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European Journal of Pharmacology 369 (1999) 43–47

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Stereoselective and endothelium-independent action of nicardipine on the isolated porcine coronary artery

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Received 1 October 1998; revised 19 January 1999; accepted 22 January 1999

Abstract

The qualitative and quantitative effects of the (+)-*S* and (−)-*R* enantiomers and of the racemic mixture of the Ca^{2+} channel antagonist, nicardipine, were compared on the isolated porcine coronary artery with intact and removed endothelium. All three forms of nicardipine inhibited the contractions induced by KCl (5–90 mM) in both vessel preparations. The potency (IC_{50}) of the (+)-*S* and (−)-*R* enantiomers and of the racemic mixture was 6.6, 31.8 and 10.9 nM in the vessel with endothelium and 6.4, 41.9 and 9.8 nM in the vessel without endothelium. The parameters of the concentration–response curves for each form of nicardipine at a submaximal KCl (60 mM) concentration and the potency ratios between the two enantiomers ((+)-*S*/(−)-*R*) were not statistically significantly different ($P > 0.05$) in the two vessel preparations. In conclusion, qualitatively, all three forms of nicardipine showed only Ca^{2+} channel antagonistic effects in both vessel preparations. Quantitatively, the inhibition of contraction was stereoselective, the (+)-*S* enantiomer being the most potent, and was endothelium-independent.

Keywords: Stereoisomerism; Ca^{2+} channel antagonist; Nicardipine; Coronary artery, pig

1. Introduction

Chiral Ca^{2+} channel antagonists of the 1,4-dihydropyridine group, nicardipine among them, have an asymmetric carbon at position 4 of the dihydropyridine ring and are synthesized as racemic mixtures. Pharmacodynamically, the enantiomers of Ca^{2+} channel antagonists of the 1,4-dihydropyridine group can show quantitative (Towart et al., 1982; Hof et al., 1986) and some qualitative differences (Hof et al., 1985; Gjörstrup et al., 1986; Bechem and Schramm, 1987). The enantiomers of nicardipine ((+)-*S* and (−)-*R* enantiomers) showed only quantitative differences in the isolated rat aorta and in in vivo experiments with cardiovascular preparations. The (+)-*S* enantiomer was always more potent than the (−)-*R* enantiomer and the potency ratios between the two enantiomers ((+)-*S*/(−)-*R*) were between two and eight (Takenaka et al., 1982; Iwatsuki et al., 1984; Brisac et al., 1988). Ca^{2+} channel antagonists relax blood vessels by acting on the voltage-operated Ca^{2+} channels in the cell membrane, reducing the influx of Ca^{2+} ions into smooth muscle cells (Ferro and Webb, 1997). However, there is evidence that the endothelium is involved in the vasorelaxation induced by Ca^{2+} channel antagonists by means of an increased release of nitric oxide (NO) (Kojda et al., 1990, 1991; Günther et al., 1992; Dhein et al., 1995; Salameh et al., 1996).

There are no data about the effects of the three forms of nicardipine on the isolated porcine coronary artery, which can be used as a model for human coronary artery (Matsumoto et al., 1993), and the contribution of the endothelium to the vasorelaxation produced by this Ca^{2+} channel antagonist has not been elucidated. Thus, we investigated the role of stereoisomerism and the endothelium in the action of all three forms of nicardipine on the isolated porcine coronary artery.

2. Materials and methods

2.1. Coronary artery preparation, protocol, measurement of the contractions

Pig hearts (250–420 g, $n = 68$) were transported from the local slaughterhouse in ice-cold Krebs–Henseleit solu-

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00049-7

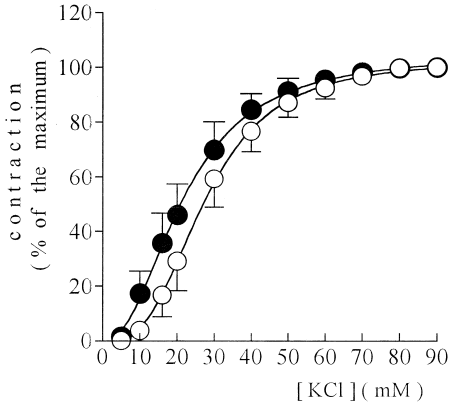

Fig. 1. Control contractions induced by KCl (5–90 mM) of the isolated porcine coronary artery with (○ $n = 29$) and without (● $n = 45$) endothelium. Each point on the graph is the arithmetic mean. Error bars are S.D.

tion of the following composition (mM): 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 2.5 $CaCl_2$, 11 glucose. The left anterior descending coronary artery was isolated and cleaned of visible connective tissue and fat. Three adjacent rings (5 mm wide) 5 mm distal from the beginning of the left anterior descending coronary artery were cut and transferred to 30-ml tissue chambers filled with Krebs–Henseleit solution oxygenated with 95% O_2 + 5% CO_2 at 37°C. In some experiments the endothelium was kept intact and in the others it was removed with a thin steel needle. The rings were stretched gradually during the first hour of equilibration to obtain 50 mN initial tension. After equilibration, 27 mM KCl was added twice successively to obtain stable contractions. The intactness of the endothelium was verified with substance P (10 nM) applied to rings precontracted for a second time with 27 mM KCl (Hussain and Mustafa, 1993). To obtain the control curve, KCl was added in a series of concentrations

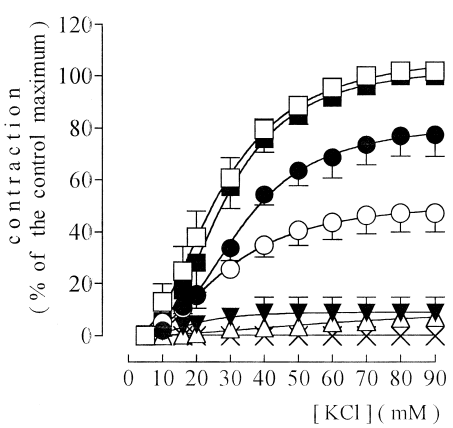

Fig. 2. Effects of the (+)-*S* enantiomer of nicardipine (□ 0.1 nM, ● 1 nM, ○ 10 nM, ▼ 0.1 μM, △ 1 μM, ×10 μM) on the contractions induced by KCl (5–90 mM) (■ control) of the isolated porcine coronary artery with intact endothelium. Each point on the graph is the arithmetic mean of the control experiments ($n = 27$) and of experiments with nicardipine in different concentrations ($n = 3$–5). Error bars are S.D.

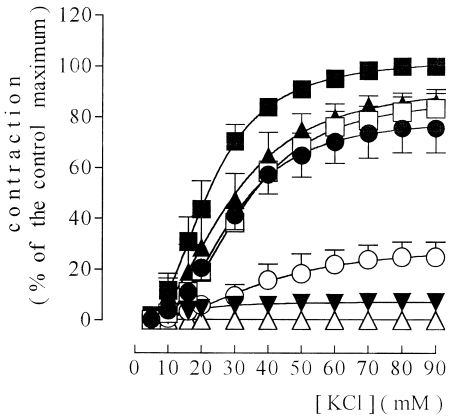

Fig. 3. Effects of the (+)-*S* enantiomer of nicardipine (▲ 10 pM, □ 0.1 nM, ● 1 nM, ○ 10 nM, ▼ 0.1 μM, △ 1 μM) on the contractions induced by KCl (5–90 mM) (■ control) of the isolated porcine coronary artery without endothelium. Each point on the graph is the arithmetic mean of the control experiments ($n = 26$) and of experiments with nicardipine in different concentrations ($n = 3$–5). Error bars are S.D.

(5–90 mM). Either racemic nicardipine or one of its enantiomers was added in increasing concentrations (10 pM–0.1 mM) 30 min before the second series of KCl (5–90 mM) additions. In each preparation only one concentration of nicardipine was used. The contractions were measured isometrically and recorded on the chart recorders.

2.2. Drugs and chemicals

All three forms of nicardipine were synthesized by the LEK Pharmaceutical and Chemical, Slovenia; (+)-*S* and (−)-*R* enantiomers were HCl salts. The specific rotation of the (+)-*S* and (−)-*R* enantiomers was $[\alpha]_D^{23} = +83.8$ ($c = 0.3$, H_2O) and $[\alpha]_D^{23} = -82.8$ ($c = 0.3$, H_2O). All three forms were prepared as 4% dimethylsulfoxide stock solutions and were further diluted for each experiment

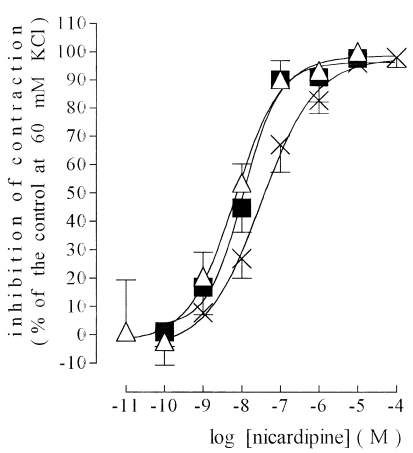

Fig. 4. Effects of the (+)-*S* enantiomer (△), the (−)-*R* enantiomer (×) and of racemic (■) nicardipine on the contractions induced by 60 mM KCl of the isolated porcine coronary artery with intact endothelium. Each point on the graph is the arithmetic mean ($n = 3$–5). Error bars are S.D.

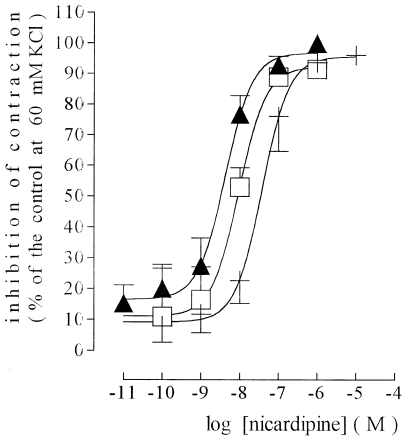

Fig. 5. Effects of the (+)-*S* enantiomer (▲), the (−)-*R* enantiomer (+) and of racemic (□) nicardipine on the contractions induced by 60 mM KCl of the isolated porcine coronary artery without endothelium. Each point on the graph is the arithmetic mean ($n = 3–5$). Error bars are S.D.

with bidistilled water. Substance P was prepared as a 3-nM solution in bidistilled water and frozen. Aliquots were thawed for each experiment. All ingredients of the Krebs–Henseleit solution were of p.a. grade.

2.3. Data and statistical analysis

The KCl (5–90 mM) concentration–response curves were fitted with three-parameter logistic equations (EC_{50} (concentration of the agonist which produces 50% of the maximal effect with this agonist), p (slope of the curve), max (maximum of the curve)). The maximal control contraction obtained with KCl was taken as 100%. The concentration–response curves for nicardipine at a submaximal KCl (at 60 mM) concentration were fitted with four-parameter logistic equations (IC_{50} (concentration of the antagonist which produces 50% inhibition of the maximal effect with agonist), p (slope of the curve), min (minimum of the curve), max (maximum of the curve)). The Prism Graph Pad program version 2.01 was used to calculate the curve parameters.

The values in the text are shown as arithmetic means ± S.D. (n = number of experiments). Parameters (EC_{50}, IC_{50}, p, min, max) of the concentration–response curves and the potency ratios between two enantiomers were statistically compared with a one-way analysis of variance (ANOVA) or two-tailed Student's t-test for the independent samples, as appropriate. Differences at $P < 0.05$ were regarded as statistically significant.

3. Results

3.1. Control contractions induced by KCl (5–90 mM)

KCl concentration dependently (5–90 mM) induced contractions of the isolated porcine coronary artery with intact and mechanically removed endothelium. In both vessel preparations the maximal control contraction was reached at 90 mM KCl. The EC_{50} of the control curve was 27.5 ± 4.2 mM ($n = 29$) for the coronary artery with endothelium and 21.8 ± 4.3 mM ($n = 45$) for the coronary artery without endothelium. The control curve for the isolated coronary artery with mechanically removed endothelium was less steep ($P < 0.05$ for p) than the control curve for the coronary artery with endothelium and was shifted to the left ($P < 0.05$ for EC_{50}) (Fig. 1).

3.2. Inhibition by nicardipine of the contractions induced by KCl (5–90 mM)

Each form of nicardipine concentration dependently (10 pM–0.1 mM) reduced the maximum of the KCl (5–90 mM) concentration–response curves in both vessel preparations without displacing these curves ($P > 0.05$ for EC_{50}) and without changing their slopes ($P > 0.05$ for p). In Figs. 2 and 3 only the curves for the (+)-*S* enantiomer are shown.

3.3. Inhibition by nicardipine of the contractions induced by a submaximal KCl (60 mM) concentration

All three forms of nicardipine concentration dependently (0.1 pM–0.1 mM) inhibited the contractions in-

Table 1
Parameters (IC_{50}, p, max, min) of the concentration–response curves for the three forms of nicardipine at a submaximal KCl (60 mM) concentration in the isolated porcine coronary artery with and without endothelium

Nicardipine	Endothelium	IC_{50} (nM)	p	Max (%)	Min (%)
(+)-*S* enantiomer	intact	6.6 (±3.4)	0.7 (±0.4)	102.3 (±12.2)	1.4 (±1.6)
	removed	6.4 (±2.7)	1.2 (±1.1)	96.8 (±7.2)	16.5 (±1.0)
Racemic nicardipine	intact	10.9 (±3.8)	0.9 (±0.4)	97.3 (±3.5)	3.7 (±3.0)
	removed	9.8 (±3.1)[a]	1.2 (±1.5)	92.2 (±13.8)	11.1 (±4.7)
(−)-*R* enantiomer	intact	31.8 (±21.2)[b]	0.6 (±0.3)	104.6 (±16.5)	2.3 (±2.1)
	removed	41.9 (±17.4)[a]	1.3 (±0.5)[c]	95.5 (±3.2)	9.3 (±2.8)

[a] $P < 0.05$ compared to the IC_{50} of the (+)-*S* enantiomer in preparations without endothelium.
[b] $P < 0.05$ compared to the IC_{50} of the (+)-*S* enantiomer in preparations with endothelium.
[c] $P < 0.05$ compared to the p value for the (−)-*R* enantiomer in preparations with endothelium.
Values are arithmetic means (±S.D.), $n = 3–5$.

duced by a submaximal KCl (60 mM) concentration from 0 to 100%. All three concentration–response curves for nicardipine were parallel ($P > 0.05$ for p) and shifted along the x-axis (Figs. 4 and 5). In both vessel preparations the (+)-*S* enantiomer was the most potent, racemic nicardipine was intermediate and the (−)-*R* enantiomer was the least potent. The (+)-*S* enantiomer was five times more potent than the (−)-*R* enantiomer in the vessels with endothelium and seven times more potent in the vessels without endothelium (Table 1). The potency ratios between two enantiomers were not statistically significantly different ($P > 0.05$) in the two vessel preparations.

4. Discussion

Qualitative and quantitative effects of the (+)-*S* and (−)-*R* enantiomers and of the racemic mixture of nicardipine were compared on the isolated porcine coronary artery with and without endothelium.

4.1. Control contractions induced by KCl (5–90 mM)

At each KCl concentration the vessels with an intact endothelium were contracted less than the vessels without an endothelium in the control experiments without nicardipine (Fig. 1). This finding could be explained by the tonic release of NO from endothelial cells (Spedding et al., 1986).

4.2. Qualitative effects of the three forms of nicardipine

All three forms of nicardipine only inhibited the contractions induced by KCl (5–90 mM) (Figs. 2 and 3) in both vessel preparations and, thus, showed only Ca^{2+} channel antagonistic effects (Hof et al., 1985). These results are in accordance with those of in vitro and in vivo experiments in which the endothelium was intact and in which none of the three forms of nicardipine had a Ca^{2+} channel agonistic or dualistic effect (Takenaka et al., 1982; Iwatsuki et al., 1984; Brisac et al., 1988). The removal of the endothelium in our experiments did not change the qualitative action of the three forms of nicardipine. The shape of the KCl (5–90 mM) concentration–response curves suggests a noncompetitive type of antagonism; however, nicardipine is not an antagonist of KCl. KCl does not bind to Ca^{2+} channels, but rather depolarizes the membrane of smooth muscle cells and in this way modulates the state (open, closed, inactivated) of Ca^{2+} channels (Bolton, 1979).

4.3. Quantitative effects of the three forms of nicardipine

In our study, the (+)-*S* enantiomer ($IC_{50} = 6.6$ nM) was five times more potent than the (−)-*R* enantiomer ($IC_{50} = 31.8$ nM) in the porcine coronary artery with an intact endothelium. The (+)-*S* enantiomer is always more potent than the (−)-*R* enantiomer in in vivo experiments with vessels with an intact endothelium: three times more potent in increasing vertebral blood flow and in decreasing mean blood pressure in dogs (Takenaka et al., 1982) and in normotensive rats (Brisac et al., 1988), five times more potent in increasing blood flow in the isolated, blood-perfused canine pancreas (Iwatsuki et al., 1984) and eight times more potent in decreasing mean blood pressure in spontaneously hypertensive rats (Brisac et al., 1988). The only available data on the potency of the (+)-*S* (3 nM) and (−)-*R* enantiomers (1.5 nM) in isolated vessels are those for the isolated rat aorta (Takenaka et al., 1982) where the (+)-*S* enantiomer was two times more potent than the (−)-*R* enantiomer. Thus, our data about the potency and the potency ratio between the two enantiomers in the isolated porcine coronary artery with intact endothelium are in accordance with those mentioned above. In the isolated porcine coronary artery without endothelium the (+)-*S* enantiomer ($IC_{50} = 6.4$ nM) was seven times more potent than the (−)-*R* enantiomer ($IC_{50} = 41.9$ nM). In the literature we could not find comparable results for endothelium-denuded vessels.

4.4. The influence of the endothelium on the quantitative effects of the three forms of nicardipine

Some studies indicate that the vasorelaxation of isolated vessels by Ca^{2+} channel antagonists may be mediated partially by an increased release of NO from endothelial cells. Inhibitors of NO synthase (Günther et al., 1992), mechanical removal of the endothelium (Kojda et al., 1990, 1991) and inhibitors cGMP synthase (methylene blue) (Kojda et al., 1991) reduce the relaxation elicited by different Ca^{2+} channel antagonists. The mechanism of action of Ca^{2+} channel antagonists on endothelial cells is not completely understood since endothelial cells do not express voltage-operated Ca^{2+} channels (Himmel et al., 1993). Others claim (Rubanyi et al., 1988; Mügge et al., 1991) that the endothelium plays no role in the relaxation elicited by Ca^{2+} channel antagonists. With the mechanical removal of the endothelium we obtained an in vitro model for the evaluation of the endothelial contribution to the inhibition of contractions by nicardipine. In the present study, the parameters of the concentration–response curves for the effect of each form of nicardipine on the contraction elicited by a submaximal KCl (60 mM) concentration were not statistically significantly different ($P > 0.05$) between the two vessel preparations, except for the slope of the (−)-*R* enantiomer curve (Table 1). The potency ratios between the two enantiomers were also not statistically significantly different ($P > 0.05$) in the two vessel preparations. Our results, thus, are in accordance with those of experiments where no contribution of the endothelium to the relaxation elicited by Ca^{2+} channel antagonists in

isolated blood vessels could be found (Rubanyi et al., 1988; Mügge et al., 1991).

In conclusion, qualitatively, all three forms of nicardipine showed only Ca^{2+} channel antagonistic effects in both vessel preparations. Quantitatively, the inhibition of contraction was stereoselective, the (+)-*S* enantiomer being the most potent, and was endothelium-independent.

Acknowledgements

The authors wish to thank the Slovenian Science Foundation for the financial support.

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