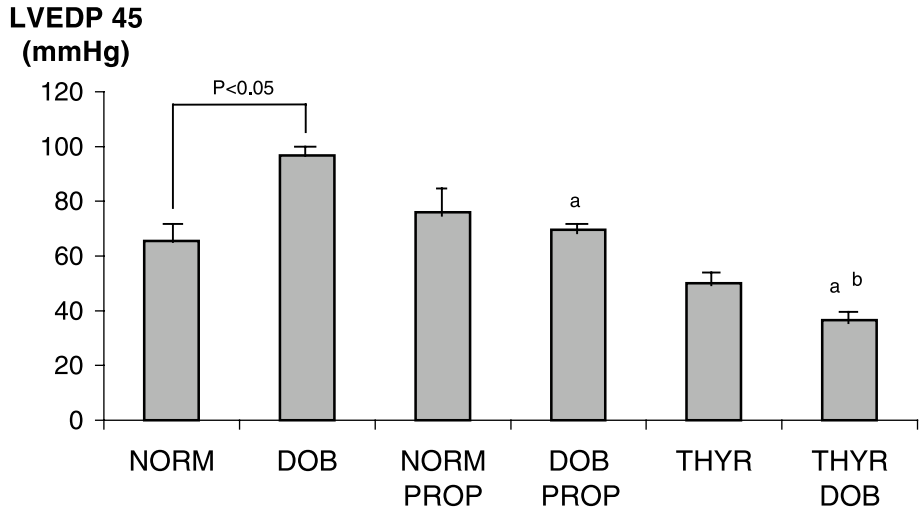


Fig. 3. Left ventricular end-diastolic pressure at 45 min of reperfusion (LVEDP 45, %) in NORM, DOB, NORM-PROP, DOB-PROP, THYR and THYR-PROP groups.

administration and after dobutamine administration at its peak value (LVDP max).

2.9. Statistics

Values are presented as means (S.E.M.). One-way analysis of variance with Bonferroni correction was used when multiple comparisons were carried out. Unpaired *t*-test was used to test for differences between groups. A two-tailed test with a P value less than 0.05 was considered significant. When it was required, one-way analysis of variance with Dunnett correction was used when multiple comparisons were carried out, and Mann–Whitney test was used for differences between groups.

3. Results

3.1. Cardiac function with dobutamine administration in hearts without ischaemia

LVDP was increased from 100.5 (4.9) mm Hg at baseline to LVDP max, 129.4 (5.7) mm Hg after dobutamine administration, $P<0.05$.

3.2. Parameters of postischaemic cardiac function after dobutamine and propranolol administration

(a) LVDP during stabilization period, at 45 min of reperfusion and LVDP% for the NORM, DOB, NORM-PROP and DOB-PROP hearts, are shown in Table 1. Dobutamine infusion at reperfusion resulted in lower LVDP% compared to nontreated hearts, $P<0.05$ (Fig. 2). NORM-PROP hearts exhibited similar LVDP% compared to NORM hearts, not significant. However, propranolol administration in dobutamine-treated hearts resulted in enhanced LVDP% compared to DOB hearts, $P<0.05$ (Fig. 2). LVDP% was not different between NORM-PROP and DOB-PROP hearts, not significant (Fig. 2).

(b) LVEDP at 45 min of reperfusion for the NORM, DOB, NORM-PROP and DOB-PROP hearts are shown in Table 1. Dobutamine infusion at reperfusion resulted in higher LVEDP compared to nontreated hearts, $P<0.05$ (Fig. 3). However, propranolol administration in dobutamine-treated hearts resulted in significantly lower LVEDP compared to DOB hearts, $P<0.05$ (Fig. 3). LVEDP was not different between NORM-PROP and DOB-PROP hearts, not significant (Fig. 3).

3.3. Parameters of postischaemic cardiac function in thyroxine-treated hearts after dobutamine administration

(a) LVDP during stabilization period, at 45 min of reperfusion and LVDP% for the THYR and THYR-DOB groups, are shown in Table 1. Dobutamine infusion at reperfusion resulted in higher LVDP% compared to thyroxine-treated hearts without dobutamine administration (Fig. 2).

(b) LVEDP at 45 min for the two groups are shown in Table 1. LVEDP was lower after dobutamine infusion but not at a statistically significant level as compared to the nontreated hearts.

4. Discussion

In the present study, we investigated the ability of dobutamine to modify the postischaemic dysfunction when

it is administered at reperfusion period in the experimental setting of zero-flow global ischaemia and reperfusion. We used an experimental model of isolated rat heart preparation, which allows measurement of indices of left ventricular function independently of loading conditions. Dobutamine was given at reperfusion at a dose of 10 μg/kg/min. This particular dose was found to exert a positive inotropic effect when was administered in perfused only hearts. Our study showed that administration of dobutamine at reperfusion can rather be detrimental than beneficial. In fact, left ventricular developed pressure at the end of reperfusion period was found to be lower in dobutamine-treated hearts than in nontreated hearts. Similarly, the end-diastolic pressure at the end of reperfusion was shown to be increased after dobutamine administration. Furthermore, the adverse effect of dobutamine was abolished by concomitant administration of propranolol, a β-adrenoceptor antagonist.

This paradoxical response can be attributed to various factors. Dobutamine is a β-receptor agonist that exerts its inotropic effects via the interaction with cellular membrane-bound β-receptors, which in turn activate the adenylate cyclase with consequent formation of cytosolic cyclic adenosine monophosphate (cAMP). cAMP activates the protein kinase A, which increases the intracellular calcium concentration and consequently the magnitude of myocardial contractility. However, this increased amount of intracellular calcium supersaturates the maximum amount of sites in the actin–myocin complex during myocardial contraction, so that some of the calcium released does not contribute to the contractile performance during systole. This extra amount of calcium, though, requires greater amounts of adenosine triphosphate to be driven back into the sarcoplasmic reticulum during diastole, since calcium resequestration is a triphosphate-dependent process (Entman et al., 1985). This is known as the oxygen-wasting effect (Ko et al., 1993). Due to this phenomenon, the inotropic effect of dobutamine has been associated with a considerable increase in oxygen consumption, elevated adenosine levels and decreased phosphorylation potential indicating enhanced metabolic stress in cardiomyocytes (Van Wylen et al., 1990). Thus, the detrimental effect of dobutamine on postischaemic myocardium, observed in our study, could be partly explained by the oxygen-wasting effect.

Alterations in Ca^{2+} handling that occur in cells subjected to ischaemia–reperfusion could also play a role in this response. Dobutamine can significantly increase intracellular Ca^{2+} as a result of impaired calcium handling at reperfusion and this is thought to be due to the reduced sarcoplasmic reticulum Ca^{2+}-ATPase activity in postischaemic myocardium (Krause et al., 1989; Ojamaa et al., 2000). Furthermore, increased cardiomyocyte apoptosis is shown to occur after postischaemic administration of dopamine via activation of calcium-dependent signalling cascades (Stamm et al., 2002). These effects could also explain the detrimental effect of dobutamine administration at reperfusion. In fact, Du Toit and Opie (1992), using a model of isolated rat heart, demonstrated that interventions, which increase cytosolic calcium, such as perfusion with high-calcium concentration, isoproterenol (a β-adrenoceptor agonist) and forskolin (an adenylate cyclase activator) worsen postischaemic myocardial dysfunction, while interventions that limit calcium influx during early reperfusion, such as perfusion with low calcium concentration, ryanodine, nisoldipine, or the inorganic blockers Mn^{2+} and Mg^{2+}, enhance postischaemic recovery. Deleterious effects of catecholamine administration in the postischaemic setting, corresponding to a prostunning effect, have been also demonstrated in dogs (Vatner and Baig, 1978) and in humans (Tsoukas et al., 1997).

The present study was further extended to investigate whether dobutamine's detrimental effect can be modified by interventions that target the preischaemic period and consequently improve postischaemic dysfunction. Thyroxine, a well-known inotrope, has been recently shown to increase postischaemic recovery of function in hearts subjected to sustained ischaemia (Pantos et al., 2000, 2002a). This effect is thought to be mediated through regulation of intracellular signalling transduction pathways related to cell death (Pantos et al., 2000, 2001, 2003 in press). Furthermore, thyroid hormone interferes with calcium regulation mechanisms in cell by upregulating specific genes. In fact, thyroid hormone is shown to increase sarcoplasmic reticulum Ca^{2+}-ATPase activity, protein and mRNA expression (Balkman et al., 1992; Arai et al., 1991; Kiss et al., 1998; Hamasaki et al., 2000) and directly increases the expression of cardiac Na^{+}–Ca^{2+} exchanger, a protein that plays a pivotal role in intracellular calcium handling (Hojo et al., 1997). Interestingly, after acute myocardial infarction in rats, it was found that the expression of several thyroid hormone responsive genes is altered such as alpha-myosin heavy chain, sarcoplasmic reticulum calcium-activated ATPase and phospholamban and these changes could be restored after long treatment with elevated doses of triiodothyronine (Ojamaa et al., 2000). In addition, recent studies show that long-term thyroxine administration can result in overexpression and activation of important cardioprotective molecules such as protein kinase C δ (PKCδ) (Pantos et al., 2002a). PKC is recently shown to regulate calcium homeostasis during ischaemia–reperfusion. In fact, increased PKC activity after ischaemia is found to contribute to calcium homeostasis whereas postischaemic administration of PKC inhibitors such as chelerythrine was shown to exacerbate postischaemic calcium overload. (Stamm et al., 2001). Moreover, diazoxide is found to attenuate the lethal injury associated with calcium overload via PKC signalling pathways (Wang and Ashraf, 1999). On the basis of this evidence, thyroxine could potentially be a suitable pharmacological modulator of the dobutamine effect on postischaemic myocardium. In fact, our study has clearly demonstrated that in thyroxine-treated hearts, the detrimental effect of dobutamine on postischaemic left ventricular function was reversed and

recovery of function was increased in those hearts in an additive way after dobutamine administration.

The role of thyroxine as a positive inotrope has already been demonstrated in clinical settings (Novitzky et al., 1989; Carrel et al., 2002). Thyroid analogs have been tested in the treatment of heart failure with few systemic effects and tachycardia (Pennock et al., 1993, 2000). Additionally, the present study shows that thyroxine can modify the effect on postischaemic contractile function of widely used inotropes such as dobutamine by its cardioprotective properties. However, more studies are needed to further characterize the mechanisms of this effect.

It is apparent that a combination of dobutamine and thyroxine pretreatment could be desirable in several conditions particularly in clinical settings in which thyroid hormone levels are low, such as heart failure (Ascheim and Hryniewicz, 2002; Manowitz et al., 1996), acute myocardial infarction and after cardiac bypass operations (Ojamaa et al., 2000; Klein, 2001). This treatment might lower the need for mechanical assisting devices for supporting haemodynamics in those conditions.

In conclusion, dobutamine administration increases postischaemic dysfunction in normal hearts, a detrimental effect that can be reversed by thyroxine pretreatment. The combination of thyroxine and dobutamine could be a new therapeutic strategy to improve postischaemic dysfunction after cardiopulmonary bypass and/or myocardial infarction.

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