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Catecholamine modulatory effects of nepicastat (RS-25560-197), a novel, potent and selective inhibitor of dopamine-β-hydroxylase

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1 Inhibitory modulation of sympathetic nerve function may have a favourable impact on the progression of congestive heart failure. Nepicastat is a novel inhibitor of dopamine-β-hydroxylase, the enzyme which catalyses the conversion of dopamine to noradrenaline in sympathetic nerves. The *in vitro* pharmacology and *in vivo* catecholamine modulatory effects of nepicastat were investigated in the present study.

2 Nepicastat produced concentration-dependent inhibition of bovine ($IC_{50} = 8.5 \pm 0.8$ nM) and human ($IC_{50} = 9.0 \pm 0.8$ nM) dopamine-β-hydroxylase. The corresponding **R**-enantiomer (RS-25560-198) was approximately 2–3 fold less potent than nepicastat. Nepicastat had negligible affinity (>10 μM) for twelve other enzymes and thirteen neurotransmitter receptors.

3 Administration of nepicastat to spontaneously hypertensive rats (SHRs) (three consecutive doses of either 3, 10, 30 or 100 mg kg^{-1}, p.o.; 12 h apart) or beagle dogs (0.05, 0.5, 1.5 or 5 mg kg^{-1}, p.o.; b.i.d., for 5 days) produced dose-dependent decreases in noradrenaline content, increases in dopamine content and increases in dopamine/noradrenaline ratio in the artery (mesenteric or renal), left ventricle and cerebral cortex. At the highest dose studied, the decreases in tissue noradrenaline were 47%, 35% and 42% (in SHRs) and 88%, 91% and 96% (in dogs) in the artery, left ventricle and cerebral cortex, respectively. When tested at 30 mg kg^{-1}, p.o., in SHRs, nepicastat produced significantly greater changes in noradrenaline and dopamine content, as compared to the **R**-enantiomer (RS-25560-198), in the mesenteric artery and left ventricle.

4 Administration of nepicastat (2 mg kg^{-1}, b.i.d, p.o.) to beagle dogs for 15 days produced significant decreases in plasma concentrations of noradrenaline and increases in plasma concentrations of dopamine and dopamine/noradrenaline ratio. The peak reduction (52%) in plasma concentration of noradrenaline and the peak increase (646%) in plasma concentration of dopamine were observed on day-6 and day-7 of dosing, respectively.

5 The findings of this study suggest that nepicastat is a potent, selective and orally active inhibitor of dopamine-β-hydroxylase which produces gradual modulation of the sympathetic nervous system by inhibiting the biosynthesis of noradrenaline. This drug may, therefore, be of value in the treatment of cardiovascular disorders associated with over-activation of the sympathetic nervous system, such as congestive heart failure.

Keywords: Nepicastat; RS-25560-197; dopamine-β-hydroxylase; catecholamines; noradrenaline; dopamine; heart failure; neurohormonal; sympathetic

Introduction

Activation of neurohormonal systems, chiefly the sympathetic nervous system, is the cardinal clinical manifestation of congestive heart failure (see Packer, 1989, 1992; Parmley, 1995 for reviews). Congestive heart failure patients have elevated concentrations of plasma noradrenaline (Thomas & Marks, 1978; Levine *et al*., 1982), increased central sympathetic outflow (Leimbach *et al*., 1986) and augmented cardio-renal noradrenaline spillover (Hasking *et al*., 1986). Prolonged and excessive exposure of myocardium to noradrenaline may lead to downregulation of cardiac β_1-adrenoceptors, remodelling of left ventricle, arrhythmias and necrosis, all of which can diminish the functional integrity of the heart (see Packer, 1989; Daly & Sole, 1990 for reviews). Indeed, congestive heart failure patients who have higher plasma concentrations of noradrenaline also have the most unfavourable long-term prognosis (Cohn *et al*., 1984). Of greater significance is the observation that plasma noradrenaline concentrations are already elevated in asymptomatic patients with no overt heart failure and can predict ensuing mortality and morbidity (Francis *et al*., 1990; Benedict *et al*., 1996). This implies that the activated sympathetic drive is not merely a clinical marker of congestive heart failure but may contribute to progressive worsening of the disease. Consequently, therapeutic interventions which inhibit sympathetic nerve function are likely to alter the natural history of congestive heart failure in a favourable direction (Packer, 1992; Remme, 1994).

Inhibition of sympathetic nerve function with adrenoceptor antagonists (pure β-blockers or mixed α,β-blockers) is a promising approach which is under clinical evaluation (see Bristow, 1993; Doughty *et al*., 1994; Hjalmarson & Waagstein, 1994 for reviews). Recent clinical trials with carvedilol, a compound having both α- and β-adrenoceptor antagonistic properties, have shown a promising reduction in mortality and morbidity in congestive heart failure patients (Packer *et al*., 1996a,b). However, a significant proportion of patients do not tolerate the immediate haemodynamic deterioration which accompanies β-blocker treatment (Pfeffer & Stevenson, 1996). An alternative strategy for directly modulating sympathetic nerve function is to inhibit the biosynthesis of noradrenaline via inhibition of dopamine-β-hydroxylase, the enzyme responsible for conversion of dopamine to noradrenaline in sympathetic nerves. This approach, on theoretical grounds, has several merits over adrenoceptor blockade which include

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gradual modulation, as opposed to abrupt inhibition, of the sympathetic system, and causing increased release of dopamine which can improve renal function.

Dopamine-β-hydroxylase-inhibitors that have been previously described include disulfiram (Goldstein & Nakajima, 1966), FLA-63 (Anden *et al*., 1972), SCH 10595 (Korduba *et al*., 1973), fusaric acid (Matta & Wooten, 1973), BRL 8242 (Claxton *et al*., 1976) and SKF 102698 (Berkowitz *et al*., 1988). The therapeutic utility and clinical development of some of the above compounds has been hampered by low potency, lack of selectivity for dopamine-β-hydroxylase and toxic side-effects. Nepicastat (RS-25560-197) (Figure 1) is a novel inhibitor of dopamine-β-hydroxylase (Hegde *et al*., 1996a) the pharmacology of which is described in the present study. The findings of this study show that nepicastat is a potent and highly selective inhibitor of dopamine-β-hydroxylase which modulates catecholamine levels in rats and dogs.

A preliminary account of the findings has been presented to the American Heart Association (Hegde *et al*., 1996a) and the British Pharmacological Society (Whiting *et al*., 1997).

Methods

In vitro *studies*

Bovine and human dopamine-β-hydroxylase activity were assayed by measuring the conversion of tyramine to octopamine (Feilchenfeld *et al*., 1982). Bovine adrenal dopamine-β-hydroxylase was obtained from Sigma Chemicals (St Louis, MO, U.S.A.) whereas human dopamine-β-hydroxylase was purified from the culture medium of the neuroblastoma cell line SK-N-SH (Li *et al*., 1995). The assay was performed at pH 5.2 and 32°C in a medium containing 0.125 M sodium acetate, 10 mM fumarate, 0.5–2 μM $CuSO_4$, 0.1 mg ml^{-1} catalase, 0.1 mM tyramine and 4 mM ascorbate. In a typical assay, 0.5–1 mu of enzyme were added to the reaction mixture and, subsequently, a substrate mixture containing catalase, tyramine and ascorbate was added to initiate the reaction (final volume of 200 μl). Samples were incubated with or without the appropriate concentration of nepicastat (RS-25560-197, **S**-enantiomer) or RS-25560-198 (**R**-enantiomer) at 37°C for 30–40 min. The reaction was quenched by the stop solution containing 25 mM EDTA and 240 μM 3-hydroxytyramine (internal standard). The samples were analysed for octopamine by reverse phase high pressure liquid chromatography (h.p.l.c.) with ultraviolet (u.v.)-detection at 280 nM. The h.p.l.c. run was carried out at a flow rate of 1 ml min^{-1} with a LiChroCART 125-4 RP-18 column and isocratic elution with 10 mM acetic acid, 10 mM 1-heptane sulphonic acid, 12 mM tetrabutyl ammonium phosphate and 10% methanol. The remaining % activity was calculated based on controls (without RS 25560), corrected with internal standards and fitted to a non-linear four-parameter concentration-response curve.

The activity of nepicastat at twelve selected enzymes and receptors was determined by use of established assays. Details of individual receptor radioligand binding assays can be found in Wong *et al*. (1993). A brief account of the principle underlying each of the enzymatic assays is given in Table 1. Binding data were analysed by iterative curve-fitting to a four parameter logistic equation. K_i values were calculated from IC_{50} values by the Cheng-Prusoff equation (Cheng & Prusoff, 1973). Enzyme inhibitory activity was expressed as IC_{50} (concentration required to produce 50% inhibition of enzyme activity).

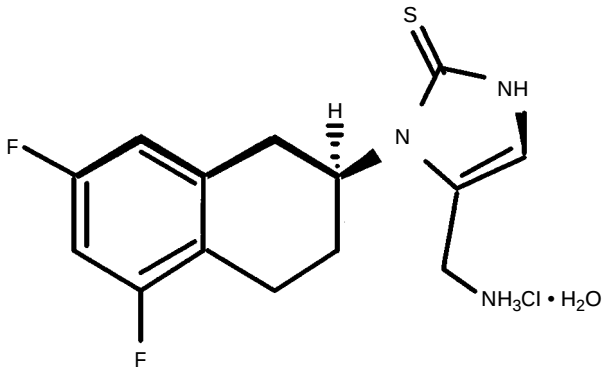

Figure 1 Chemical structure of nepicastat (**S**-5-aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-yl)-1,3-dihydroimidazole-2-thione hydrochloride).

In vivo *studies*

Effects on tissue catecholamimes in spontaneously hypertensive rats (SHRs) Male SHRs (15–16 weeks old, Charles River, Wilmington, MA, U.S.A.) were used in the study. On the day of the study, animals were weighed and randomly assigned to be dosed with either vehicle (control) or the appropriate dose of nepicastat (3, 10, 30 or 100 mg kg^{-1}, p.o.) or RS-25560-198 (30 mg kg^{-1}, p.o.) three consecutive times, twelve hours apart. At six hours after the third dose, the rats were anaesthetized with halothane, decapitated and tissues (cerebral cortex, mesenteric artery and left ventricle) were rapidly harvested, weighed, placed in iced perchloric acid (0.4 M), frozen in liquid nitrogen and stored at −70°C until subsequent analysis. To quantify noradrenaline and dopamine concentrations, tissues were homogenized by brief sonication

Table 1 Details of the individual enzymatic assays

Enzyme	*Assay*
Tyrosine hydroxylase	Release of ^3H_2O associated with conversion of L-[3,5-^{3}H]-tyrosine to DOPA
Acetyl CoA synthetase	Utilization of [^{3}H]-acetic sodium acetate
Acyl-CoA, cholesterol acyltransferase	Formation of cholesteryl ester from [1-^{14}C]-palmitoyl-CoA and endogenous cholesterol
Ca^{2+}/calmodulin-protein kinase II	Phosphorylation ^{32}P of BB40 (a synthetic peptide substrate)
Cyclo-oxygenase-I	Oxidation of arachidonic acid followed by spectrophotometric quantitation of malondialdehyde
HMG-CoA reductase	Formation of [^{14}C]-mevalonic acid fom [^{14}C]-HMG-CoA
Neutral endopeptidase (human)	Formation of 4-methoxy-2-naphthylamine from glutaryl-Ala-Ala-Phe-4-methoxy-2-naphthylamide
Nitric oxide synthase (constitutive)	Conversion of [^{3}H]-arginine to [^{3}H]-citrulline
Nitric oxide synthase (inducible)	Measurement of iNOS reaction products (NO_2 and NO_3 in cytosol preparation from mouse macrophages induced by interferon-γ and lipopolysaccharide
Phosphodiesterase III (human)	Conversion of [^{3}H]-cyclicAMP to [^{3}H]-AMP which is subsequently converted to [^{3}H]-adenosine
Phospholipase A_2	Formation of [^{14}C]-palmitate from [^{14}C]-3-phosphatidylcholine
Protein kinase C (non-selective)	Phoshorylation ^{32}P of histone H1

and centrifuged at 13,000 r.p.m. for 30 min at 4°C. The supernatant, spiked with 3,4-dihydroxybenzylamine (internal standard) was assayed for noradrenaline and dopamine by h.p.l.c. analysis (reverse phase column, isocratic mobile phase consisting of Cat-A-PhaseII with 0.25% acetonitrile) with electrochemical detection.

Effects on tissue catecholamimes in beagle dogs Male beagle dogs (10–16 kg, Marshall Farms U.S.A. Inc, North Rose, NY, U.S.A.) were used in the study. On the day of the study, dogs were weighed and randomly assigned to be orally dosed with either empty capsules (control) or the appropriate dose of nepicastat (0.05, 0.5, 1.5 or 5 mg kg^{-1}; p.o., b.i.d.) for 5 days. At six hours following the first dose on day 5, the dogs were killed by an overdose of pentobarbitone and the tissues (cerebral cortex, renal artery, left ventricle) were rapidly harvested. The tissues were subsequently processed and analysed for noradrenaline and dopamine as described above.

Effects on plasma catecholamimes in beagle dogs Male beagle dogs were grouped randomly and orally dosed with either empty capsules (control) or nepicastat (2 mg kg^{-1}, p.o., b.i.d.) for 15 days. Daily venous blood samples were drawn (phlebotomy) six hours after the first dose, for measurement of plasma concentrations of dopamine and noradrenaline. The samples were collected in tubes containing heparin and glutathione, centrifuged at −4°C and the separated plasma was stored at −70°C until analysis.

Drugs

Nepicastat (**S**-5-aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-1,3-dihydroimidazole-2-thione hydrochloride) and the corresponding **R**-enantiomer (RS-25560-198) were synthesized at Roche Bioscience. In studies involving SHRs, the drugs were dissolved in distilled water and dosed orally with a gavage needle. In the dog studies, the drugs were filled in capsules and dosed orally. All doses are expressed as free base equivalents.

Statistical analysis

All data are expressed as mean ± s.e.mean. Tissue and plasma catecholamine data were analysed by a non-parametric one-way analysis of variance (ANOVA) or two-way ANOVA, respectively, followed by pairwise comparison with Fisher LSD test. $P<0.05$ was considered statistically significant.

Results

In vitro *studies*

Nepicastat (**S**-enantiomer) and RS-25560-198 (**R**-enantiomer) produced concentration-dependent inhibition of bovine and human dopamine-β-hydroxylase activity. The calculated IC_{50}s for nepicastat were 8.5 ± 0.8 nM and 9.0 ± 0.8 nM for the bovine and human enzyme, respectively. RS-25560-198 was slightly less potent (IC_{50}s of 25.1 ± 0.6 nM and 18.3 ± 0.6 nM for the bovine and human enzyme, respectively) than nepicastat.

Nepicastat had negligible affinity (IC_{50}s or K_is > 10 μM) for a range of other enzymes (tyrosine hydroxylase, acetyl CoA synthetase, acyl CoA-cholesterol acyl transferase, Ca^{2+}/calmodulin protein kinase II, cyclo-oxygenase-I, HMG-CoA reductase, neutral endopeptidase, nitric oxide synthase, phosphodiesterase III, phospholipase A_2, and protein kinase C) and neurotransmitter receptors (α_{1A}, α_{1B}, α_{2A}, α_{2B}, β_1 and β_2 adrenoceptors, M_1 muscarinic receptors, D_1 and D_2 dopamine receptors, μ opioid receptors, 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} 5-hydroxytryptamine receptors).

In vivo *studies*

Effects on tissue catecholamimes in spontaneously hypertensive rats (SHRs) Basal tissue catecholamine content (μg g^{-1} wet weight) in control animals were as follows: mesenteric artery (noradrenaline, 10.40 ± 1.03; dopamine, 0.25 ± 0.02) left ventricle (noradrenaline, 1.30 ± 0.06; dopamine, 0.02 ± 0.00) and cerebral cortex (noradrenaline, 0.76 ± 0.03; dopamine, 0.14 ± 0.01). When compared to control animals, nepicastat produced dose-dependent reduction in noradrenaline content and enhancement of dopamine content and dopamine/noradrenaline ratio in the three tissues which were studied (Figures 2 and 3). These changes attained statistical significance ($P<0.05$) at doses ⩾3 mg kg^{-1} in the mesenteric artery and left ventricle but only at doses of 30 and 100 mg kg^{-1} in the

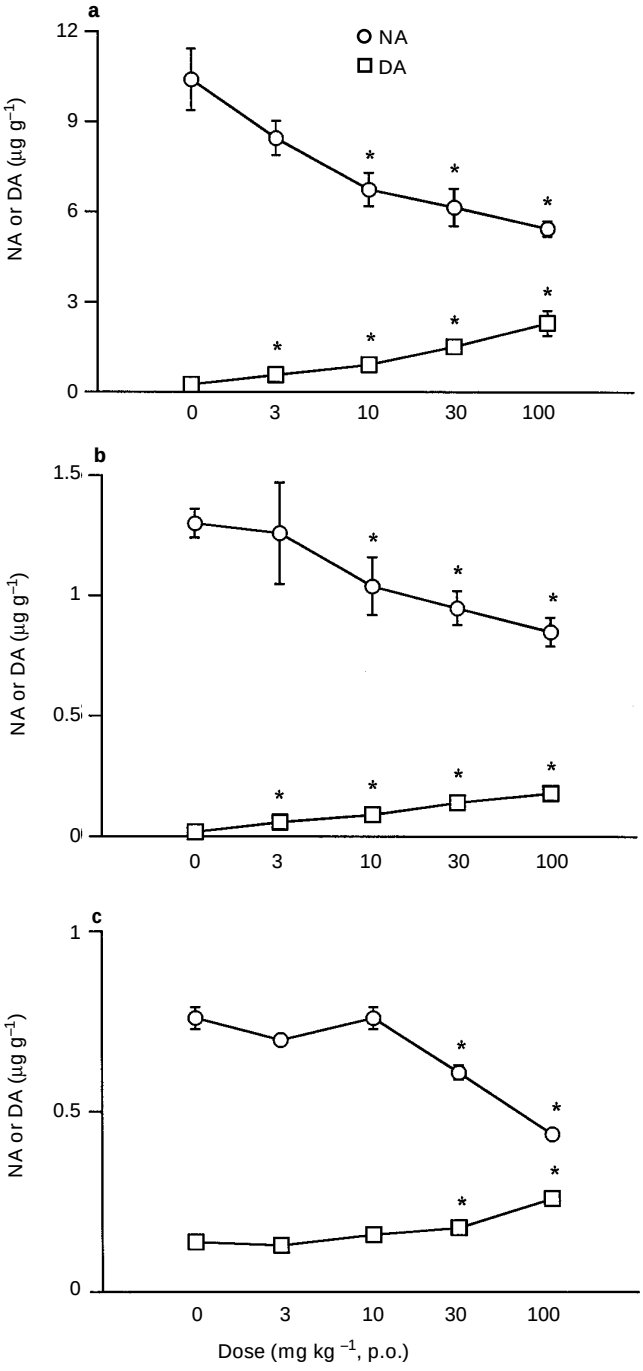

Figure 2 Effects of nepicastat on tissue noradrenaline (NA) and dopamine (DA) content in the mesenteric artery (a), left ventricle (b) and cerebral cortex (c) of SHRs. Data are expressed as mean and vertical lines show s.e.mean; $n=7-9$ per group. *$P<0.05$ vs control (0).

cerebral cortex. At the highest dose studied (100 mg kg^{-1}, p.o.), the decreases in noradrenaline were 47%, 35%, 42% and increases in dopamine were 820%, 800% and 86% in the mesenteric artery, left ventricle and cerebral cortex, respectively. When tested at 30 mg kg^{-1}, p.o., the **S**-enantiomer (nepicastat) produced significantly greater changes in catecholamine content, as compared to the **R**-enantiomer (RS-25560-198), in the mesenteric artery and left ventricle (Table 2).

Effects on tissue catecholamimes in beagle dogs Basal tissue catecholamine content ($\mu g\ g^{-1}$ wet weight) in control animals was as follows: renal artery (noradrenaline, 10.7 ± 1.05; dopamine, 0.22 ± 0.01), left ventricle (noradrenaline, 2.11 ± 0.18; dopamine, 0.07 ± 0.03) and cerebral cortex (noradrenaline, 0.26 ± 0.02; dopamine, 0.03 ± 0.00). When compared to control animals, nepicastat produced a dose-dependent reduction in noradrenaline content and enhancement of dopamine content and dopamine/noradrenaline ratio in the three tissues which were studied (Figures 4 and 5). These changes attained statistical significance ($P<0.05$) at doses $\geqslant 0.1$ mg kg^{-1} day^{-1} in the three tissues. At the highest dose studied (10 mg kg^{-1} day^{-1} p.o.), the decreases in noradrenaline were 88%, 91% and 96% and increases in dopamine were 627%, 700% and 166% in the renal artery, left ventricle and cerebral cortex, respectively.

Effects on plasma catecholamimes in beagle dogs Baseline concentrations of catecholamines in two groups of animals were not significantly different from each other: plasma noradrenaline and dopamine concentrations were 460.3 ± 59.6 and

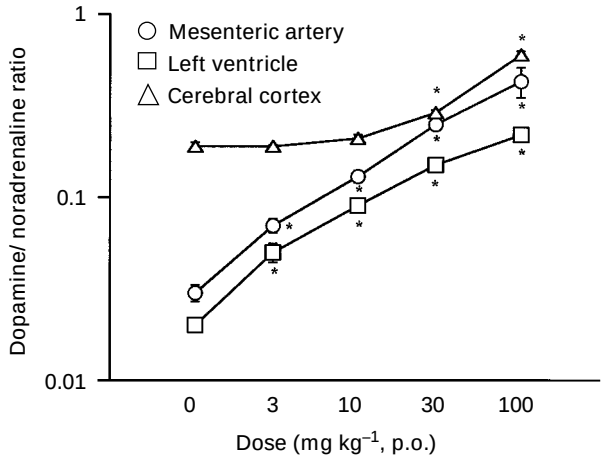

Figure 3 Effects of nepicastat on tissue dopamine/noradrenaline ratio in the mesenteric artery, left ventricle and cerebral cortex of SHRs. Data are expressed as mean and vertical lines show s.e.mean; $n=7-9$ per group. $^*P<0.05$ vs control (0).

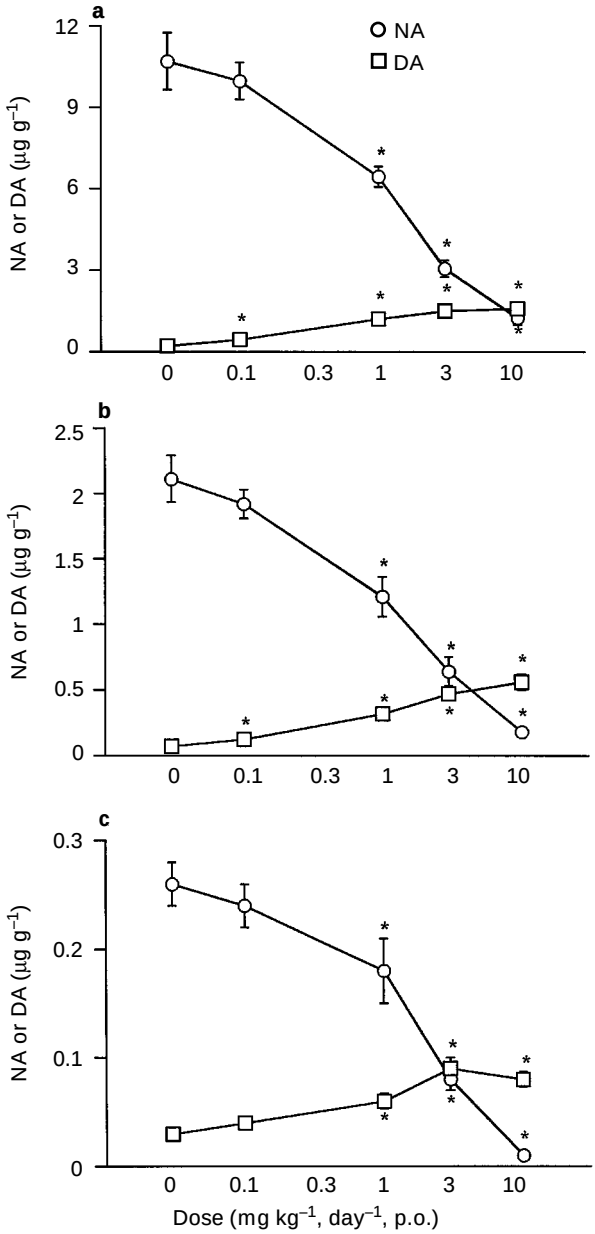

Figure 4 Effects of nepicastat on tissue noradrenaline (NA) and dopamine (DA) content in renal artery (a), left ventricle (b) and cerebral cortex (c) of beagle dogs. Data are expressed as mean and vertical lines show s.e.mean; $n=8$ per group. $^*P<0.05$ vs control (0).

Table 2 Effect of nepicastat and RS-25560-198 (30 mg kg^{-1}, p.o.) on noradrenaline content, dopamine content and dopamine/noradrenaline ratio in mesenteric artery, left ventricle and cerebral cortex of SHRs

Treatment	*Tissue*	*Noradrenaline* ($\mu g\ g^{-}$)	*Dopamine* ($\mu g\ g^{-1}$)	*Dopamine/ noradrenaline ratio*
Control (vehicle)	Mesenteric artery	10.4 ± 1.03	0.25 ± 0.02	0.03 ± 0.00
Nepicastat		6.15 ± 0.62*	1.51 ± 0.16*	0.25 ± 0.01*
RS-25560-198		8.91 ± 0.9#	0.67 ± 0.09*#	0.08 ± 0.01*#
Control (vehicle)	Left ventricle	1.30 ± 0.06	0.02 ± 0.00	0.02 ± 0.00
Nepicastat		0.95 ± 0.07*	0.14 ± 0.01*	0.15 ± 0.01*
RS-25560-198		1.16 ± 0.08#	0.07 ± 0.00*#	0.06 ± 0.01*#
Control (vehicle)	Cerebral cortex	0.76 ± 0.03	0.14 ± 0.01	0.19 ± 0.01
Nepicastat		0.61 ± 0.02*	0.18 ± 0.00*	0.29 ± 0.01*
RS-25560-198		0.88 ± 0.02*#	0.18 ± 0.00*	0.20 ± 0.01*

All data are expressed as mean $\pm$ s.e.mean, $n=9$ per group. $^*P<0.05$ vs control, $\#P<0.05$ vs nepicastat.

34.4 ± 11.9 pg ml^{-1}, respectively, in the control group and 401.9 ± 25.5 and 41.1 ± 8.8 pg ml^{-1}, respectively, in the nepicastat-treated group. When compared to the control group, nepicastat (2 mg kg^{-1}, b.i.d, p.o.) produced significant decreases in plasma concentrations of noradrenaline and increases in plasma concentrations of dopamine and dopamine/noradrenaline ratio (Figure 6). The peak reduction (52%) in plasma concentration of noradrenaline was observed on day 6 of dosing whereas the peak increase (646%) in plasma concentration of dopamine was observed on day 7 of dosing.

Discussion

Inhibitory modulation of sympathetic nerve function, through pharmacological means, is an attractive therapeutic strategy for the management of congestive heart failure, inasmuch as elevated activity of this system has been implicated in the progressive worsening of the disease. The aim of this study was to characterize pharmacologically the effects of nepicastat, a compound which modulates noradrenaline synthesis in sympathetic nerves by inhibiting the enzyme dopamine-β-hydroxylase.

Nepicastat was shown to be an inhibitor ($IC_{50} \sim 9$ nM) of human and bovine dopamine-β-hydroxylase *in vitro* and was more potent than previous inhibitors such as BRL 8242 ($IC_{50} = 5$ μM), FLA-63 ($IC_{50} = 7.5$ μM), SKF 102698 ($IC_{50} = 40$ nM), fusaric acid ($IC_{50} > 0.1$ μM) and SCH 10595 ($IC_{50} > 0.1$ μM). The inhibitory effects of the compound were stereospecific since the **S**-enantiomer (nepicastat) was marginally, but significantly, more potent than the **R**-enantiomer (RS-25560-198). Nepicastat displayed a high degree of selectivity for dopamine-β-hydroxylase but possessed negligible affinity for twelve other enzymes and thirteen neurotransmitter receptors.

Inhibition of dopamine-β-hydroxylase *in vivo* would be expected to result in elevated levels of the substrate (dopamine) and diminished levels of the product (noradrenaline) in tissues which receive noradrenergic innervation. This expectation was borne out in experiments which investigated the effects of nepicastat on catecholamine levels in central and peripheral tissues *in vivo*. In both SHRs and beagle dogs, nepicastat produced dose-dependent reductions in noradrenaline content and increases in dopamine content in peripheral (mesenteric or renal artery, left ventricle) and central (cerebral cortex) tissues. In this respect, RS 25560-198 was less potent than nepicastat which is consistent with the higher IC_{50} of the former enantiomer for the enzyme. Although dopamine/noradrenaline ratio was also elevated, there did not appear to be stoichiometric replacement of noradrenaline with dopamine. The most likely explanation for this finding is that tissue levels of dopamine may have been underestimated due to intraneuronal metabolism of dopamine.

The ability of nepicastat to alter catecholamine levels in the cerebral cortex suggests that the drug does penetrate the blood brain barrier. In dogs, the magnitude of the changes in catecholamines in the cerebral cortex appeared comparable to those in peripheral tissues. However, in SHRs, nepicastat, at low doses ($\leqslant 10$ mg kg^{-1}), produced significant changes in noradrenaline and dopamine content in peripheral tissues without affecting catecholamines in the cerebral cortex. This suggests that, at least in SHRs, the drug does possess modest peripheral selectivity. This is in contrast to SKF 102698 which produces similar changes in catecholamine levels in the central nervous system and peripheral tissues (Berkowitz *et al*., 1988).

Attenuation of noradrenaline synthesis would be expected to result in haemodynamic changes *in vivo* especially in a setting of elevated sympathetic tone. Accordingly, nepicastat attenuates sympathetically mediated cardiovascular responses and lowers blood pressure in SHRs (Hegde *et al*., 1996a,b).

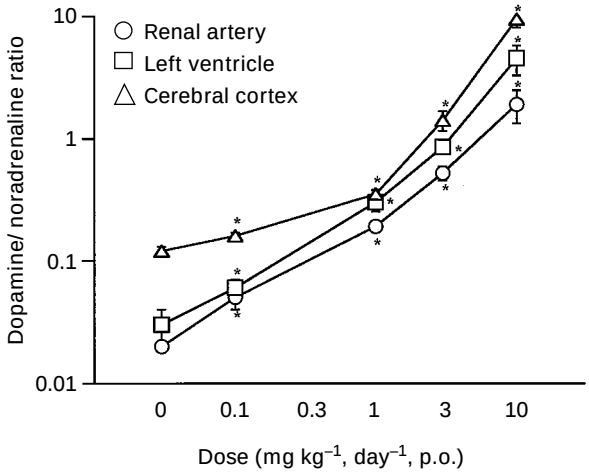

Figure 5 Effects of nepicastat on tissue dopamine/noradrenaline ratio in the renal artery, left ventricle and cerebral cortex of beagle dogs. Data are expressed as mean and vertical lines show s.e.mean; $n = 8$ per group. $^*P < 0.05$ vs control (0).

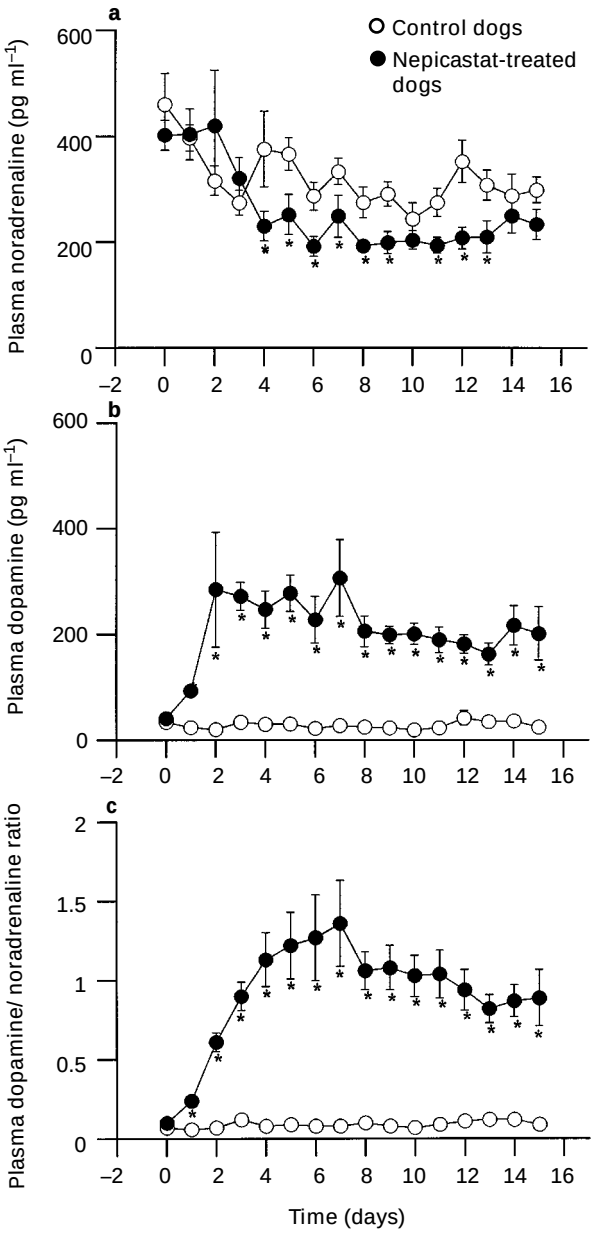

Figure 6 Effects of nepicastat on plasma concentrations of noradrenaline (a), dopamine (b) and dopamine/noradrenaline ratio (c) in beagle dogs. Data are expressed as mean and vertical lines show s.e.mean; $n = 8$ per group. $^*P < 0.05$ vs control dogs.

Plasma noradrenaline concentrations provide a useful measure of overall sympathetic nerve activity although this parameter may be influenced by alterations in neuronal uptake and metabolic clearance of the catecholamine (Esler, 1982). Baseline concentrations of noradrenaline in the plasma were surprisingly elevated in the dogs and are, perhaps, a reflection of the initial stress induced by the phlebotomy procedure. Nevertheless, compared to the control group, nepicastat produced significant decreases in plasma noradrenaline concentrations consistent with reduced transmitter synthesis and release although an indirect effect, secondary to facilitation of neuronal uptake or metabolic clearance, cannot be discounted. Since released noradrenaline represents a small fraction of the total neuronal noradrenaline stores, an inhibitor of noradrenaline biosynthesis would affect noradrenaline release only after existing stores of the catecholamine have been sufficiently depleted. Accordingly, the decreases in plasma noradrenaline concentrations did not attain statistical significance until 4 days of dosing with nepicastat suggesting gradual modulation of the sympathetic nervous system. It should be recognized that measurements of plasma noradrenaline concentrations alone do not account for regional differences in noradrenaline release (Esler *et al*., 1984), which underscores the need for making measurements of organ-specific noradrenaline spillover rates in future studies.

A growing body of evidence suggests that chronic activation of the sympathetic nervous system in congestive heart failure is a maladaptive response (see Packer, 1989, 1992; Parmley, 1995 for reviews). This contention is supported by recent clinical trials which have shown a beneficial effect of carvedilol in congestive heart failure patients with respect to long-term morbidity and mortality (Packer *et al*., 1996a,b). However, it should be noted that most patients do require some level of sympathetic drive to support cardiovascular homeostasis (Packer, 1989). Indeed, the therapeutic value of β-blockers, including carvedilol, may be limited by their propensity to cause haemodynamic deterioration especially during initiation of therapy (Pfeffer & Stevenson, 1996). This unwanted effect, which results from abrupt withdrawal of sympathetic support, necessitates careful dose-titration. Inhibitors of dopamine-β-hydroxylase, such as nepicastat, may be devoid of this undesirable effect for the following reasons. First, this class of drugs would attenuate, but not abolish, noradrenaline release and, second, they produce gradual modulation of the system thereby obviating the need for dose-titration. Another advantage of nepicastat over β-blockers is that it enhances dopamine levels which, via agonism of dopamine receptors, may have salutary effects on renal function such as renal vasodilatation, diuresis and natriuresis (Lokhandwala & Hegde, 1991). Indeed, nepicastat produces renal vasodilatation and preserves renal function in anaesthetized SHRs (Hegde *et al*., 1996a,b).

In summary, nepicastat is a potent, selective and orally active inhibitor of dopamine-β-hydroxylase which may be of value in the treatment of cardiovascular disorders associated with over-activation of the sympathetic nervous system. The compound appears to be well tolerated in animal toxicity studies thus far and, in this respect, differs from earlier dopamine-β-hydroxylase inhibitors, such as fusaric acid, the toxicity of which (Matsuzaki *et al*., 1976) precluded extensive clinical evaluation. Consequently, nepicastat is currently being clinically evaluated for the treatment of congestive heart failure.

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