SPECIAL REPORT

Evidence that agonist and antagonist enantiomers of the dihydropyridine PN 202-791 act at different sites on the voltage-dependent calcium channel of vascular muscle

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S(+)-PN 202-791, a calcium channel agonist, and its optical isomer R(–)-PN 202-791, a calcium channel antagonist, respectively increased and decreased inward current carried by barium through voltage-dependent calcium channels in isolated ear artery cells of the rabbit in a concentration-dependent manner. The EC₅₀ or IC₅₀ derived from the concentration-response relationship was unaltered in the presence of the other enantiomer indicating that no competitive antagonism exists between these enantiomers and suggesting that the actions of these two enantiomers involve two separate binding sites.

Introduction Dihydropyridine drugs exert actions on voltage-operated calcium channels (VOCs). Some dihydropyridines, such as PN 202-791, exist as two different enantiomers: S(+)-PN 202-791 is an agonist but its enantiomer, R(–)-PN 202-791, acts as a calcium antagonist (Hamilton *et al.*, 1987). Generally, both agonist and antagonist dihydropyridines are believed to act by binding to a single site associated with the VOCs (Triggle & Rampe, 1989). If this site is responsible for both actions of dihydropyridines then agonist and antagonist dihydropyridines should compete for binding to this site and hence display competitive antagonism. We have studied this issue in single voltage-clamped vascular smooth muscle cells.

Methods Single ear artery cells of the rabbit were prepared as previously described (Benham & Bolton, 1986). Whole cell voltage clamp technique (Hamill *et al.*, 1981) was used with an extracellular solution of the following composition (mM): BaCl₂ 110, tetraethylammonium Cl 10, HEPES 10, pH 7.2 (with NaOH), and intracellular pipette solution of composition (mM): NaCl 126, Mg₂SO₄ 3.2, NaH₂PO₄ 1, EGTA 2, HEPES 5, tetraethylammonium Cl 10, adenosine 5'-triphosphate 2, pH 7.2 (with NaOH). These solutions optimised recording of inward current through VOCs (Aaronson *et al.*, 1988). Pipette resistance was 3–6 MΩ. S(+) and R(–)-PN 202-791 (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridinecarboxylate, gifts from Sandoz) were prepared as 1 mM stock solutions in ethanol and diluted appropriately. The maximum concentration of ethanol used (0.3%) had no effect on currents. Inward currents were evoked every 1 s by a 20 ms step to +10 mV from a holding potential of –60 mV. With this protocol there was negligible 'run down' of current. Concentration-response data were analysed by fitting to a logistic function ($E/E_{\max} = C/(EC_{50} + C)$), where $E/E_{\max}$ is the relative effect of drug (agonist or antagonist) on the inward current and C is the molar concentration of drug, using non-linear regression analysis. Data are presented as mean $-\log(EC_{50})$ or $-\log(IC_{50})$ (i.e. pEC₅₀ or pIC₅₀) $\pm$ s.e.mean of *n* observations.

Results S(+)-PN 202-791 increased and R(–)-PN 202-791 decreased inward barium current in a concentration-dependent fashion (Figures 1 and 2). However, the pEC₅₀ for S(+)-PN 202-791 (6.91 ± 0.08 ; *n* = 6) was not significantly different from its pEC₅₀ in the presence of 3 μ M R(–)-PN 202-791 (7.02 ± 0.12 ; *n* = 4). Likewise, the pIC₅₀ for R(–)-PN

202-791 (6.34 ± 0.03 ; *n* = 5) was not significantly different from its pIC₅₀ in the presence of 1 μ M S(+)-PN 202-791 (6.42 ± 0.08 ; *n* = 5). However when 3 μ M R(–)-PN 202-791 was present, the currents when S(+)-PN 202-791 was added were smaller than when R(–)-PN 202-791 was absent, although the relative increases produced by various concentrations of S(+)-PN 202-791 were similar (Figure 1a,c and 2a,c). R(–)-PN 202-791 also had similar relative effects in the presence of 1 μ M S(+)-PN 202-791 as in its absence (Figure 1b,d and 2b,d).

Discussion The concentration-response relationships of S(+) and R(–)-PN 202-791 were not appreciably shifted by the presence of the other enantiomer in concentrations which would be anticipated to cause approximately a ten fold increase in the concentration necessary to produce half maximal effects from considerations of classical competitive antagonism. As can be seen from Figure 2 both enantiomers acted as non-competitive antagonists of the action of the other enantiomer. The failure of R(–)-PN 202-791 to block responses to S(+)-PN 202-791 has also recently been reported in cardiac myocytes (Kamp *et al.*, 1989).

These electrophysiological results contrast with most, but not all (Kokubun *et al.*, 1986; Lee *et al.*, 1987), ligand binding studies which show a single binding site for ³H-labelled dihydropyridines (Triggle & Rampe, 1989). The reason for this apparent discrepancy is unclear. Others (Brown *et al.*, 1986; Kokubun *et al.*, 1986) have proposed the existence of two dihydropyridine binding sites on the basis of whole cell and unitary calcium current studies in cardiac cells. Kokubun *et al.* (1986) concluded that co-operative interactions could occur between enantiomers and Brown & Lacerda (1989) have also recently proposed a model involving co-operative interactions between dihydropyridine binding sites regulating VOCs from single channel data. Our data do not provide support for co-operative interactions between enantiomers of PN 202-791, as none of the Hill slopes of the concentration-response curves was found to differ significantly from unity. A simple interpretation of our data is that the action of agonist and antagonist enantiomers is at two independent sites, one to which the agonist, the other to which the antagonist, bind. These two sites respectively increase and decrease current through VOCs in vascular smooth muscle. If so, this finding has important implications for understanding the mechanisms of action of dihydropyridine drugs and for the development of new modulators of voltage-dependent calcium channels.

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Figure 1 The effect of the enantiomers of PN 202-791 on calcium channel current, with 110 mM Ba^{2+} as charge carrier. Current was evoked from a holding potential of -60 mV by a 20 ms step to $+10$ mV every 1 s in single ear artery cells. (a) S(+)-PN 202-791 (100 nM–3 μM). (b) R(-)-PN 202-791 (100 nM–3 μM). (c) S(+)-PN 202-791 (100 nM–3 μM) in the presence of R(-)-PN 202-791 (3 μM). (d) R(-)-PN 202-791 (100 nM–3 μM) in the presence of S(+)-PN 202-791 (1 μM). C is the current before drug application (a,b) or in the presence of the opposite enantiomer (c,d).

Figure 2 Concentration-response relationships for enantiomers of PN 202-791. ($\square$) S(+)-PN 202-791 ($n = 6$); ($\circ$) R(-)-PN 202-791 ($n = 5$); ($\blacksquare$) S(+)-PN 202-791 in the presence of R(-)-PN 202-791 (3 μM) ($n = 4$); ($\bullet$) R(-)-PN 202-791 in the presence of S(+)-PN 202-791 (1 μM) ($n = 5$). The effect of S(+)-PN 202-791 in the absence and presence of R(-)-PN 202-791 is shown in (a) and after normalization in (c). The effect of R(-)-PN 202-791 in the absence and presence of S(+)-PN 202-791 is shown in (b) and after normalization in (d). Points represent means and vertical bars s.e.mean of n observations.

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