Paradoxical reversion of the inhibitory effects of dihydropyridine enantiomers on the calcium current in frog heart by CGP 28861

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A dihydropyridine CGP 28861 (5×10^{-6} M) did not change slow inward Ca current as measured by the double sucrose gap method in frog atrial fibres but decreased the agonist effects of Bay K 8644, CGP 28392, (+)-(S)-202-791 and the antagonist effects of nifedipine and (+)-(PN)-200-110. Paradoxically, the weak antagonists (–)-(R)-202-791, (–)-(PN)-200-110 and (+)-Bay K 8644 increased Ca-current after wash-out of CGP 28861. These data suggest that CGP 28861 can convert the dihydropyridine Ca-channel receptor from an antagonistic site into an agonistic one.

Introduction Competition between dihydropyridine Ca channel antagonists and agonists was shown in binding and pharmacological experiments (see for example review by Glossmann *et al.*, 1985; Williams *et al.*, 1985) supporting the idea of one high affinity binding site for agonists and antagonists. On the other hand, cooperative interaction between dihydropyridine agonists and antagonists was demonstrated recently in cardiac cells, supporting the idea of two sites, one for agonists and another for antagonists (Kokubun *et al.*, 1986). Hence the questions of the number of sites and of the interaction between effects of different dihydropyridines remain open. Another question is whether a dihydropyridine receptor can be shielded from the action of agonists or antagonists by related compounds which themselves do not influence Ca current?

Compound CGP 28861, the derivative of the calcium agonist CGP 28392 (OCHF₂ substituent in the *para*-instead of the *ortho*-position on the phenyl ring), binds with low affinity to the dihydropyridine site in cardiac membranes (Erne *et al.*, 1984). However, its electrophysiological effect is unknown. We studied the influence of this compound on the calcium channel current and on the effects of dihydropyridine agonists and antagonists on frog atrial fibres.

Methods Frogs (*Rana ridibunda*) weighing 50–100 g of either sex were killed by destroying the spinal

cord. Trabeculae 0.07–0.15 mm in diameter were voltage clamped by the double sucrose gap method as described previously (Filippov *et al.*, 1983). Fast sodium channels were blocked by 2×10^{-6} M tetrodotoxin (Sankyo, Japan). Slow inward calcium current was measured from the peak to the steady value which was reached at the end of 100 ms voltage pulse. Pulses were measured relative to the resting potential. Stimulation of the preparation and data recording were performed automatically with a computer system SM-3 and CAMAC-modules (Polon, Poland). Sampling interval was 0.05 ms.

Dihydropyridines used were: (+)-or(–)-(PN)-200-110, (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridine-carboxylate); (+)-(S)-or(–)-(R)-202-791, (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridinecarboxylate) (Sandoz Ltd); (±)-Bay K 8644, (+)-Bay K 8644, (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) (Bayer); CGP 28392, (4-(2-difluoromethoxy)-phenyl)-1,4,5,7-tetrahydro-2-methyl-5-oxo-fluoro(3,4-b)pyridine-3-carboxylic acid ethylester; CGP 28861 (Ciba Geigy). Drugs were dissolved in ethanol or dimethylsulphoxide (DMSO) to prepare 1×10^{-2} M stock solutions which were diluted with the appropriate Ringer solution. Ringer solution of the following composition (mM) was used: NaCl 110, KCl 2.5, CaCl₂ 1.8, MgCl₂ 1, NaHCO₃ 2.4, glucose 5.5; isotonic sucrose solution (sucrose dissolved in twice distilled water); pH = 7.5 at $18 \pm 2^\circ\text{C}$.

Results CGP 28861 (up to 5×10^{-6} M) did not influence the slow inward calcium channel current ($n = 8$) (Figure 1a as an example). In spite of this the compound decreased the effect of the dihydropyridine agonists, Bay K 8644, CGP 28392 and (+)-(S)-202-791 and of the antagonists, nifedipine and (+)-(PN)-200-110, on the calcium current (Figure 1b,c for example). An unexpected action was observed with enantiomers (–)-(R)-202-791, (–)-(PN)-200-110 and (+)-Bay K 8644. They have been reported to be weak antagonists (Williams *et al.*,

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Figure 1 Current-voltage relations of the slow inward calcium channel current: (○) control (a); (●) CGP 28861, 5×10^{-6} M, 10 min (a-d); (□) CGP 28861 + CGP 28392, 5×10^{-6} M (b); (+)-(PN)-200-110, 5×10^{-8} M (c); (—)(R)-202-791, 2×10^{-6} M (d), 10 min; (■) dihydropyridines 10 min after wash-out of CGP 28861 (b, c, d) V, mV, is the amplitude of the depolarizing voltage step used. Is_i, μA, is the amplitude of the slow inward calcium channel current. In all these experiments there were no changes in resting potential.

1985; Franckowiak *et al.*, 1985). In our experiments ($n = 5$) they decreased Ca current no more than by 25% (at 2×10^{-6} M) compared with control. However in a series of experiments, when CGP 28861 was removed from the bathing solution, a paradoxical increase of calcium channel current was observed in spite of the fact that the antagonist still remained in the solution (e.g. Figure 1d). Thus antagonistic effects were converted into agonistic effects. This was seen in 3 experiments with (—)(R)-202-791, and in 3 experiments with (—)(PN)-200-110 and (+)-Bay K 8644. The effect was not related to the solvents used since it was not observed when in control experiments ethanol or DMSO were used instead of CGP 28861.

Discussion These experiments suggest that CGP 28861 interacts with dihydropyridine Ca channel receptors for agonists and antagonists without producing any effect on the slow inward calcium channel current, while influencing the effects of other dihydropyridines. The compound decreases the effects of the strong Ca channel agonists and antagonists. Moreover the compound seems to modify the dihydropyridine receptors so that weak antagonists become agonists after wash-out of CGP 28861. How

can these results be interpreted? We think that they are in favour of the hypothesis for different binding sites for dihydropyridine agonists and antagonists. We suggest that CGP 28861 competes for binding sites with dihydropyridine agonists and antagonists. However, the compound probably increases the effects of Ca agonists even after washing out. Since dihydropyridines may have dual effects, agonistic and antagonistic (Brown *et al.*, 1986), one can suggest that the weak antagonists are converted into agonists if their binding to agonistic sites increases. However, the results do not exclude the possibility that the site on the calcium channel for dihydropyridine agonists and antagonists is the same and some dihydropyridine receptor modification can convert it from an antagonistic into an agonistic one.

The molecular mechanism of the effect observed is unknown. Recently, activation of G_1 protein of adenylate cyclase was shown to convert Ca antagonists into Ca agonists (Scott & Dolphin, 1987). Whether a similar mechanism occurred in our experiments remains to be shown.

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