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Haemodynamic effects of dopexamine and nitric oxide synthase inhibition in healthy and endotoxaemic sheep

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Abstract

Chronically instrumented awake healthy sheep ($n = 6$) received the synthetic catecholamine, dopexamine, during or without a background infusion of the nitric oxide synthase inhibitor, L-nitro-arginine-methylester (L-NAME). Three days later, hypotensive-hyperdynamic circulation was induced and maintained by continuous infusion of *Salmonella typhosa* endotoxin (10 ng/kg per min). After 24 h of continuous endotoxin infusion, the dopexamine L-NAME protocol was repeated. In healthy and endotoxaemic animals with and without nitric oxide synthase inhibition dopexamine caused the same haemodynamic changes: heart rate and cardiac output increased, mean arterial pressure and systemic vascular resistance decreased. L-NAME infusion induced normalisation of the hypotonic-hyperdynamic circulation in endotoxaemic animals. Dopexamine reduced some adverse effects of L-NAME treatment, like increased pulmonary vascular resistance and decreased oxygen delivery. In conclusion the haemodynamic effects of dopexamine are independent of the amount of nitric oxide production. Dopexamine may attenuate some of the adverse effects of nitric oxide synthase inhibition. © 1997 Elsevier Science B.V.

Keywords: Endotoxaemia; Hyperdynamic circulation; Nitric oxide (NO) synthase inhibition; Dopexamine

1. Introduction

Dopexamine is a synthetic catecholamine that causes vasodilatation by stimulation of β_2-adrenoceptors with a drop in mean arterial pressure. The effect has been observed in animal studies as well as in humans (Smith et al., 1987; VanDerStarre and Rosseel, 1988; Foulds, 1988; Smith and O'Connor, 1988; Stephan et al., 1990). In clinical practice, dopexamine is usually given to patients with severe left ventricular failure.

The positive inotropic and chronotropic effects of dopexamine, which lead to an increase in cardiac output, contractility and rate, are antagonized by β_2-adrenoceptor-antagonists (Smith et al., 1987). In addition to these β_2-adrenoceptor-mediated effects, dopexamine stimulates the heart by two other mechanisms: baroceptor-mediated release of norepinephrine, resulting in hypotension produced by β_2-adrenoceptor- and dopamine D_1-receptor-mediated vasodilatation; and potentiation of the released norepinephrine due to the prevention of norepinephrine uptake by sympathetic nerves (Goldberg and Bass, 1988). The role of dopexamine and myocardial β_2-adrenoceptor stimulation is the subject of controversy (Smith et al., 1987; Goldberg and Bass, 1988).

Investigating the haemodynamic effects of β-adrenergic drugs during nitric oxide (NO) synthase inhibition, Graves and Poston (1993) showed that the NO synthase inhibitor, L^G-nitro-arginine-methylester (L-NAME), did not change the vasodilatator properties of the β_2-adrenoceptor agonist, salbutamol, in vitro, but reversed the vasodilatator properties of the β_1-adrenoceptor agonist, dobutamine. The authors suggested that these different reactions of β-adrenoceptor agonists during nitric oxide synthase inhibition were related to the different pathways by which β-adrenoceptor agonists cause vasodilatation (Graves and Poston, 1993). β_1-adrenoceptor agonists are supposed to cause vasodilatation by activating adenylate cyclase with subsequent release of nitric oxide, whereas β_2-adrenoceptor agonists should act partially independently of nitric oxide. It is still unknown whether dopexamine's haemodynamic properties are mediated at least in part by NO. To evaluate the interactions of dopexamine and NO, we used an estab-

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lished awake chronically instrumented large animal model in which we investigated haemodynamic properties of dopexamine during or without inhibition of the NO synthase with L-NAME.

Additionally, we studied haemodynamic characteristics of dopexamine during endotoxaemia as well as interactions between dopexamine and NO synthase inhibition during endotoxaemia.

Colardyn et al. (1989) investigated the effects of dopexamine in septic patients. They found that the heart rate was further increased and systemic vascular resistance further decreased after dopexamine infusion. Thus, they did not recommend the use of dopexamine for the treatment of sepsis. However, in an experiment with endotoxaemic rats, the infusion of dopexamine maintained intestinal villus blood flow in contrast to that in untreated endotoxaemic animals (Schmidt et al., 1996). Therefore, the use of dopexamine during endotoxaemia and sepsis may limit gut ischaemia and prevent the development of multi-organ failure.

Increased production of NO by the inducible calcium-independent NO synthase is thought to be the main cause of the hypotensive-hyperdynamic circulation and the reduced responsiveness to catecholamines during sepsis or endotoxaemia (Moncada et al., 1991; Meyer et al., 1994; Gomez-Jimenez et al., 1995; Szabo, 1995). In several studies it was possible to reverse these NO mediated changes by the application of a NO synthase inhibitor (Hollenberg et al., 1993; Landin et al., 1994; Petros et al., 1994; Booke et al., 1995). Adverse effects of this NO synthase inhibition were an increased mean pulmonary artery pressure and a decreased cardiac output (Gibson et al., 1994; Wang et al., 1995). Therefore, we investigated the combination of a NO synthase inhibitor with dopexamine, a drug that increases cardiac output and reduces pulmonary artery pressure.

2. Material and methods

2.1. Animal model

The study was approved by the State Animal Protection Committee. Eight female sheep of the merino breed with an average weight of 46.8 ± 2.6 kg (S.E.M., range 40–60 kg) were utilised for the study. After intramuscular premedication with ketamine (15–20 mg/kg Ketanest®; Parke-Davis, Berlin, Germany) the sheep were chronically instrumented with a pulmonary artery catheter positioned via a jugular vein (8.5 F Catheter Introducer Set; pvb Medizintechnik, Kirchseeon; 7.5 F Edwards Swan Ganz-Catheter®; Edwards Critical Care Division, Irvine, CA, USA) and with a femoral artery catheter (18 G Leader Cath; Vygon, Aachen, Germany). During instrumentation, anaesthesia was maintained with repeated intravenous injections of propofol (2 mg/kg Disoprivan; Zeneca, Schwetzingen, Germany), when necessary. After 24 h of recovery, the catheters were connected to pressure transducers (DTX™ Druckwandler kit; Ohmeda, Erlangen, Germany) and a monitor (Hellige Servomed; Hellige, Freiburg, Germany). A continuous intravenous infusion of Ringer's lactate (2 ml/kg per h) was started. All the following measurements were performed in awake and spontaneously breathing animals held in metabolic cages for the duration of the experiment with free access to water and food.

2.2. Experimental protocol

After baseline haemodynamic measurements, intravenous dopexamine (Dopacard®; Speywood Pharmaceuticals, Berkshire, UK) infusion was started at 1, 5 and 10 μg/kg per min. Each dose was given for at least 15 min or until the haemodynamic situation was stable, then haemodynamic and oxygen transport data were obtained. Cardiac output measurements were performed by the thermodilution technique, using the average of three injections of cold (2–5°C) saline solution (9520 A Cardiac Output Computer; Edwards Laboratories). Mixed venous and arterial blood gas samples were analysed with an ABL 520 (Radiometer Copenhagen, Copenhagen, Denmark). The dopexamine infusion was interrupted and a bolus of 2.5 mg/kg L-NAME was given intravenously followed by continuous infusion of 0.5 mg/kg per h. After 1 h of L-NAME-infusion the dopexamine treatment described above was repeated.

Following this set of determinations in healthy animals, the sheep were allowed to recover for two days. After another determination of baseline data, hypotonic–hyperdynamic circulation was induced by intravenous continuous infusion of 10 ng/kg per min *Salmonella typhosa* endotoxin (*Salmonella typhosa* LPS; Sigma, Deisenhofen, Germany). During the endotoxaemic period of the experiment, the infusion rate of Ringer's lactate was adjusted to keep central venous pressure and pulmonary capillary wedge pressure at their baseline level ± 3 mmHg. After 24 h of continuous endotoxin infusion, the hyperdynamic circulatory situation had stabilized. Following the measurement of endotoxaemic baseline data, the administration of dopexamine and then L-NAME was started as described above. Finally, the surviving animals were anaesthetized and killed with a lethal dose of potassium chloride.

2.3. Statistical analysis

The data were compared as absolute values. Differences between the different timepoints of the experiments were tested using an analysis of variance (ANOVA) followed by Fisher's protected least significant difference test (PLSD) with Bonferroni correction for multiple comparisons. Significance was defined as $P < 0.05$. All data are presented as means ± S.E.M.

3. Results

Two sheep died during the first 24 h of endotoxaemia. The data from these animals were excluded from the analysis.

3.1. Haemodynamic effects of dopexamine and L-NAME in healthy sheep

Infusion of dopexamine in healthy animals leads to an increased heart rate, cardiac output and oxygen delivery. While pulmonary vascular resistance remained statistically unchanged, elevated cardiac output caused a higher mean pulmonary arterial pressure. Systemic vascular resistance, mean arterial pressure and left ventricular stroke work index were significantly reduced. After infusion of L-NAME, vascular resistance in systemic and pulmonary circulation increased significantly, resulting in elevated mean arterial and mean pulmonary arterial pressure. The decrease in heart rate, cardiac output, and oxygen delivery and the increase in left ventricular stroke work index were not statistically significant. Dopexamine induced similar changes, whether or not NO synthase was inhibited. Heart rate, cardiac output and oxygen delivery increased while mean arterial pressure and systemic vascular resistance decreased and the left ventricular stroke work index remained unchanged. In the pulmonary circulation vascular resistance was decreased but there were no effects on mean pulmonary arterial pressure. These haemodynamic effects of dopexamine are shown in Fig. 1a–d and Fig. 2a–d.

3.2. Haemodynamic changes due to continuous endotoxaemia

After 24 h of continuous infusion of *Salmonella typhosa* endotoxin, heart rate, cardiac output and oxygen delivery were significantly elevated above their nonendotoxaemic baseline values (Fig. 1a and b, Fig. 2d), while systemic vascular resistance, mean arterial pressure and left ventricular stroke work index were significantly decreased (Fig. 1c and d, Fig. 2c). Endotoxaemia also led to a significant increase in mean pulmonary arterial pressure, while pulmonary vascular resistance was not significantly elevated (Fig. 2a and b).

3.3. Haemodynamic effects of dopexamine and L-NAME in endotoxaemic sheep

As in healthy animals, dopexamine infusion during endotoxaemia increased heart rate, cardiac output and oxygen delivery (Fig. 1a and b, Fig. 2d). Systemic vascular resistance and mean arterial pressure further decreased during dopexamine infusion (Fig. 1c and d). Pulmonary vascular resistance also decreased, and the elevated cardiac output caused an almost unchanged mean pulmonary artery pressure (Fig. 1a, Fig. 2a and b). L-NAME application in endotoxaemic animals led to an increase of mean arterial pressure and systemic vascular resistance, while heart rate and oxygen delivery, but not cardiac output, decreased significantly. In the pulmonary circulation, there was a further increase in pulmonary vascular resistance with a nonsignificant elevation in mean pulmonary arterial pressure. Infusion of dopexamine to endotoxaemic animals in

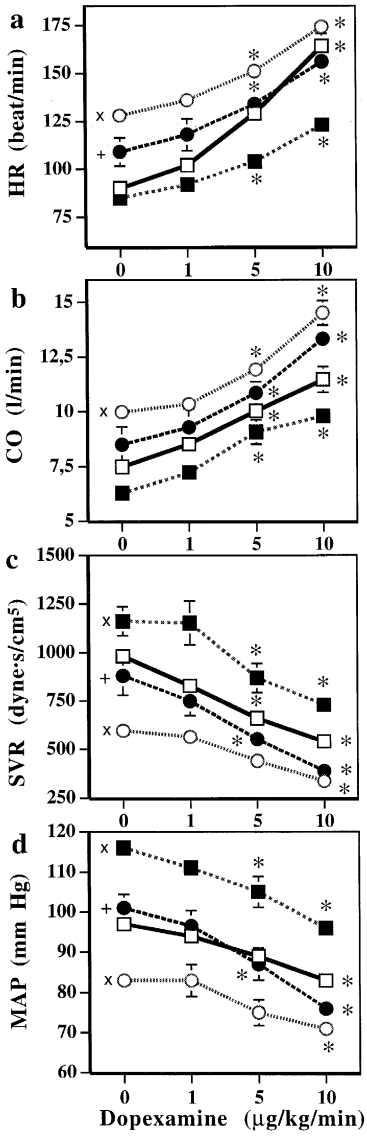

Fig. 1. Changes in heart rate (HR, a), cardiac output (CO, b), systemic vascular resistance (SVR, c) and mean arterial pressure (MAP, d) during the infusion of different doses of dopexamine. Six sheep received doses of dopexamine before (□) and during continuous infusion of L-NAME (■) and after 24 h of continuous infusion of endotoxin before (○) and after (●) infusion of L-NAME. The dose of L-NAME was 2.5 mg/kg as a bolus, followed by continuous infusion at a rate of 0.5 mg/kg per h. The dose of Salmonella typhosa endotoxin was 10 ng/kg per min. All results are expressed as means ± standard error of the mean (S.E.M.). (∗) denotes $P < 0.05$ vs. predopexamine baseline, (×) denotes $P < 0.05$ vs. healthy in the absence of L-NAME, (+) denotes $P < 0.05$ vs. endotoxaemic in the absence of L-NAME.

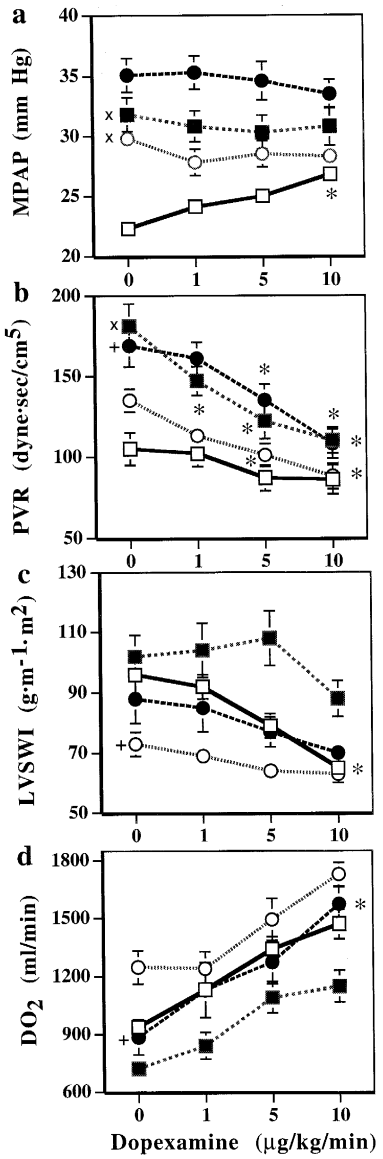

Fig. 2. Changes in pulmonary artery pressure (MPAP, a), pulmonary vascular resistance (PVR, b), left ventricular stroke work index (LVSWI, c) and oxygen delivery (DO_2, d) during the infusion of different doses of dopexamine. Six sheep received doses of dopexamine before (□) and during continuous infusion of L-NAME (■) and after 24 h of continuous infusion of endotoxin before (○) and after (●) infusion of L-NAME. The dose of L-NAME was 2.5 mg/kg as a bolus, followed by continuous infusion with a rate of 0.5 mg/kg per h. The dose of Salmonella typhosa endotoxin was 10 ng/kg per min. All results are expressed as means ± standard error of the mean (S.E.M.). (*) $P < 0.05$ vs. predopexamine baseline, (×) = $P < 0.05$ vs. healthy in the absence of L-NAME, (+) = $P < 0.05$ vs. endotoxaemic in the absence of L-NAME.

presence of L-NAME had the same effects as in the absence of NO synthase inhibition. Heart rate, cardiac output and oxygen delivery were increased while mean arterial pressure and systemic vascular resistance were decreased. Dopexamine reduced pulmonary vascular resistance without changes in mean pulmonary arterial pressure. Left ventricular stroke work index was not altered by the interventions in this part of the study.

During the whole experimental period, right ventricular stroke volume index (baseline value: 84 ± 6.5 ml m^2), right ventricular stroke work index (baseline value: 18.1 ± 1.3 g m^{-1} m^2), oxygen consumption (baseline value: 331 ± 55 ml min^{-1}) and oxygen extraction (baseline value: 0.35 ± 0.02) did not change significantly.

4. Discussion

In healthy sheep, dopexamine caused a dose-dependent increase in heart rate, cardiac output and oxygen delivery, while mean arterial pressure and systemic vascular resistance index decreased. These haemodynamic effects are consistent with previous experimental and clinical data (Foulds, 1988; VanDerStarre and Rosseel, 1988; Stephan et al., 1990). Smith et al. (1987), using β_2-blockers, showed that these effects are caused by the stimulation of β_2-adrenoceptors.

The haemodynamic effects of dopexamine were not modified by NO synthase inhibition. These results confirm the in vitro study of Graves and Poston (1993) but contradict the results of an in vivo study by Wang et al. (1993). Graves and Poston (1993) found that L-NAME had no influence on salbutamol-mediated vasorelaxation, partially attenuated the effects of isoprenaline and abolished vasodilatation due to dobutamine. They concluded that NO is produced in response to the β_1-adrenoceptor-induced elevation of cyclic AMP in the endothelial cell and that β_2-adrenoceptors can produce vasorelaxation via pathways other than those mediated via cyclic AMP. In contrast, Wang et al. (1993) observed that the relaxant response to salbutamol was attenuated in part by L-NAME and postulated that NO is involved in β_2-adrenoceptor-mediated vasodilatation. MacGregor et al. (1996) investigated changes in levels of cAMP after application of different β-adrenoceptor agonists to human lymphocytes and found increased cAMP levels only with epinephrine and isoprenaline, but not with dopexamine. The authors concluded that dopexamine has only a minimal β_2-sympathomimetic effect. This conclusion is in contrast to results of Smith et al. (1987), who antagonized the cardiovascular effects of dopexamine with a β_2-adrenoceptor antagonist. However, the observations of MacGregor et al. (1996) may be explained by the assumption of Graves and Poston (1993) who found that β_2-adrenoceptor agonists cause vasodilatation through a cAMP-independent mechanism, perhaps via elevation of cyclic guanylate monophosphate (cGMP).

In the present animal model the haemodynamic effects of dopexamine were unchanged during endotoxaemia as described previously for patients in septic shock (Colardyn et al., 1989). Our results however contradicted those of De Wit et al. (1996), who found a supra-additive effect of the NO donor, sodium nitroprusside, and isoprenaline on the maximum dilator response in a hamster cremaster model.

The authors suggested that this supra-additive effect was caused by increased levels of cGMP due to NO-mediated stimulation of the soluble guanylate cyclase. Increased levels of cGMP cause vasodilatation by decreasing the amount of intracellular calcium and also by inhibition of the cAMP-degrading enzyme, phosphodiesterase, which would increase intracellular levels of cAMP and enhance vasodilation. On the basis of this prior investigation, we expected that vasodilatator properties of dopexamine would be increased during endotoxaemia. However, we found that the effects of dobutamine remained unchanged.

Our study and those cited above have several limitations. In particular, different species or tissue cultures were investigated. Because of the differences in the distribution of receptors between species or tissues, no conclusion can be drawn from these experiments regarding the molecular actions of β_2-adrenoceptor agonists in humans.

Interactions of NO synthase inhibition and β-sympathomimetics have been investigated before (Kilbourn et al., 1994), but to our knowledge this is the first study that investigated the interaction between NO synthase inhibition and a β-adrenoceptor agonistic drug during hypotensive–hyperdynamic endotoxaemia. As observed in a previous study using an ovine animal model (Meyer et al., 1994) L-NAME infusion increased mean arterial pressure and systemic vascular resistance during endotoxaemia to such an extent that no significant difference from the pre-endotoxaemic baseline levels could be detected. Adverse effects of the treatment of endotoxaemic sheep with L-NAME were a significant increase in pulmonary vascular resistance and a significant decrease in oxygen delivery (Fig. 2). Also, cardiac output showed a trend to a decrease after L-NAME infusion in endotoxaemia. However, this change failed to reach statistical significance (Fig. 1b). A similar side-effect pattern was observed in several studies which investigated NO synthase inhibition in different animal models of sepsis (Gibson et al., 1994; Meyer et al., 1994; Wang et al., 1995) or in septic patients (Petros et al., 1994). Infusion of dopexamine partly reversed these side effects. Cardiac output and oxygen delivery increased in healthy and endotoxaemic animals when dopexamine was given after NO synthase inhibition. The same decrease in pulmonary vascular resistance was seen in healthy and endotoxaemic animals. Whether the elevation of oxygen delivery by dopexamine in L-NAME-treated endotoxaemic animals is beneficial or not, was not answered by our study. Further studies are necessary to evaluate this issue.

In summary, dopexamine has comparable haemodynamic effects in healthy and endotoxaemic sheep with and without NO synthase inhibition. Infusion of L-NAME normalized the hypotensive-hyperdynamic systemic circulation during ovine endotoxaemia. Dopexamine ameliorated some of the effects of L-NAME, namely the decrease of oxygen delivery and the increase in pulmonary vascular resistance.

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