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Short communication

Stereoselectivity of Ca^{2+} channel block by dihydropyridines: no modulation by the voltage protocol

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Abstract

The L-type Ca^{2+} current inhibition by the enantiomers of the dihydropyridine niguldipine was investigated at various holding potentials (−40 to −120 mV) and stimulus frequencies (0.1–1 Hz), using guinea-pig ventricular myocytes. Block of whole-cell current is both voltage- and concentration-dependent. (*S*)-Niguldipine is more potent than its (*R*)-enantiomer. However, the extent of enantioselectivity is rather small (≤ ×4.4). Importantly, this value does not increase when stimulus conditions favour the inactivated channel state, although this leads to more potent block. This is in contrast to our expectation based on modulated receptor hypothesis, and to the high enantioselectivity of niguldipine binding found in guinea-pig heart membranes (×40). We conclude that the common modulated receptor hypothesis has to be refined to explain the effects of niguldipine enantiomers.

Keywords: Ca^{2+} current, L-type; Niguldipine; Whole-cell recording; Voltage dependence; Enantioselectivity

1. Introduction

Stereoselectivity is an important property of chiral dihydropyridine antagonists acting on their receptors, i.e. the L-type Ca^{2+} channels. This is an indication of the high structural complementarity between the appropriate enantiomer of the drug and its binding site. Voltage dependence of action is another hallmark property of L-type Ca^{2+} channel inhibition by dihydropyridines. It means that the drug-receptor interaction is governed by structural rearrangements of the ion channel itself, caused by the intrinsic gating properties of the voltage-operated channel protein. Astonishingly, no quantitative study is available where these two important aspects of dihydropyridine action have been assessed in conjuction.

Niguldipine and its enantiomers, (*S*)-niguldipine and (*R*)-dexniguldipine, are potent dihydropyridine Ca^{2+} channel blockers in various types of cells (Klöckner and Isenberg, 1989; Romanin et al., 1992). The (*S*)-enantiomer has subnanomolar affinity and is 40-fold more potent than (*R*)-niguldipine, as measured in binding studies with isolated cardiac membranes (Boer et al., 1989). Remarkably, Ca^{2+} channel blockade and negative inotropic effects in cardiac preparations were characterised by an enantioselectivity factor (*r*, (*S*) again more potent than (*R*)) of only about 4 (Romanin et al., 1992; Schröder and Herzig, 1993). This quantitative discrepancy, however, might be easily reconciled within the framework of the modulated receptor hypothesis, first applied to dihydropyridines by Bean (1984): here, high-affinity (nanomolar) binding is a special property of the inactivated channel state, which predominates in isolated membranes or in (partially) depolarized cells. In cells or tissues with a sizeable resting membrane potential, low-affinity (micromolar) block of the resting channel state predominates and thus governs the potency of the drugs. It is very reasonable to assume that this low-affinity binding, being less dependent on the exact structural complementarity between drug molecule and the binding site, also displays a much lower (if any) extent of enantioselectivity.

The purpose of the present study was to define quantitatively the relationship between enantioselectivity and voltage dependence of dihydropyridine action on L-type channels in guinea-pig cardiomyoctes. Our specific aim was to check whether, in the case of niguldipine enantiomers, the amount of stereoselectivity *increases in parallel* with absolute potency over an appropriate range of conditions,

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as postulated by the above-mentioned, well-accepted modulated receptor hypothesis.

2. Materials and methods

Isolation of guinea-pig ventricular myocytes and measurement of whole-cell L-type Ca^{2+} currents have been described in detail (Herzig et al., 1995b). Bath solution contained (mM) NaCl 135, KCl 4, $CaCl_2$ 2, NaH_2PO_4 0.3, $MgCl_2$ 1, Hepes 10, dextrose 10, pH 7.3, 22–23°C. Pipettes (1.5–2.5 MW) were filled with (mM) K-aspartate 80, KCl 50, KH_2PO_4 10, $MgCl_2$ 0.5, MgATP 3, Hepes 5, EGTA 1, pH 7.4. During the experiment bath chambers, disposable Petri dishes (2 ml), were continuously superfused with the bath solution or the niguldipine-containing solution (120 $ml \times h^{-1}$). L-type Ca^{2+} currents were elicited by depolarising voltage steps to +10 mV. T-type Ca^{2+} currents and Na^+ currents were inactivated by prepulses from holding potential to −40 mV for 100 ms. Concentration-response curves of both niguldipine enantiomers were obtained at varying holding potentials and stimulus frequencies. Currents were recorded using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) connected to an AT-386 personal computer. Data analysis was done by the use of the pClamp software (version 5.5; Axon instruments, USA). Ca^{2+} currents were determined as difference between peak inward current and current level after 200 ms at +10 mV. Ca^{2+} current values were analysed after normalization, setting current (−40 mV, 0.1 Hz) immediately before drug addition 100%. Each cell was exposed to only a single niguldipine concentration, and the whole set of stimulus conditions was applied (holding potentials of −40, −75, −90, −120 mV, frequencies of 0.1, 0.33 and 1 Hz; see Fig. 1). Data are given as mean values ± S.E.M. of $n = 3$–8 experiments.

The enantiomers of niguldipine × HCl were a kind gift of Byk Gulden (Konstanz, Germany). They were prepared as 10 mM stock solutions in dimethyl sulfoxide (DMSO, final concentration ≤ 1% (v/v)). Niguldipine gets highly adsorbed to plastic and glass materials. The indicated concentrations refer to 'nominal' applied concentrations of niguldipine; the 'true' values may amount to only one-third of these concentrations (Klöckner and Isenberg, 1989). We confirmed this in fluid samples taken from our own set-up, measuring displacement of [^{3}H](*S*)-isradipine binding in guinea-pig cardiac membranes (see Herzig et al., 1995a), and using high-performance liquid chromatography (data not shown).

3. Results

The experimental protocol used to study the action of niguldipine enantiomers is illustrated in Fig. 1. Cardiac L-type Ca^{2+} current at various stimulus conditions is exemplified in the presence of (*R*)-niguldipine $3 \cdot 10^{-6}$ M. Application of this concentration resulted in a pronounced inhibition of Ca^{2+} current at a holding potential of −40

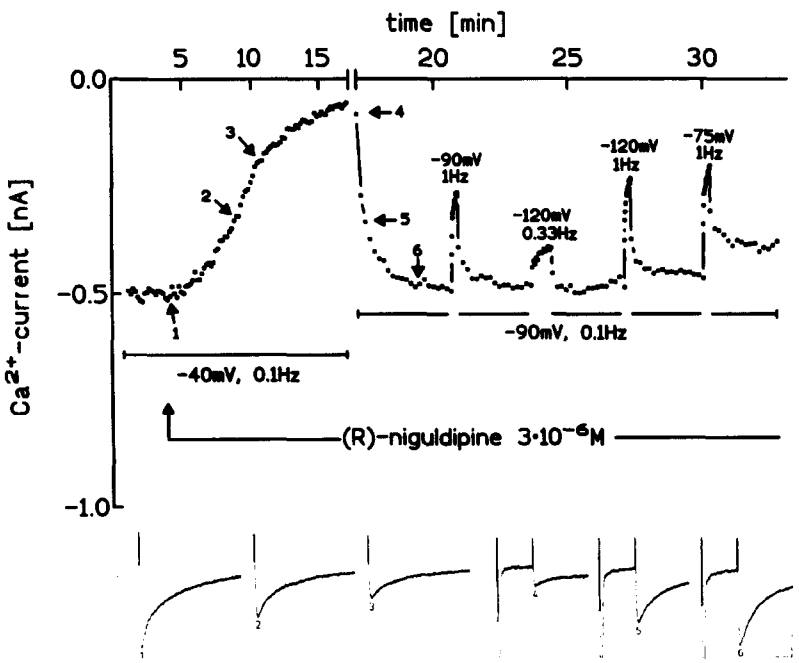

Fig. 1. Protocol used to study cardiac L-type Ca^{2+} currents at different holding potentials and stimulus frequencies in the presence of niguldipine enantiomers. An example with (*R*)-niguldipine $3 \cdot 10^{-6}$ M is shown. Original traces are displayed below. They were obtained at the time points 1 to 6, indicated by the arrows in the upper part. Scale bars denote 50 ms and 0.2 nA. T-type Ca^{2+} currents and Na^+ currents were inactivated by prepulses to −40 mV for 100 ms. Test potential was always +10 mV, total test pulse duration 300 ms.

mV and a frequency of 0.1 Hz. After reaching steady state of block, holding potential was switched to −90 mV without changing stimulus frequency, leading to a nearly complete recovery of block. This condition was always applied in between the following different protocols. Increase in stimulus frequency from 0.1 to 1 Hz markedly enhanced the potency of (*R*)-niguldipine. Hyperpolarisation to −120 mV at 0.33 Hz exerted little block, compared with −90 mV, 0.1 Hz. However, raising frequency to 1 Hz at −120 mV, or at −75 mV holding potential, led to a more pronounced inhibition. This protocol had a duration of about 30–40 min and spontaneous run-down of Ca^{2+} current was on an average 18%.

Concentration-response curves for (*S*)- and (*R*)-niguldipine under all applied stimulus conditions can be seen in Fig. 2. Half-filled symbols represent values of control experiments, which were obtained in bath solution with 1% DMSO by application of the same protocol. Both niguldipine enantiomers inhibit the L-type Ca^{2+} current in a concentration-dependent manner. Channel block by both enantiomers is clearly voltage-dependent. Inhibition of current is more pronounced at depolarised holding potential. Maximal block was achieved at −40 mV with $3 \cdot 10^{-6}$ M (*S*)- and (*R*)-niguldipine, resulting in Ca^{2+} current values of 10.7 ± 1.3, and 13.9 ± 1.4% of control, respectively. (Higher concentrations of niguldipine were insoluble in our bath solution (1% DMSO).) Frequency dependence of niguldipine action can be seen as well. Increasing the stimulus frequency at a given holding potential enhances the potency of both enantiomers. Ca^{2+} channel block by niguldipine is enantioselective. Under all tested stimulus conditions (*S*)-niguldipine is more potent than the (*R*)-enantiomer. The IC_{50} values of both enantiomers at each stimulus condition are illustrated below the respective dose-response curves. However, as evident from these data, enantioselectivity is not very pronounced. The ratios of the IC_{50} values of (*R*)- and (*S*)-niguldipine, which represent a measure of the extent of enantioselectivity, are in a narrow range of 2.4 to 4.4. Changes in holding potential (even to −40 mV, where absolute potency is ≥ 20-fold the corresponding value at −90 mV) or in pulse frequency lead to neither marked nor systematic changes of enantioselectivity.

4. Discussion

Our results demonstrate for the niguldipine enantiomers all well-known qualitative features of dihydropyridine action, i.e. concentration dependence, voltage dependence,

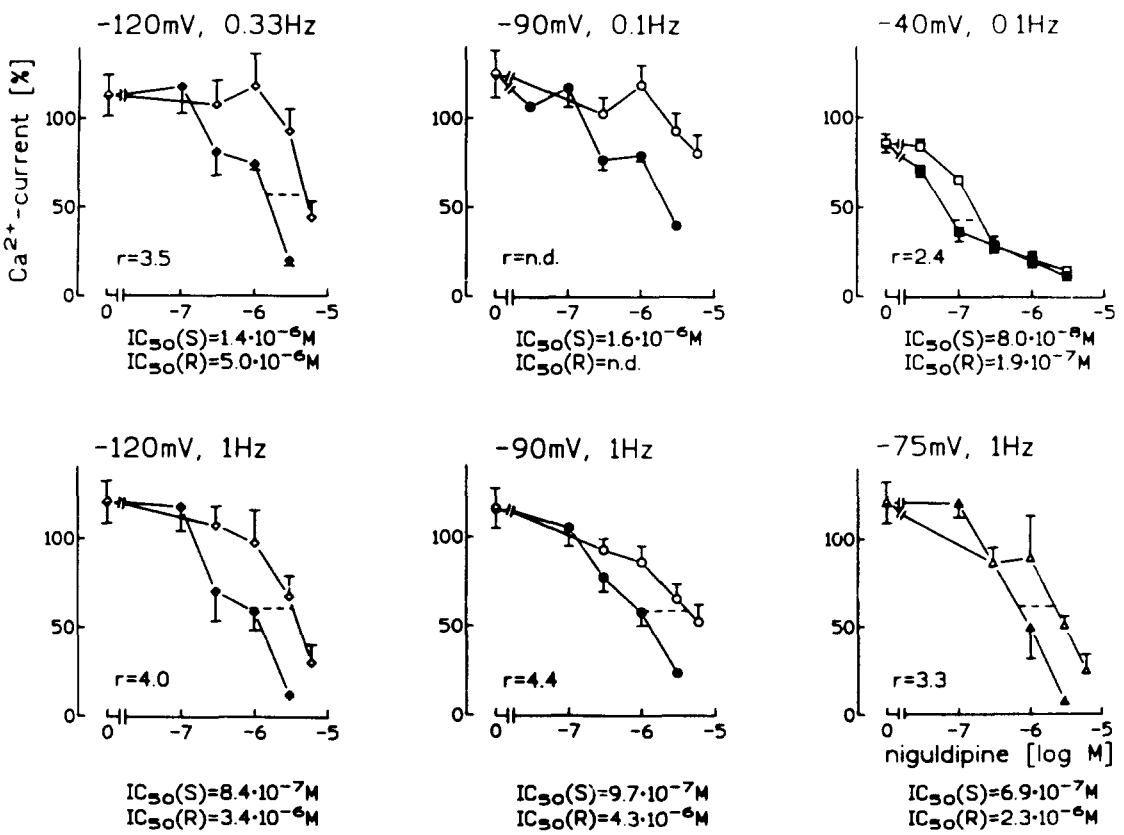

Fig. 2. Concentration-response curves of niguldipine enantiomers at the investigated stimulus conditions. Filled symbols represent (*S*)-niguldipine, open symbols (*R*)-niguldipine. Half-filled symbols on the left are from similar experiments without drug. Dashed lines within the graphs show level of half-maximal effects (relative to matched drug-free controls). Each point represents data from $n = 3$–8 cells, indicated as mean ± S.E.M. r = ratio (IC_{50} (*R*)-niguldipine/IC_{50} (*S*)-niguldipine), corresponds to the enantioselectivity factor; n.d., not determined.

and enantioselectivity (e.g. Bean, 1984; Bean et al., 1986; Romanin et al., 1992). However, the crucial hypothesis guiding our experiments, i.e. that an increase in absolute potency should be accompanied by an increase in stereoselectivity, has to be rejected. Let us first consider whether the lack of change of enantioselectivity may be due to methodological problems. The actual niguldipine concentrations in the bath can be reduced due to adsorption to plastic material (Boer et al., 1989; Klöckner and Isenberg, 1989). We could reproduce this finding (not shown), but both enantiomers were affected to a similar extent, such that the principal interpretation of our data is not violated. We were also able to reproduce the high amount (factor 44) of enantioselectivity in binding studies with guinea-pig cardiac membranes, eliminating the possibility that different sources of tissue confound the comparison between binding experiments and the functional data. Finally, one may ask whether the appropriate experimental conditions were chosen to demonstrate an increase in enantioselectivity. This can be ascertained using model calculations for K_{app}, the apparent potency of a dihydropyridine to block channels in cells where a mixture of rested channels (K_R) and inactivated channels (K_I) exists, with the inactivation parameter h representing the fraction of rested channels. According to Bean (1984),

$$K_{app} = \frac{1}{(h/K_R) + (1-h)/K_I}$$

It shall be assumed that K_I is correctly reflected by the binding experiments (Boer et al., 1989), i.e. $1.5 \cdot 10^{-10}$ M for the (*S*)-enantiomer and $6 \cdot 10^{-9}$ M for the (*R*)-enantiomer. For K_R, 10^{-5} M was chosen for both enantiomers as a lower limit estimate of potency. Then, both potency and enantioselectivity can be computed for any level of inactivation (h value). For instance, in a situation where only 1% of the channels are inactivated ($h = 0.99$), K_{app} for (*S*)- and (*R*)-niguldipine amount to $1.5 \cdot 10^{-8}$ M and $5.7 \cdot 10^{-7}$ M, i.e. the enantioselectivity ratio (r) amounts to alread a factor of 38. Only at very low levels of inactivation (e.g. $h = 0.99995$), K_{app} is in the micromolar range for both enantiomers ($2.3 \cdot 10^{-6}$ M and $9.2 \cdot 10^{-6}$ M), and r is 4.0, matching our data obtained under polarized conditions. r rises sharply together with increasing potency, such that for instance at $h = 0.9995$, (*S*)-niguldipine ($K_{app} = 2.9 \cdot 10^{-7}$ M) is 19-fold more potent than (*R*)-niguldipine ($K_{app} = 5.5 \cdot 10^{-6}$ M (or for $h = 0.9997$: $K_{app} = 4.8 \cdot 10^{-7}$ M and $6.7 \cdot 10^{-6}$ M, respectively, $r = 14.0$). Thus, in the range of conditions and potencies covered in our experiments, a dramatic increase in enantioselectivity should have occurred. Therefore, it can be concluded that the lack of observation of a change in enantioselectivity is not due to an inappropriate experimental protocol.

Given that the modulated receptor hypothesis, at least in its very simple form considered here, cannot account for the voltage dependence of enantioselectivity, how can the change in potency be explained? In fact, the so-called guarded receptor hypothesis (Starmer et al., 1984) would predict exactly what we found (see also Gödicke et al., 1992): here, the binding characteristics of the drug at the binding site itself is taken to be constant, and voltage-dependent gating modulates the access to and/or escape from the immediate vicinity of this site. Then, it would be natural to assume that enantioselectivity (a function of the binding site) remains the same, while potency – determined by channel gating and the (identical) physicochemical properties of the enantiomers – varies. This idea is also in line with the observation that structural variations in the channel protein remote from the primary binding site (Grabner et al., 1996) still affect the amount of voltage dependence of dihydropyridine action (Soldatov et al., 1995).

We therefore draw the – originally unanticipated – conclusion that the pattern of enantioselectivity of niguldipine argues in favour of the guarded, rather than the conventional modulated receptor hypothesis. It must be admitted that the discrepancy between functional and binding data cannot be resolved at present. This question could be resolved using parallel binding and electrophysiological studies in intact cells (e.g. Wei et al., 1989).

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