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Effects of Nicardipine on Collar-induced Intimal Thickening and Vascular Reactivity in the Rabbit

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Abstract

The effects of nicardipine treatment on collar-induced intimal thickening and on accompanying reactivity changes in rabbit carotid artery have been investigated.

Treatment for three weeks with subcutaneous nicardipine ($20\,mg\,kg^{-1}$ per day) significantly inhibited the intimal thickening caused by perivascular application of a silicone rubber collar. Potassium chloride (KCl), phenylephrine and 5-hydroxytryptamine (5-HT) induced concentration-dependent contractions in both sham-operated and collared arteries. Collar-induced attenuation of maximum KCl-, phenylephrine- and 5-HT-induced contraction was not affected by nicardipine. Collaring caused the means of pD_2 values (the negative logarithm of EC50 values, 50% effective concentration) of 5-HT and phenylephrine to increase and decrease, respectively. Nicardipine did not affect the altered sensitivity to these agonists. Neither collar implantation nor nicardipine treatment altered the pD_2 values for acetylcholine- and nitroglycerine-induced relaxations.

These results demonstrate that nicardipine inhibits collar-induced intimal thickening in rabbit carotid artery without affecting the accompanying changes in vascular reactivity, indicating a possible lack of association between the development of intimal thickening and altered reactivity.

Intimal thickening is considered a susceptible site for atherosclerosis (Stary et al 1992). Mechanisms involved in the development of intimal thickening can include the transformation of medial smooth muscle cells from contractile to synthetic phenotype and the migration of smooth muscle cells from the medial layer to the intimal layer, then cell proliferation (Ross 1993). On the basis of studies of hypercholesterolaemic patients (Vrints et al 1992) and hypercholesterolaemic animals (Verbeuren et al 1986; Jayakody et al 1988), this early stage of atherosclerosis is usually accompanied by alterations in vascular reactivity. Similar observations have also been made after perivascular manipulation of rabbit carotid arteries (De Meyer et al 1990; Üstünes et al 1996), a model previously described by Booth et al (1989). Perivascular application of a flexible silicone collar around the carotid artery resulted in a diffuse intimal thickening as a result of the proliferation of smooth muscle cells, most of which migrated from the medial layer (Kockx et al 1992).

Many calcium-channel blockers have anti-atherosclerotic activity (Paoletti et al 1995). Nicardipine, an L-type dihydropyridine calcium-channel blocker, has also been shown to inhibit the development of atherosclerosis in man and in animal models (Paoletti et al 1995), although the effectiveness of nicardipine on collar-induced intimal thickening and altered vascular reactivity was not tested.

This study has investigated whether nicardipine prevents the intimal thickening and the altered vascular reactivity caused by the collar.

Materials and Methods

Drugs

Acetylcholine hydrochloride, phenylephrine hydrochloride and 5-hydroxytryptamine creatinine sul-

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phate were from Sigma (St Louis, MO), indomethacin sodium and nitroglycerine solution from Merck, Sharp & Dohme (Munich, Germany), sodium pentobarbital from Psyphac (Brussels, Belgium), heparin solution from Roche (Istanbul, Turkey), and silicone (MED-4011) from Nusil Silicone Technology (Anglet, France). Nicardipine was a gift from Novartis (Istanbul, Turkey). 5-Hydroxytryptamine creatinine sulphate monohydrate was dissolved in an aqueous solution of ascorbic acid (0·01%) and diluted with distilled water. Nicardipine was dissolved in ethanol (10%, v/v). Other drug solutions were prepared in distilled water.

Treatment of animals

White rabbits, 2–2·5 kg, of either sex ($n = 20$) were divided into two groups. The first group ($n = 10$) received a single subcutaneous injection of nicardipine (20 mg kg^{-1} per day). The second group (placebo, $n = 10$) received only the vehicle (10%, v/v, ethanol, 1 mL kg^{-1} per day). Throughout the three-week treatment period each rabbit was kept in a separate cage and allowed free access to regular food and tap water.

Induction of intimal thickening

After the 7th day of treatment with nicardipine or placebo the rabbits were anaesthetized with intravenous sodium pentobarbital (30 mg kg^{-1}). Subsequently, the left carotid artery was surgically accessed and surrounded by a non-occlusive, flexible silicone collar 2 cm long (Booth et al 1989). The right carotid artery was sham-operated (i.e. separated from surrounding connective tissue and vagus nerve and received a similar stretch as the contralateral collared artery). The carotid arteries were then returned to their original positions and the incisions closed. After recovery from the anaesthesia, the animals were kept in their individual cages for a further two weeks before tissue isolation.

Morphometry

After anticoagulation with intravenous heparin (150 units kg^{-1}), the rabbits were killed by sodium pentobarbital overdose. Isolated vessel segments from sham-operated and collared arteries were cut into two 4-mm-long rings, one for morphometry and the other for organ-bath experiments. The first ring was immediately placed in formalin fixative solution (4%) for 24 h, dehydrated in a graded series of isopropyl alcohol (60–100%), then toluene, before being embedded in paraffin. Transverse sections were cut and stained with Sirius red haematoxylin. Two transverse sections from each artery ring were randomly chosen and their video images recorded with a video-camera (JVC Colour Video Camera, Head Model No. TK-890E, Japan) connected to a light microscope (Olympus BH-2, Japan). Intimal and medial cross-sectional areas were measured by use of a computerized system. In brief, video images (Sony VCR SL-C6E) of each segment were captured via a video-card (Video Blaster SE, Creative Labs, USA). Intimal and medial cross-sectional areas were marked (CorelDraw, Version 4.00.A5, Corel Corporation 1993, USA) and measured (AutoCAD, release 12-cl, 1993, Autodesk, USA). The ratios of intimal to medial cross-sectional areas were also calculated (index).

Vascular reactivity

The two remaining rings from both the right (sham) and left (collared) carotid arteries were used in organ-chamber experiments to study vascular reactivity. After careful removal of loose connective tissue the tissue rings were suspended in organ chambers filled with physiological salt solution (PSS; 25 mL) at 37°C continuously oxygenated with 95% O_2–5% CO_2 (De Meyer et al 1991). PSS contained (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 25; and glucose, 11·1. Indomethacin (3×10^{-6} M) was included in all experiments to inhibit cyclooxygenase activity. Isometric contractile force development was measured by means of a Grass FT3 force transducer and recorded (Polywin95 1.0, Commat, Ankara, Turkey) by means of a microcomputer (IBM PS/1). After 15-min equilibration, tissues were gradually stretched to a tension of 7 g, a previously determined optimum resting tension based on the length–tension relationship, and left to equilibrate for 45 min longer. During the equilibration period, the bath solution was changed every 15 min.

Acetylcholine-induced endothelium-dependent vasorelaxant responses resulting from the release of nitric oxide were tested at the end of the equilibration period. For this purpose, tissues were contracted with phenylephrine (10^{-6} M) and during steady-state contraction, acetylcholine (10^{-9}–10^{-4} M) was added cumulatively. Tissues relaxing more than 40% of the initial contraction (acceptable level of intact endothelium) were washed out three times with PSS, recontracted with phenylephrine (10^{-6} M), and then exposed to cumulative concentrations of nitroglycerine (10^{-9}–

3×10^{-6} M). Later, tissues were washed three times and treated with cumulative concentrations of phenylephrine (10^{-9}–10^{-4} M) and 5-HT (10^{-9}–3×10^{-5} M). Each agonist was washed out by changing the bath solution three times within 30 min before addition of the next agonist. Concentration–response relationships for 5-HT-, phenylephrine-, acetylcholine- and nitroglycerine-induced responses were constructed for each preparation. At the end of each experiment, tissues were washed out three times and contracted with 120 mM KCl (with equimolar replacement of NaCl) to determine the contractility.

Statistical methods

Statistical analyses, i.e. factorial analysis of variance (SPSS/PC+, Chicago, IL), were performed to determine the effect of drug treatment (two levels, nicardipine or placebo) and that of the collar (two levels, with and without collar). The Wilcoxon matched-pairs signed rank test and the Mann-Whitney *U*-test were performed for paired and unpaired comparisons when an interaction was observed between the factors.

Shown are means ± s.e.m.; n indicates the number of animals. Significance was accepted at $P = 0{\cdot}05$. Values of maximum effect (E_{max}) and 50% effective concentration (EC50) were derived for each cumulative concentration–response curve by iterative non-linear curve fitting (KaleidaGraph 3.06; Synergy Software). Means of the negative logarithm of EC50 values (pD_2) were compared. Acetylcholine- and nitroglycerine-induced relaxations were normalized to the initial phenylephrine contraction.

Results

Survival and body weight

One rabbit from each group died during the treatment period. Nicardipine did not have any visible side effects. The body weights of animals of both groups were not changed by the treatment protocol.

Intimal thickening

Intimal cross-sectional area and the ratio of intimal area to medial area (index) of collared arteries were significantly higher than those of sham arteries (Table 1). Nicardipine treatment significantly inhibited intimal thickening (Table 1) and significantly reduced the index (Table 1). Collar or nicardipine treatment did not alter the medial cross-sectional area (Table 1).

Vascular reactivity

Contractions

Contractions induced by 120 mM KCl were significantly reduced in collared arteries. Nicardipine treatment did not significantly affect the KCl-induced contractions in either sham or collared arteries (Table 2).

Table 1. Effects of nicardipine (20 mg kg^{-1} per day) on collar-induced intimal thickening.

	Placebo (n = 8)	Nicardipine (n = 7)
Intima (mm^2)		
Sham	0·005 ± 0·001	0·009 ± 0·003
Collared	0·067 ± 0·010*	0·040 ± 0·009*†
Significance of factors in analysis of variance:		
Collar	$P < 0{\cdot}001$	
Nicardipine	$P > 0{\cdot}05$	
Interaction: nicardipine by collar	$P < 0{\cdot}05$	
Media (mm^2)		
Sham	0·358 ± 0·014	0·413 ± 0·038
Collared	0·367 ± 0·017	0·380 ± 0·033
Significance of factors in analysis of variance:		
Collar	$P > 0{\cdot}05$	
Nicardipine	$P > 0{\cdot}05$	
Interaction: nicardipine by collar	$P > 0{\cdot}05$	
Index (intima/media)		
Sham	0·014 ± 0·002	0·024 ± 0·009
Collared	0·182 ± 0·030*	0·110 ± 0·028*†
Significance of factors in analysis of variance:		
Collar	$P < 0{\cdot}001$	
Nicardipine	$P > 0{\cdot}05$	
Interaction: nicardipine by collar	$P < 0{\cdot}05$	

Results are means ± s.e.m.; n denotes the number of animals in each group. †$P < 0{\cdot}05$, placebo compared with nicardipine (Mann-Whitney *U*-test). *$P < 0{\cdot}05$, sham compared with collared (Wilcoxon matched-pairs signed-ranks test).

Table 2. Effects of collar and of nicardipine (20 mg kg^{-1} per day) on maximum contractile responses to KCl.

	Placebo (n = 5)	Nicardipine (n = 6)
Maximum contractile responses to KCl (g)		
Sham	2·24±0·39	3·06±0·32
Collared	0·26±0·04	0·50±0·19
Significance of factors in analysis of variance:		
Collar	$P<0·001$	
Nicardipine	$P>0·05$	
Interaction: nicardipine by collar	$P>0·05$	

Results are means±s.e.m.; n denotes the number of animals in each group.

Table 3. Effects of collar and of nicardipine (20 mg kg^{-1} per day) on pD_2 values for 5-HT- and phenylephrine-induced contractions.

	Placebo	Nicardipine
5-HT	(n=6)	(n=9)
Sham	6·90±0·12	6·85±0·08
Collared	7·11±0·13	7·23±0·08
Significance of factors in analysis of variance:		
Collar	$P=0·003$	
Nicardipine	$P>0·05$	
Interaction: nicardipine by collar	$P>0·05$	
Phenylephrine	(n=7)	(n=8)
Sham	6·06±0·12	6·12±0·05
Collared	5·55±0·1	5·67±0·15
Significance of factors in analysis of variance:		
Collar	$P=0·001$	
Nicardipine	$P>0·05$	
Interaction: nicardipine by collar	$P>0·05$	

Results are means±s.e.m.; n denotes the number of animals in each group.

5-HT induced concentration-dependent contractions in both sham and collared arteries. Maximum contractile response (E_{max}) was significantly reduced in collared arteries (Figure 1). Collar placement significantly increased the sensitivity to 5-HT as indicated by higher pD_2 values (Table 3). Neither the increased pD_2 nor the reduced E_{max} was altered by nicardipine treatment (Table 3, Figure 1).

Phenylephrine induced concentration-dependent contractions in both sham and collared arteries. E_{max} and pD_2 values for collared arteries were significantly lower than those for sham arteries (Figure 2, Table 3). Nicardipine treatment did not significantly affect either E_{max} or pD_2 (Figure 2, Table 3).

Initial phenylephrine (10^{-6} M) contractions were reduced in collared arteries. Nicardipine treatment did not influence these contractions (Figures 3 and 4).

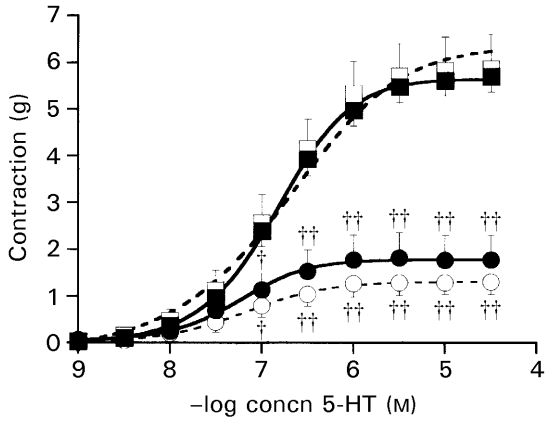

Figure 1. Effects of collar and nicardipine treatment on 5-HT-induced contractions. Concentration–response relationships are shown for 5-HT-induced contraction performed in placebo-treated sham (□), placebo-treated collared (○), nicardipine-treated sham (■), and nicardipine-treated collared (●) arteries. Results are means± s.e.m. (placebo group, n = 6; nicardipine group, n = 9). †$P < 0·001$, sham compared with collared in placebo- or nicardipine-treated groups.

Relaxations

Acetylcholine induced concentration-dependent relaxations in both sham and collared arteries pre-contracted with 10^{-6} M phenylephrine (Figure 3). The sensitivity to acetylcholine was not altered significantly, whereas the maximum acetylcholine relaxation was significantly enhanced in collared arteries (pD_2 values 6·87± 0·14 and 6·64± 0·13 in placebo-treated sham and collared arteries, respectively, n = 7) (Figure 3). Nicardipine treatment did not significantly alter either pD_2 or E_{max} for acetylcholine-induced relaxations in sham and collared arteries (pD_2 values 6·87± 0·14 and

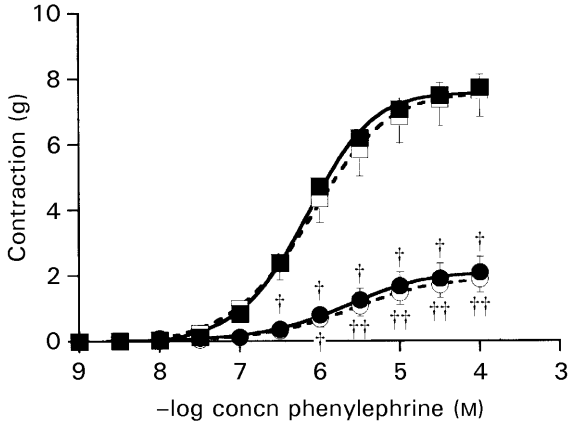

Figure 2. Effects of collar and nicardipine treatment on phenylephrine-induced contractions. Concentration–response relationships are shown for phenylephrine-induced contraction performed in placebo-treated sham (□), placebo-treated collared (○), nicardipine-treated sham (■), and nicardipine-treated collared (●) arteries. Results are means± s.e.m.(placebo group, n = 7; nicardipine group, n = 8). †$P < 0·001$, sham compared with collared in placebo- or nicardipine-treated groups.

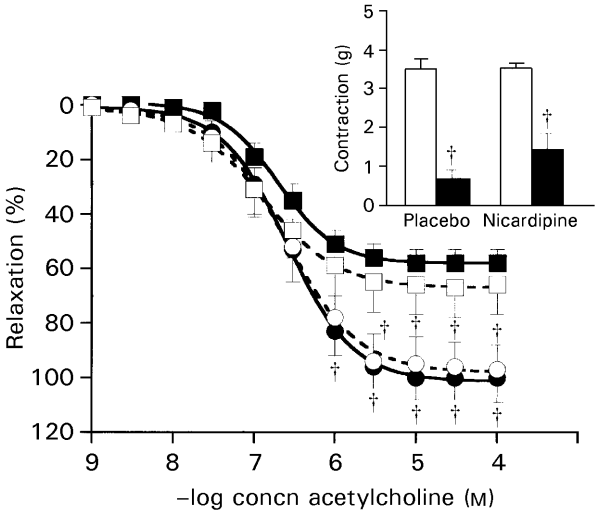

Figure 3. Effects of collar and nicardipine treatment on acetylcholine-induced relaxations. Concentration–response relationships are shown for acetylcholine-induced relaxation performed in placebo-treated sham (□), placebo-treated collared (○), nicardipine-treated sham (■), and nicardipine-treated collared (●) arteries precontracted with 10^{-6} M phenylephrine. Results are means ± s.e.m. (placebo group, n = 7; nicardipine group, n = 9). Relaxation data are expressed as a percentage of the initial contraction induced by 10^{-6} M phenylephrine. †$P < 0.001$, sham compared with collared in placebo- or nicardipine-treated groups. The inset shows initial contractions elicited by 10^{-6} M phenylephrine in sham (□) and collared (■) arteries. †$P < 0.001$, sham compared with collared in placebo- or nicardipine-treated groups.

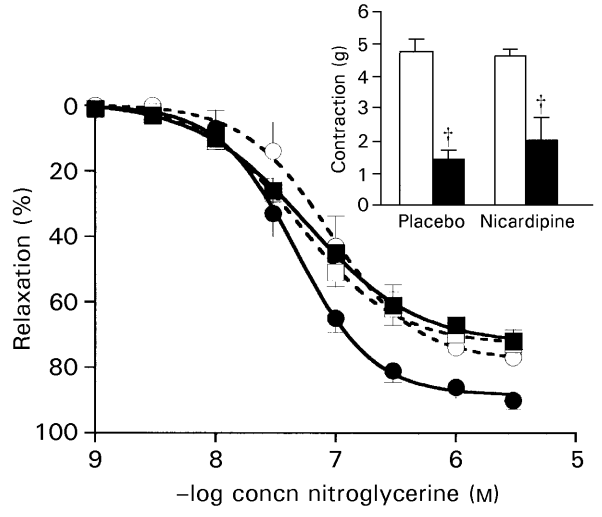

Figure 4. Effects of collar and nicardipine treatment on nitroglycerine-induced relaxations. Concentration–response relationships are shown for nitroglycerine-induced relaxation performed in placebo-treated sham (□), placebo-treated collared (○), nicardipine-treated sham (■), and nicardipine-treated collared (●) arteries precontracted with 10^{-6} M phenylephrine. Results are means ± s.e.m. (placebo group, n = 4; nicardipine group (n = 5). Relaxation data are expressed as a percentage of the initial contraction induced by 10^{-6} M phenylephrine. The insert shows initial contractions elicited by 10^{-6} M phenylephrine in sham (□) and collared (■) arteries. †$P < 0.001$, sham compared with collared in placebo- or nicardipine-treated groups.

6·64 ± 0·13 in nicardipine-treated sham and collared arteries, respectively, n = 9) (Figure 3).

Nitroglycerine induced concentration-dependent relaxations in both sham and collared arteries precontracted with 10^{-6} M phenylephrine (Figure 4). Neither collar nor nicardipine treatment significantly influenced E_{max} and pD_2 values for nitroglycerine-induced relaxations (pD_2 values 7·36 ± 0·07 and 7·20 ± 0·16 for placebo-treated sham and collared arteries, respectively, n = 4, and 7·28 ± 0·07 and 7·29 ± 0·08 for nicardipine-treated sham and collared arteries, respectively, n = 5) (Figure 4).

Discussion

Inhibition of intimal thickening

This study demonstrates that nicardipine treatment prevents collar-induced intimal thickening in rabbit carotid artery. This suppression of intimal thickening by nicardipine is a novel finding and is possibly involved in the anti-atherosclerotic effectiveness of the drug reported in previous studies using different models (Catapano 1992).

Although the exact mechanism(s) by which nicardipine (these results) or other L-type Ca^{2+}-channel blockers inhibit intimal thickening is not clear, it might include inhibition of smooth muscle cell migration (Nakao et al 1983) or proliferation (Nilsson et al 1985), or both, and extracellular matrix synthesis (Weinstein & Heider 1987). Indeed, lacidipine, another dihydropyridine, has been shown to inhibit collar-induced intimal thickening in the cholesterol-fed rabbit model (Soma et al 1994).

Lacidipine also effectively inhibited collar-induced intimal thickening in a series of experiments in our laboratory (unpublished observations) and nilvadipine, a potent inhibitor of smooth muscle cell migration in-vitro, has been shown to inhibit intimal thickening resulting from balloon catheterization in the coronary artery of miniature pigs (Arakawa et al 1992). Nilvadipine also suppressed the intimal thickening induced by a rigid collar whereas verapamil, a phenylalkylamine, was without effect (Nomoto et al 1987). Verapamil also failed to prevent collar-induced intimal thickening (Üstünes et al 1996). Local perivascular application of diltiazem, a benzothiazepine, inhibited intimal thickening resulting from balloon denudation in rat carotid artery (Hadeishi et al 1994). These results imply that the effects of L-type Ca^{2+}-channel blockers on intimal thickening are quite heterogeneous. Therefore, it can be suggested that the

anti-atherosclerotic effectiveness of a calcium-channel blocker depends on its individual mechanism(s) of action, irrespective of its chemical class, i.e. different agents in the same class of calcium-channel blockers might not be as effective as expected (Catapano 1992). Delineation of downstream events of Ca^{2+}-entry blockade by different agents in intimal thickening would explain the role of calcium-channel blockers in the treatment of atherosclerosis.

Nicardipine and vascular reactivity

Contractile responses

Consistent with previous results (De Meyer et al 1994; Üstünes et al 1996), collar placement suppressed KCl-, 5-HT- and phenylephrine-induced maximum contraction in rabbit carotid artery. Attenuation of contractions has been discussed in detail (Kockx et al 1992; De Meyer et al 1994; Üstünes et al 1996). The possibility that the collar might cause mechanical damage of medial vascular smooth muscle cells is unlikely in this study, because no mechanical damage or necrosis of the medial layer was found by histological examination and the medial cross-sectional area did not change in collared arteries either with or without nicardipine. The possibility that an increase in nitric oxide release might prevent the agonist-induced contractions in collared arteries has also been eliminated because contractility studies failed to demonstrate the induction of inducible nitric oxide synthase in collared arteries (De Meyer et al 1994). It is more likely that a reduced number of contractile smooth muscle cells in the medial layer might account for the diminished agonist-induced contractions (Beesley et al 1992).

Nicardipine treatment did not significantly affect KCl-, 5-HT- and phenylephrine-induced contractions either in sham or collared-arteries. Verapamil treatment also had no effect on KCl-induced contractions in the collar model (Üstünes et al 1996).

The respective leftward and rightward shifts observed in 5-HT and phenylephrine concentration–response relationships are characteristic in the collar model (Üstünes et al 1996) and were not affected by nicardipine treatment.

Thus, the current results suggest that nicardipine treatment does not alter receptor sensitivity to phenylephrine and 5-HT.

Relaxations

In contrast with previous observations (De Meyer et al 1991; Üstünes et al 1996), acetylcholine-induced maximum relaxation was significantly increased in collared arteries. This conflicting result might not be caused by different levels of initial phenylephrine-induced contractions observed in sham-operated and collared arteries, a conclusion based upon the findings that initial contractions at different magnitudes induced by different concentrations of phenylephrine did not affect E_{max} and pD_2 values for acetylcholine-induced relaxations in untreated rabbit carotid arteries (van Put et al 1995). Also consistent with the findings of van Put et al (1995) is that the collaring did not alter pD_2 values of acetylcholine. Nicardipine treatment did not affect the endothelium-dependent relaxation in either sham-operated or collared arteries, results consistent with those of Jayakody et al (1987), suggesting that nicardipine does not interact with muscarinic receptors or constitutive nitric oxide production.

In addition, nitroglycerine was used to induce cGMP-mediated relaxations directly acting on smooth muscle cells. Consistent with the previous reports, neither pD_2 nor E_{max} values for nitroglycerine-induced relaxations were affected in collared arteries (De Meyer et al 1991). Chronic nicardipine treatment did not affect the nitroglycerine-induced relaxations in sham and collared arteries, suggesting that nicardipine does not interfere with the downstream effects of nitric oxide in which cGMP-mediated responses are involved.

In summary, this study demonstrates that in rabbit carotid artery nicardipine treatment prevented collar-induced intimal thickening without affecting the accompanying changes in vascular reactivity, also suggesting that the collar-induced alterations in vascular reactivity develop independently of intimal thickening in this model.

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