Physiol. Res. 44: 1–6, 1995

Vasorelaxation by Pinacidil in Isolated Perfused Lungs is Enhanced in Rats with Hypoxic Pulmonary Hypertension but is Dependent on the Constrictor

J.C. WANSTALL (with the technical assistance of A. Gambino)

Pulmonary Pharmacology Group, Department of Physiology and Pharmacology, University of Queensland, Brisbane, Australia

Received October 25, 1994
Accepted December 1, 1994

Summary

The potassium channel opening drug, pinacidil, has been examined in isolated perfused lungs taken from rats with hypoxic pulmonary hypertension (housed in 10 % oxygen for 7 days) and control rats. Inhibition by pinacidil (1 to 30 μM) of noradrenaline (NA)-induced vasoconstriction (NA infusions; β-adrenoceptors blocked) and of hypoxic pulmonary vasoconstriction (HPV; ventilation for 3.5–4.5 min with 0–1 % oxygen) were compared. The vasoconstrictor responses in preparations from control and hypoxic rats, respectively, were (mm Hg) NA 6.6±0.68 (6); 8.2±1.45 (9); HPV 7.8±1.03 (12); 8.8±0.93 (13). These responses were reversibly inhibited by pinacidil. In lungs from control rats pinacidil was 10-fold less potent against NA than against HPV, but in lungs from hypoxic rats it was equipotent against NA and HPV. When tested against NA, but not HPV, pinacidil was significantly more potent in lungs from hypoxic rats than control rats. It is postulated that NA-induced vasoconstriction in lungs from hypoxic rats, and HPV in both groups of rats, involve calcium influx through voltage-operated calcium channels. Consequently, these responses are readily inhibited by drugs such as pinacidil which open potassium channels and hyperpolarise the cell membrane. In contrast in lungs from control rats, NA-induced constriction may involve mainly intracellular calcium release and thus be less readily inhibited by the hyperpolarising effect of pinacidil.

Key words

Pinacidil – Rat perfused lungs – Pulmonary hypertension – Hypoxic pulmonary vasoconstriction – Noradrenaline

Introduction

Treatment with vasodilator drugs is one of the few therapeutic options that is available for patients with pulmonary hypertension. The most successful drugs have been prostacyclin (Long and Rubin 1987) and the calcium entry blocking drugs (Rich *et al.* 1992), but the "ideal" pulmonary vasodilator for pulmonary hypertension has yet to be found. Potassium channel opening drugs may be a valuable alternative to the current drugs. To date this group of drugs has not been systematically evaluated in pulmonary hypertension although there are isolated reports of the successful use of diazoxide in some patients with this disease (Chan *et al.* 1987). In animal experiments various potassium channel opening drugs have been shown to be good vasorelaxants in isolated preparations of large (conduit) pulmonary arteries (Kay *et al.* 1990, Wanstall and O'Donnell 1992, Rodman 1992), but it is also important to determine the pharmacological properties of these drugs on the resistance vessels of the pulmonary circulation, especially in experimental models of pulmonary hypertension.

The aim of this study was to examine the pulmonary vasorelaxant effects of the potassium channel opening drug, pinacidil, in isolated perfused lungs (pulmonary resistance vessel preparation) from normal rats and rats with chronic hypoxic pulmonary hypertension. The effects of pinacidil have been determined against two different vasoconstrictor responses, i.e. hypoxic pulmonary vasoconstriction (HPV) and noradrenaline (NA)-induced vasoconstriction.

Methods

Treatment of rats

Male Wistar rats (6 weeks, 170–235 g) were housed for 1 week in normobaric hypoxic chambers (10 % oxygen; hypoxic rats) or in room air (21 % oxygen; control rats) as described previously (Wanstall *et al.* 1992). They were then anaesthetized with pentobarbitone (90 mg kg^{-1} i.p.), the thorax was opened and heparin (2500 IU kg^{-1}) was administered directly into the right ventricle. A blood sample was removed for determination of the haematocrit. The lungs were removed, together with the heart and trachea, and were set up for perfusion of the pulmonary circulation (see below).

At the end of the experiment, the heart was separated from the lungs, divided into right ventricle (RV) and left ventricle plus septum (LV+S), blotted and weighed. The ratios RV/(LV+S) and RV/body weight were significantly higher ($P<0.001$) in hypoxic rats (0.52 ± 0.02 mg/mg and 1.15 ± 0.03 mg/g, respectively, $n=22$) than in control rats (0.31 ± 0.01 and 0.71 ± 0.02, respectively, $n=17$). Thus the hypoxic rats had right ventricular hypertrophy. This provided evidence that the hypoxic rats had pulmonary hypertension since the presence of right ventricular hypertrophy is a reliable indicator of elevated pulmonary artery pressure (Ghodsi and Will 1981, Wanstall and O'Donnell 1992, Wanstall *et al.* 1992). The hypoxic rats also had elevated haematocrit (65 ± 1.4 %) when compared with control rats (47 ± 0.6 %), i.e. the hypoxic rats had polycythemia.

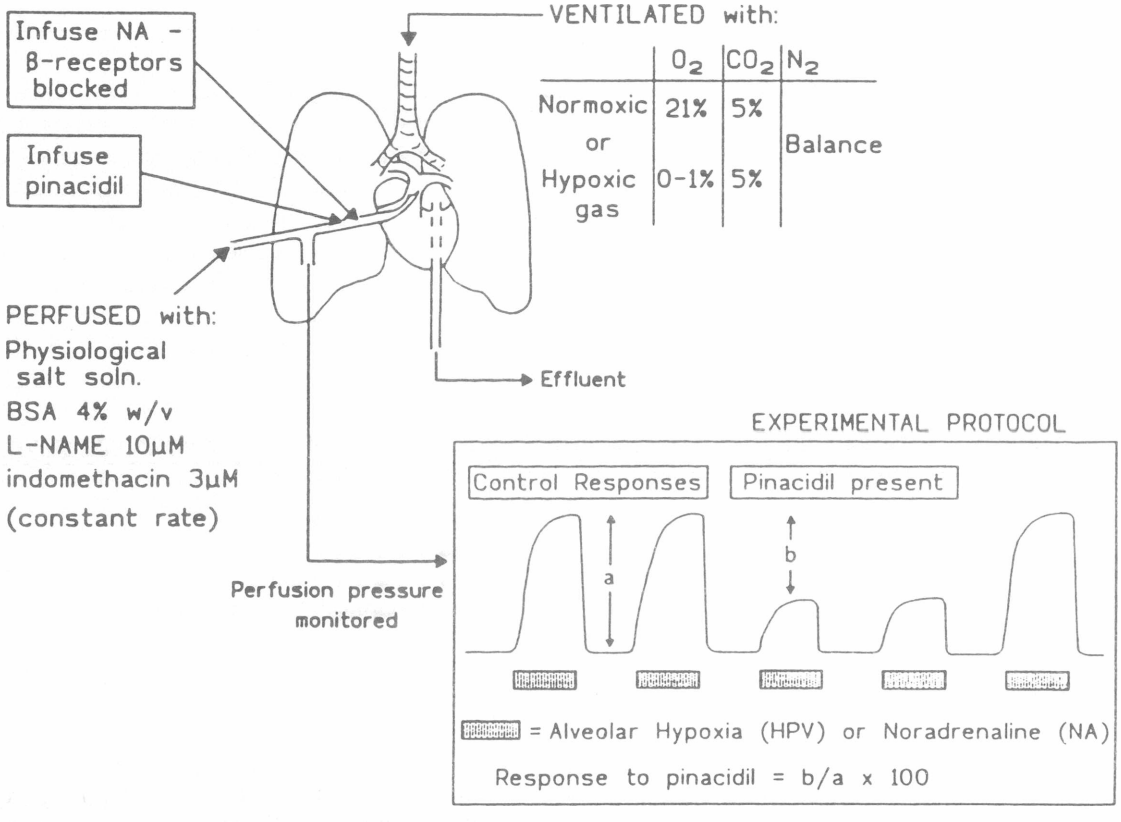

Fig. 1
Method for perfusing rat lungs *in vitro* and experimental protocol for determining the inhibitory effect of pinacidil on vasoconstrictor responses induced by alveolar hypoxia (HPV) or by noradrenalin infusion (NA).

Isolated perfused lung preparations

The lungs were set up in a humidified chamber maintained at 37 °C. They were ventilated *via* a cannula in the trachea with a normoxic gas mixture containing 21 % O_2/5 % CO_2/balance N_2 (60 strokes x min^{-1}; inspiratory pressure 9 cm H_2O; end expiratory pressure 2.5 cm H_2O; Ugo Basile rodent ventilator). The pulmonary circulation was perfused at constant rate (0.035 ml x g body $weight^{-1}$ x min^{-1}), *via* a cannula in the pulmonary artery, with physiological salt solution (PSS) containing 4 % w/v bovine serum albumin (BSA), 10 μM N^G-nitro-L-arginine methyl ester (L-NAME; nitric oxide synthase inhibitor), and 3 μM indomethacin (cyclooxygenase inhibitor). The effluent perfusate was not recirculated. Perfusion

pressure was measured *via* a side arm in the perfusion line (Trantec pressure transducer Model 60-800, Ugo Basile Gemini recorder). A diagrammatic representation of the method is shown in Fig. 1. The composition of the PSS was (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $CaCl_2$ 3.2, KH_2PO_4 1.18, $NaHCO_3$ 22.6, glucose 5.5, sucrose 50 (McMurtry 1984).

Experimental protocol and analysis of data

In one series of experiments, hypoxic pulmonary vasoconstrictor (HPV) responses were induced by replacing the normoxic gas mixture ventilating the lungs with a hypoxic gas mixture containing 0–1 % O_2/5 % CO_2/balance N_2 (Fig. 1). In these experiments angiotensin II (2.5 nM; a concentration just below the threshold for vasoconstriction) was included in the PSS. In a second series of experiments vasoconstrictor responses were induced by infusion of noradrenaline (NA) into the perfusate just proximal to the pulmonary artery cannula (Fig. 1). The rate of infusion was ≤ 2 % of the perfusion rate and the final concentration of NA in the perfusate was 1 μM in experiments on lungs from control rats and 0.3 μM in lungs from hypoxic rats. The different concentrations were selected in order to match the size of the constrictor responses to NA in lungs from the two groups of rats. In the NA experiments propranolol (1 μM) was included in the perfusate PSS to block any effect of NA on β-adrenoceptors. Each vasoconstrictor response (HPV or NA) took 3.5 to 4.5 min to reach equilibrium; 4 min was allowed to elapse between successive responses.

The experimental protocol for examining the vasorelaxant effect of pinacidil is shown in Fig. 1. Vasoconstrictor responses (HPV or NA) were repeated until two consecutive control responses were reproducible. Two further vasoconstrictor responses were then obtained in the presence of pinacidil which was infused into the perfusion line (infusion rate ≤ 2 % of the perfusion rate) to give a final concentration in the perfusate of 1, 3, 10 or 30 μM pinacidil. The pinacidil infusion was then switched off and the vasoconstrictor responses were repeated in the absence of pinacidil to check the reversibility of the effect of pinacidil (Fig. 1). In the experiments where pinacidil was tested against HPV responses only one concentration of pinacidil was studied on each lung preparation, but in the experiments with NA up to three concentrations of pinacidil were tested on each lung preparation.

Vasoconstrictor responses were measured as increases in perfusion pressure (Δ mm Hg). The effect of pinacidil was determined as "percentage inhibition" of the vasoconstrictor response as shown in Fig. 1. All values are expressed as mean values ± S.E.M. Values of n represent the number of different animals. Statistical differences between mean values have been assessed by Mann-Whitney U-test for values of "percentage inhibition" and by Student's t test for all other values.

Drugs and solutions

The following drugs and chemicals were used: angiotensin II (Sigma); bovine albumin (Fraction V; Sigma); heparin sodium (Commonwealth Serum Laboratories, Australia); indomethacin (Sigma); N^G-nitro-L-arginine methyl ester (L-NAME, Sigma); (−) noradrenaline acid tartrate (Sigma); pentobarbitone sodium (Nembutal, Boehringer Ingelheim); pinacidil (gift from Leo Pharmaceuticals); propranolol hydrochloride (Zeneca Pharmaceuticals). Solutions of L-NAME (10 mM) and propranolol (10 mM) were prepared in deionized water, of angiotensin (1 mM) and noradrenaline (100 mM) in 10 mM HCl, of pinacidil (100 mM) in 100 mM HCl and of indomethacin (10 mM) in absolute ethanol. Dilutions were prepared in PSS.

Table 1
Resting perfusion pressure and vasoconstrictor responses to hypoxia (HPV) and noradrenaline (NA) in isolated perfused lungs from control and hypoxic rats.

	Control rats	Hypoxic rats
Resting perfusion pressure (mm Hg)	8.2 ± 0.51 (18)	12.6 ± 0.39*** (22)
Vasoconstrictor responses (Δ mm Hg):		
HPV[a]	7.8 ± 1.03 (12)	8.8 ± 0.93 (13)
NA[b]	6.6 ± 0.68 (6)	8.2 ± 1.45 (9)

Values are means ± S.E.M. Numbers of lung preparations are in parentheses. All lung preparations were perfused with PSS containing 4 % w/v BSA, 10 μM L-NAME and 3 μM indomethacin. [a] *hypoxic pulmonary vasoconstriction induced by ventilation of lungs with hypoxic gas mixture (0–1 % oxygen). AII (2.5 nM) present in the PSS.* [b] *vasoconstriction induced by infusion of noradrenaline into lung perfusate (noradrenaline concentration in perfusate: control rats 1 μM, hypoxic rats 0.3 μM). Propranolol (1 μM) present in the PSS.* *** *Value in hypoxic rats significantly higher than value in control rats P < 0.001 (Student's t test)*

Results

The resting perfusion pressure was significantly higher in lungs from rats exposed to chronic hypoxia than in those from control rats (Table 1). The vasoconstrictor responses were the same whether induced by ventilation with a hypoxic gas mixture (HPV) or by NA infusion, and whether obtained in lungs from control rats or hypoxic rats (Table 1).

Pinacidil caused concentration-dependent, reversible inhibition of HPV or NA-induced vasoconstriction. Concentration-response (inhibition) curves for pinacidil are shown in Fig. 2. This figure allows data for pinacidil to be compared (a) between the two different types of vasoconstriction and (b) between lungs from control and hypoxic rats.

In lungs from control rats, pinacidil was significantly less effective in inhibiting NA than HPV; the difference in potency was about 10-fold (Fig. 2). In contrast, in lungs from hypoxic rats pinacidil was equally potent against NA and HPV (Fig. 2). When tested against NA, the effects of pinacidil were significantly greater in lungs from hypoxic rats than in those from control rats but no significant difference between the two groups of rats was seen when pinacidil was tested against HPV (Fig. 2).

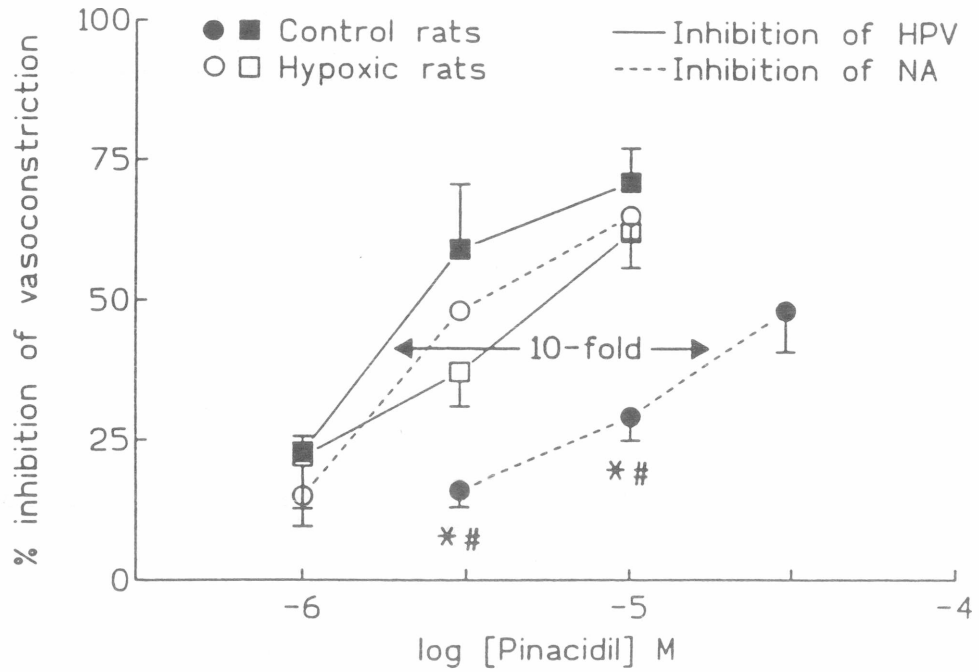

Fig. 2
Mean concentration-response curves for pinacidil in inhibiting vasoconstriction induced by hypoxia (HPV) or noradrenaline (NA) in isolated perfused lungs from control rats (solid symbols) or hypoxic rats (open symbols). * inhibition of NA (control rats, full dot) significantly less than inhibition of HPV (control rats, full square) by the same concentration of pinacidil ($P<0.05$, Mann-Whitney U-test). × inhibition of NA (control rats, full dot) significantly less than inhibition of NA (hypoxic rats, open circle) by the same concentration of pinacidil ($P<0.05$, Mann-Whitney U-test). Data are mean ± S.E.M. values from 4–5 different lungs.

Discussion

There were two main observations from this study on the vasorelaxant effects of pinacidil in perfused lungs from rats. Firstly, in lungs from control rats, the potency of pinacidil in inhibiting vasoconstrictor responses was dependent on the method of inducing the vasoconstriction, i.e. pinacidil was less potent when constriction was induced with NA than when it was induced with acute alveolar hypoxia (HPV). Secondly, any difference in the vasorelaxant effects of pinacidil between lungs from pulmonary hypertensive and control rats was also dependent on the vasoconstrictor, because the potency of this drug was enhanced in lungs from pulmonary hypertensive rats when it was tested against NA but not when tested against HPV.

These data in perfused lungs reflect previous data obtained in ring preparations of the main pulmonary artery. In arteries from control rats the

potency of pinacidil was found to be dependent on the vasoconstrictor spasmogen used to contract the preparations, i.e. it was less potent against NA and endothelin than against $PGF_{2\alpha}$ (O'Donnell *et al.* 1991) or U46619 (Wanstall, unpublished data). Furthermore, in arteries from pulmonary hypertensive rats the potency of pinacidil was enhanced, when compared with control rats. This enhancement was also spasmogen-dependent, i.e. it was seen if arteries were contracted with NA or endothelin but not if contracted with $PGF_{2\alpha}$ or U46619 (Kay *et al.* 1990, Wanstall and O'Donnell 1992, Wanstall and Kay, unpublished data). Responses to another potassium channel opening drug, levcromakalim, have also been shown to be enhanced in pulmonary arteries from pulmonary hypertensive rats (Rodman 1992); in that study only one vasoconstrictor spasmogen was used, i.e. phenylephrine (α-adrenoceptor agonist).

The differences in the potency of pinacidil between NA and HPV, or between lungs from the two groups of rats, could not be attributed to differences in the magnitude of the various vasoconstrictor responses because care was taken to match these for size. For NA-induced vasoconstriction this necessitated the use of different concentrations of NA in lungs from the two groups of rats since we found in preliminary studies that responses to a fixed concentration of NA were larger in lungs from hypoxic rats than in those from control rats. For HPV the presence of L-NAME in the PSS ensured that the constrictions were of the same magnitude in lungs from the two groups of rats. We have previously shown that, in the absence of L-NAME, HPV responses were larger in lungs from hypoxic rats than in those from control rats. However L-NAME potentiated HPV responses in lungs from control, but not hypoxic, rats, thereby eliminating the difference in the size of the HPV response in the two groups of rats (Wanstall, unpublished data).

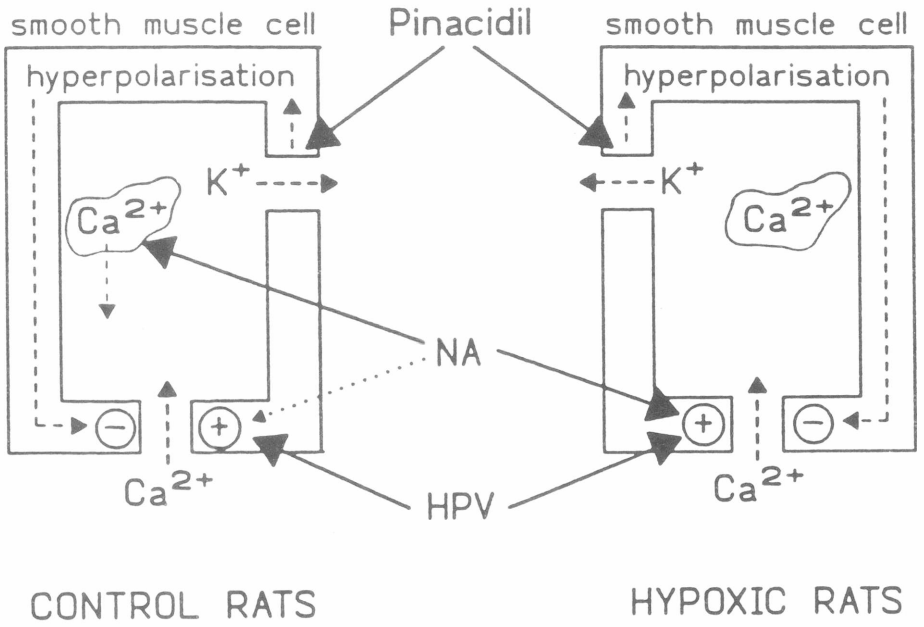

Fig. 3

Diagram of pulmonary vascular smooth muscle cells to illustrate the inhibitory effect of pinacidil on vasoconstriction induced by hypoxia (HPV) or noradrenaline (NA) in lungs from control and hypoxic rats. It is postulated that both (a) HPV (in lungs from control or hypoxic rats) and NA-induced vasoconstriction (in lungs from hypoxic rats) involve calcium influx through VOCs and are therefore readily inhibited by pinacidil which opens potassium channels and causes hyperpolarisation; (b) NA-induced vasoconstriction in lungs from control rats involves mainly intracellular calcium release and hence is comparatively resistant to the hyperpolarising effects of pinacidil.

A hypothesis which could explain the findings of this study, and which takes into consideration the mechanisms of action of pinacidil, NA and HPV, is illustrated in Fig. 3. The primary mechanism whereby pinacidil relaxes smooth muscle cells is through the opening of potassium channels; this leads to potassium efflux and hyperpolarisation of the cell membrane (Southerton *et al.* 1988). We have previously shown that inhibition of HPV in isolated lungs by pinacidil is prevented by the potassium channel blocking drug, glibenclamide, confirming an action of pinacidil on potassium channels in these preparations (Wanstall, unpublished data). Drugs, such as pinacidil, that act by hyperpolarisation are most effective against vasoconstrictor responses that involve calcium influx through voltage-operated calcium channels (VOCs).

The pulmonary vasoconstriction that is induced by alveolar hypoxia (HPV) is known to involve calcium influx through VOCs (McMurtry 1985). Thus our observation that HPV was readily inhibited by pinacidil was predictable. Since pinacidil was equally effective against HPV in lungs from hypoxic rats as it was in control rats we suggest that the mechanism of HPV remains the same in rats with hypoxic pulmonary hypertension (Fig. 3). On the other hand, NA- induced vasoconstriction may involve mainly intracellular calcium release in lungs from control rats, but calcium influx through VOCs in lungs from hypoxic (pulmonary hypertensive) rats (Fig. 3). This hypothesis would explain why pinacidil was less effective against NA than against HPV in control rats, but equally effective against the two types of vasoconstriction in lungs from hypoxic rats.

In summary, pinacidil is an effective pulmonary vasodilator in rat perfused lungs. Since, in control rats, it was more effective against HPV than against NA pinacidil might preferentially dilate hypoxic regions of the lung in these rats, thereby exacerbating any ventilation-perfusion mismatch. This disadvantage may not exist in pulmonary hypertensive rats where pinacidil was found to be just as effective against NA as against HPV. If it should be equally effective against other endogenous pulmonary vasoconstrictors then, in pulmonary hypertension, pinacidil should cause vasodilatation throughout the whole lung (i.e. in both hypoxic and normoxic regions), and ventilation-perfusion mismatch would be minimized. It remains to be established whether the findings of this study on rat lungs are reflected in lungs from humans.

Acknowledgement

The financial support of the National Health and Medical Research Council of Australia is gratefully acknowledged.

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Reprint Requests

Dr. Janet C. Wanstall, Department of Physiology and Pharmacology, The University of Queensland, Brisbane, Queensland 4072, Australia.