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S-Nitrosocaptopril: acute in-vivo pulmonary vasodepressor effects in pulmonary hypertensive rats

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Abstract

The effects of S-nitrosocaptopril (SNOcap), administered either intravenously or by oral gavage, on pulmonary artery pressure (PAP) were examined in anaesthetised normotensive rats and rats with hypoxic pulmonary hypertension (10% oxygen for 1 week). Mean PAP (MPAP) values in hypoxic and normoxic rats were (mmHg) 26 ± 1.7 and 15 ± 1.1, respectively. When given intravenously, 1 mg kg^{-1} SNOcap reduced MPAP by 28 and 32% in hypoxic and normoxic rats, respectively. The effects of 2 mg kg^{-1} were no greater than those of 1 mg kg^{-1}. Pulmonary vasodepressor responses reached equilibrium in 1.7 ± 0.18 min following intravenous administration. When given orally 30 min before the measurement of PAP, 30 mg kg^{-1}, but not 10 mg kg^{-1}, significantly reduced MPAP in hypoxic rats to 17 ± 1.5 mmHg. These in-vivo data are consistent with previous in-vitro data showing that SNOcap has direct pulmonary vasorelaxant properties in both large and small pulmonary arteries and also show that SNOcap causes pulmonary vasodepression in the setting of pulmonary hypertension. Since SNOcap also inhibits pulmonary vascular angiotensin converting enzyme (ACE) in pulmonary blood vessels (previous study), it would be an interesting drug with which to assess the benefits of direct pulmonary vasodilatation combined with ACE inhibition (which attentuates pulmonary vascular remodelling) in a long-term study in pulmonary hypertension.

Introduction

S-Nitrosocaptopril (SNOcap) is an S-nitrosothiol in which the thiol component is the angiotensin converting enzyme (ACE) inhibitor, captopril (Jia et al 1999). Hence it has the properties of a nitric oxide (NO) donor and also inhibits ACE (Cooke et al 1989; Loscalzo et al 1989; Tsui et al 2003). Because it is an NO donor, it is a direct vasodilator. Pulmonary hypertension is a disease that requires drugs to inhibit pulmonary vascular remodelling (e.g. ACE inhibitors, endothelin antagonists), and also to cause pulmonary vasodilatation (Wanstall & Jeffery 1998; Jeffery & Wanstall 2001a; Pass & Dusing 2002). Therefore, SNOcap could be potentially valuable in treating this disease.

The in-vitro pulmonary vasorelaxant effects of SNOcap have recently been described in both main and intralobar pulmonary arteries from rats (Tsui et al 2003), complementing previous studies in isolated systemic vessels (Cooke et al 1989; Loscalzo et al 1989). However, SNOcap has not been studied on the pulmonary circulation in-vivo, each of the previous in-vivo studies having been confined to the systemic (Shaffer et al 1991; Jia et al 1999) or coronary circulations (Nakae et al 1995). Furthermore, there are no studies (in-vivo or in-vitro) in which SNOcap has been investigated in an animal model of pulmonary hypertension.

In view of this, the aim of this study was to examine the effects of acute administration of SNOcap on pulmonary artery pressure (PAP) in two groups of rats – normotensive and pulmonary hypertensive. Pulmonary hypertension was induced by exposure of rats to hypoxia (Jeffery & Wanstall 2001b). Two different routes of administration were used – intravenous and oral gavage. Doses selected for the study were within the range previously shown to cause systemic vasodepression when administered by each of these routes. (Jia & Blantz 1998; Jia et al 1999; Jia & Wong 2001).

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Materials and Methods

Drugs

Heparin sodium was from David Bull Laboratories (Mulgrave, Victoria, Australia), pentobarbitone sodium from Merial (Parramatta, NSW, Australia) and S-nitrosocaptopril (SNOcap) from Calbiochem (La Jolla, CA). Heparin (5000 IU ml^{-1}) was diluted in normal saline (0.9% NaCl in deionised water). SNOcap was dissolved and diluted in de-ionised water (oral administration) or normal saline (i.v. administration) immediately before use.

Rats

The experiments in this study conformed to the Code of Practice for Animal Experiments of the National Health and Medical Research Council of Australia and were approved by the University of Queensland Animal Ethics Committee. Male Wistar rats aged 7–9 weeks were used. Some of the rats were housed for 1 week in hypoxic chambers (10% O_2; hypoxic rats) to induce pulmonary hypertension, as described in detail previously (Jeffery & Wanstall 2001b). All other rats were housed in room air (21% O_2; normoxic rats).

Intravenous administration of S-nitrosocaptopril (SNOcap)

Rats were anaesthetised with sodium pentobarbitone (90 mg kg^{-1}, i.p), and 250 IU heparin sodium was injected intraperitoneally. They were ventilated with room air via a tracheal cannula connected to a rodent ventilator (Ugo Basile, Comerio-Varese, Italy) (60 strokes min^{-1}; stroke volume 2.3 mL). The right jugular vein was cannulated for administration of SNOcap or saline. The thoracic cavity was opened and a blunted, heparin-filled hypodermic needle was inserted into the right ventricle and then guided into the main pulmonary artery to measure PAP, as described previously (Jeffery & Wanstall 1999, 2001b). The successful positioning of the cannula in the pulmonary artery was confirmed by the difference in wave-form from that obtained when the cannula was in the right ventricle. Pressure (mmHg) was measured with a Bentley Trantec pressure transducer (Model 60-800; American Edwards Laboratories, Santa Ana, CA) and recorded on a Gemini chart recorder (Ugo Basile, Comerio-Varese, Italy). After recording resting pressure, bolus doses of SNOcap or saline were administered intravenously in volumes ≤ 0.25 mL and responses were recorded at equilibrium. Where two doses of SNOcap were administered to any one rat, the second (higher) dose was given cumulatively once the response to the previous dose had reached equilibrium.

At the completion of the experiment, a blood sample was taken to measure the haematocrit and the heart was removed, divided into right ventricle (RV) and left ventricle plus septum ([LV + S]), blotted and weighed.

Oral administration of SNOcap

Normoxic and hypoxic rats were starved for 4 h and orally gavaged with SNOcap (10 or 30 mg kg^{-1}) or corresponding volume of vehicle (water: 0.4–1.0 mL). The surgical procedure and measurement of pressures, haematocrit and heart weights were the same as described above for intravenous administration, except that the jugular vein was not cannulated. The time between drug administration and pressure measurements was designed to be as close as possible to 30 min, based on the time to peak plasma concentration after oral administration reported by Jia et al (1999); the mean time was 30 ± 1.5 min ($n = 34$). In some of the experiments in this series, systemic artery pressure (SAP) was recorded via a cannula in the left carotid artery.

Data assessment

Mean pulmonary artery pressure (MPAP) and mean systemic artery pressure (MSAP) were calculated from the formula [diastolic pressure $+ \frac{1}{3}$(systolic pressure – diastolic pressure)]. The ratios of the weights of the RV/[LV + S] (mg mg^{-1}) and RV/body weight (mg g^{-1}) were calculated.

Statistical tests

Mean values from a number (n) of different rats are given together with their standard errors (s.e.m.). The significance of differences between mean values was determined by either *t*-test (comparison of 2 values; Student's *t*-test or paired *t*-test as appropriate) or one-way analysis of variance followed by Student Newman Keuls post test (comparison of more than 2 values). Statistical analyses were performed using Instat (Graphpad Software, San Diego, CA). Differences were considered significant at $P < 0.05$.

Results

Characteristics of pulmonary hypertensive rats

Rats exposed to hypoxia for 1 week, when compared with normoxic rats, had significantly elevated MPAP (mmHg: hypoxic 26 ± 1.7, $n = 12$; normoxic 15 ± 1.1, $n = 12$; $P < 0.05$; Student's *t*-test); this was indicative of pulmonary hypertension. These rats also had right ventricular hypertrophy (i.e., there were increases in both RV/[LV + S] (mg mg^{-1}: hypoxic 0.49 ± 0.01; normoxic 0.32 ± 0.01; $P < 0.05$; Student's *t*-test) and RV/body weight (mg g^{-1}: hypoxic 1.12 ± 0.04; normoxic 0.73 ± 0.03; $P < 0.05$; Student's *t*-test)). In addition, the hypoxic rats had polycythaemia (haematocrit values, %: hypoxic rats 61 ± 1.0; normoxic rats 45 ± 1.2; $P < 0.05$; Student's *t*-test). The body weights of the rats were (g): hypoxic 299 ± 9.3; normoxic 323 ± 6.7; $P < 0.05$; Student's *t*-test).

Intravenous administration of SNOcap

In this series of experiments, values of MPAP were determined in each rat before and after administration of either SNOcap or vehicle (saline).

An intravenous bolus dose of $1\,mg\,kg^{-1}$ SNOcap reduced MPAP in all normoxic and hypoxic rats. Mean values of MPAP before and after SNOcap are shown in Figure 1. The reductions in MPAP were: hypoxic rats $28 \pm 1.8\%$, $n = 6$; normoxic rats $32 \pm 5.4\%$, $n = 4$. The responses to SNOcap reached equilibrium rapidly (time to equilibrium after intravenous bolus injection: 1.7 ± 0.18 min, $n = 10$). Intravenous administration of saline did not affect MPAP in either group of rats (range: 1% decrease to 3% increase; $n = 5$). The effects of $2\,mg\,kg^{-1}$ SNOcap were no greater than the effects of $1\,mg\,kg^{-1}$ (% reduction in MPAP: hypoxic rats 31 ± 1.0, $n = 5$; normoxic rats 28 ± 5.6, $n = 3$).

Oral administration of SNOcap

In this series of experiments, data after treatment with SNOcap (10 or $30\,mg\,kg^{-1}$) and data after treatment with vehicle (water) were obtained in separate groups of rats. In normoxic rats, neither $10\,mg\,kg^{-1}$ nor $30\,mg\,kg^{-1}$ SNOcap, administered by oral gavage, had any effect on MPAP (Figure 2). In hypoxic rats, $10\,mg\,kg^{-1}$ likewise had no effect on MPAP but, in contrast to normoxic rats, the higher dose of SNOcap ($30\,mg\,kg^{-1}$, p.o.) significantly reduced MPAP (20% reduction; $P < 0.05$; one-way analysis of variance and Student Newman Keuls post test). As a result, the MPAP in hypoxic rats treated with $30\,mg\,kg^{-1}$ SNOcap (17 ± 1.5 mmHg, $n = 5$) approached the normal value, viz. the value in water-treated normoxic rats (15 ± 1.8 mmHg, $n = 7$). In some of the hypoxic rats, MSAP was also measured; the value in hypoxic rats treated with $30\,mg\,kg^{-1}$ SNOcap (49 ± 3.3 mmHg, $n = 4$) was 38% lower than the value in vehicle-treated hypoxic rats (79 ± 11.3 mmHg, $n = 4$; $P < 0.05$; Student's t-test).

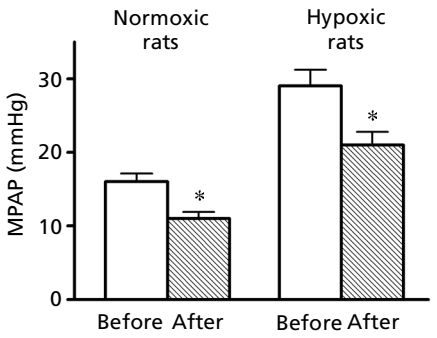

Figure 1 Effects of intravenous administration of S-nitrosocaptopril (SNOcap; $1\,mg\,kg^{-1}$) on mean pulmonary artery pressure (MPAP) in normoxic ($n = 4$) and hypoxic ($n = 6$) rats. Data before and after administration of SNOcap ($1\,mg\,kg^{-1}$) were obtained in each rat. Values depicted by the bars are mean values with s.e.m. shown by vertical lines. $^*P < 0.05$, compared with the corresponding value before SNOcap (paired t-test).

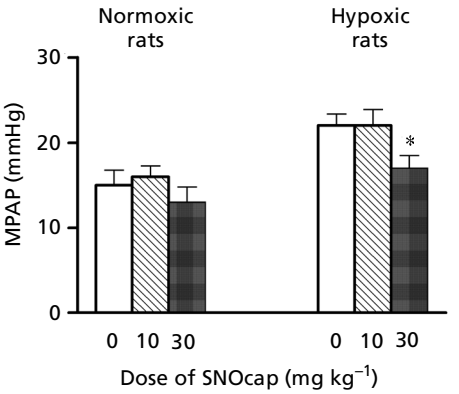

Figure 2 Effects of oral administration of S-nitrosocaptopril (SNOcap) on mean pulmonary artery pressure (MPAP) in normoxic and hypoxic rats. In both normoxic and hypoxic rats, treatment was with either water (control rats; $0\,mg\,kg^{-1}$ SNOcap), $10\,mg\,kg^{-1}$ SNOcap or $30\,mg\,kg^{-1}$ SNOcap. Values depicted by the bars are mean values with s.e.m. shown by vertical lines. n values for rats treated with 0, 10 and $30\,mg\,kg^{-1}$ SNOcap, respectively, were: normoxic rats, 7, 8, 4; hypoxic rats, 6, 4, 5. $^*P < 0.05$, compared with the corresponding control value ($0\,mg\,kg^{-1}$ SNOcap) (one-way analysis of variance and Student Newman Keuls post test).

As expected, this acute treatment with SNOcap (at either dose) had no effect on RV/[LV + S], RV/body weight or haematocrit in either normoxic or hypoxic rats ($P > 0.05$; one-way analysis of variance). Values for these parameters in hypoxic rats treated with the higher dose of SNOcap, $30\,mg\,kg^{-1}$, were ($n = 5$): RV/[LV + S], $0.53 \pm 0.01\,mg\,mg^{-1}$; RV/body weight, $1.06 \pm 0.09\,mg\,g^{-1}$; haematocrit, $59 \pm 2.4\%$ ($P > 0.05$ when compared with corresponding values in untreated hypoxic rats given in the first section of the Results).

Discussion

In this study, we have shown that the novel compound SNOcap, which is reported to have the properties of an NO donor and ACE inhibitor, can reduce PAP in-vivo in rats. This is the first time that the effects of SNOcap on the pulmonary circulation have been examined in-vivo and the results are consistent with the ability of SNOcap to relax pulmonary arteries (both large and small) in-vitro (Tsui et al 2003). SNOcap was shown to reduce PAP whether given intravenously or orally and was effective at doses within the ranges previously shown to reduce SAP in rats. A reduction in PAP was seen not only in normal rats but also in rats with hypoxic pulmonary hypertension.

When SNOcap was given intravenously, the drop in PAP occurred rapidly (i.e., the response reached equilibrium within 2 min). This observation was consistent with the speed of the response seen in-vitro in isolated pulmonary arteries where relaxation likewise reached equilibrium in approximately 2 min (Tsui et al 2003). It is also consistent with the rapid increase in cerebral blood flow in rats

and reduction in aortic pressure in dogs reported for SNOcap in the in-vivo study of Jia & Wong (2001). Our previous in-vitro study provided evidence to suggest that dissociation of SNOcap into NO and captopril occurred much more slowly than this, even in the presence of vascular tissue (Tsui et al 2003). Hence, it was concluded that the presence of free NO may not be required for the vasorelaxant action of the drug but, instead, vasorelaxation may occur by a transnitrosation reaction. Undissociated SNOcap can readily cross the endothelium to the underlying vascular smooth muscle and it does this more rapidly than captopril (Jia & Wong 2001). Therefore, it is quite possible that the in-vivo depressor effect, like in-vitro vasorelaxation, is due to undissociated SNOcap (i.e., does not necessarily require the presence of free NO).

To test the effects of SNOcap on PAP in pulmonary hypertensive rats, a model of early pulmonary hypertension was chosen (i.e., exposure to hypoxia for 1 week) (Jeffery & Wanstall 2001b). This was because pulmonary vasodilators can be expected to be most effective in the early stages of the disease before pulmonary vascular remodelling becomes too advanced (Reeves et al 1986; Wanstall & Jeffery 1998). The data obtained in this study confirmed that the chosen hypoxic regime caused three key characteristics of hypoxic pulmonary hypertension, namely, an increase in PAP, right ventricular hypertrophy and polcythaemia. Since only the acute effects of SNOcap were examined in this study, the increase in PAP was the only one of these parameters to be reduced by the drug treatment.

The finding that SNOcap was able to reduce MPAP after acute administration in rats with early pulmonary hypertension is consistent with the direct vasodilator properties of the drug. At a later stage of pulmonary hypertension, when pulmonary vascular remodelling is more pronounced, a drug that has only vasodilator properties would not be expected to be so effective. However, SNOcap may have an advantage over other vasodilators in that it is also an ACE inhibitor and can therefore be expected to inhibit pulmonary vascular remodelling, as described for captopril (Morrell et al 1995) and perindopril (Jeffery & Wanstall 1999). Hence, long-term treatment with SNOcap in rats with more severe pulmonary hypertension is worthy of investigation in the future.

A potential disadvantage of SNOcap as a vasodilator in pulmonary hypertension is its lack of selectivity for the pulmonary, compared with the systemic, circulation. The doses of SNOcap found to be effective in lowering PAP ($1\,mg\,kg^{-1}$ i.v.; $30\,mg\,kg^{-1}$ p.o.) were within the ranges previously shown to reduce SAP in rats. In two previous studies, intravenous SNOcap was shown to dose-dependently reduce SAP in rats within the dose ranges 0.125–$12.5\,mg\,kg^{-1}$ (Jia & Blantz 1998) and 0.25–$12.7\,mg\,kg^{-1}$ (Jia & Wong 2001, calculated from data provided in $\mu mol\,kg^{-1}$). When given orally, 10 and $50\,mg\,kg^{-1}$ SNOcap reduced SAP in normotensive rats (Jia & Blantz 1998) and 5 and $50\,mg\,kg^{-1}$ reduced SAP in spontaneously hypertensive rats (Jia et al 1999). The lack of pulmonary selectivity of SNOcap was confirmed in this study where $30\,mg\,kg^{-1}$, the lowest oral dose to be effective on PAP, caused a 38% drop in SAP. Hence, if SNOcap were to be useful as a vasodilator in pulmonary hypertension it, like other NO donors, would need to be formulated so that it can be given directly into the lungs by inhalation.

Conclusion

In conclusion, this study has shown that the in-vitro pulmonary vasorelaxant properties of SNOcap, reported previously, translate into a pulmonary vasodepressor effect in-vivo, in both normotensive rats and rats with hypoxic pulmonary hypertension. Since SNOcap also inhibits ACE in pulmonary blood vessels (Tsui et al 2003), it is concluded that SNOcap would be an interesting drug to test in a chronic study in more advanced pulmonary hypertension. This would allow the benefits of pulmonary vasodilatation (as demonstrated in this study) combined with chronic ACE inhibition (which attentuates pulmonary vascular remodelling) to be assessed.

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