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Effect of nifedipine on the collar-induced intimal thickening and vascular reactivity changes in rabbits

Tavşanlarda yaka ile oluşan intimal kalınlaşma ve vasküler reaktivite değişiklikleri üzerine nifedipinin etkisi

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Abstract

The positioning of a soft and nonocclusive silicon collar around the rabbit carotid artery induces intimal thickening and characteristic vascular reactivity changes. In the present study, we investigated whether the treatment with nifedipine, a dihydropyridine derivative calcium channel blocker (CCB) (40 mg/kg/day, p.o.), inhibit development of intimal thickening and accompanying vascular reactivity changes in this model. Neither intimal thickening nor vascular reactivity changes were effected by nifedipine treatment in this model.

Key Words: Calcium channel bloker, intimal tickening, collar, vascular reactivity

Introduction

Intimal thickening which is considered to be an early and essential step in the development of atherosclerosis and restenosis is characterized by smooth muscle cell proliferation and migration (Stary *et al.*, 1992). These early stages are generally associated with changes in vascular reactivity of the artery, even if atherosclerotic lessions are angiographically not detectable (Vrints *et al.*, 1992). In order to examine the mechanism of intimal thickening various experimental models of intimal hyperplasia were developed (John *et al.*, 1990, De Meyer *et al.*, 1997 a, Bayes-Genis *et al.*, 2000).

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These models include those that use intraluminal (e.g. balloon catheter denudation) and those that use perivascular (e.g. soft silicone collar) manipulations. In the model of perivascular soft collar placement which is also used in the present study (Booth *et al.*, 1989), the positioning of a nonocclusive and soft silicon collar around the carotid artery of the rabbit induces intimal thickening accompanied by vascular reactivity changes such as increased sensitivity to serotonin and decreased maximum force development to contractile agents.

On the other hand, it is known that calcium ions play a key role in the initial and chronic development of atherosclerotic lesions (Motro *et al.*, 2001). Several studies both in experimental animal models and in humans have demonstrated the potential antiatherosclerotic effects of calcium channel blockers (CCBs) (Hirata *et al.*, 2000, Yamashita *et al.*, 2001, Bae *et al.*, 2005). CCBs reduce the influx of calcium into the the cells acting on voltage-activated calcium channels. Thus, CCBs primarily inhibit many calcium-dependent events in the formation of atherosclerosis such as the localized accumulation of exracellular matrix and calcium, together with monocyte infiltration and smooth muscle proliferation and migration particularly occuring in the early phases (Haller *et al.* 1996). Besides, it was reported that CCBs enhance bioavailability of endothelial nitric oxide (NO) and eNOS expression, and protect against free-radical induced cell damage by reducing LDL oxidation process (Mak *et al.*, 1992, Kitakaze *et al.*, 1999, Mason *et al.*, 2003). Furthermore recent evidences showed that CCBs able to modulate matrix metalloproteinase (MMP) expression/activity and contribute to prevent smooth muscle cell proliferation and migration (Wada *et al.*, 2001). However, the precise mechanisms of CCBs in the development of atherosclerosis have not been yet fully elucidated.

The aim of the present study was, therefore, to investigate the potential effectiveness of nifedipine (40 mg/kg/day, p.o), the prototype of dihydropyridine CCBs, on collar-induced intimal thickening and on accompanying vascular reactivity changes.

Materials and Methods

Acethylcholine chloride, phenylephrine hydrochloride, 5-hydroxytryptamine creatinine sulphate, nitroglycerin solution (1 %), potassium chloride and indomethacin sodium were purchased from Merck, Darmstad, Germany; sodium pentabarbital from Psyphac, Brussels, Belgium; silicone (MED-4011) from Nusil Silicone Technology, Anglet, France and heparin solution from Roche, Istanbul, Turkey. Nifedipine was kindly provided by Fako İlaç Sanayii A.Ş., Istanbul, Turkey.

Animals

The animal experiments were carried out in accordance with guidelines described by the Ethics Committee of the Faculty of Pharmacy, Ege University.

White rabbits of either sex (1.8-2.5 kg; n=20) were divided into two groups. The first group (n=10) received nifedipine (40 mg/kg/day, p.o) and the second group (n=10) received only the vehicle (0.5 % methylcellulose solution, 2.5 ml/kg/day, p.o). Throughout the 3-week treatment period each rabbit was kept in a separate cage and allowed free access to regular rabbit chow and tap water.

Model

After the 7th day of treatment with nifedipine or placebo the rabbits were anaesthetized with sodium pentobarbital (30 mg/kg i.v.). Subsequently, the left carotid artery was surgically accessed and dissected from surrounding tissues. A nonocclusive, flexible silicone collar of 2 cm length was positioned around the left carotid artery as described by Booth *et al.* (1989). The right carotid artery was sham-operated (i.e. it was separated from surrounding connective tissue and vagus nerve, and received a similar stretch as the contralateral carotid artery). The carotid arteries were then returned to their original positions and the incisions were sutured. After recovery from the anesthesia, all rabbits were kept in their individual cages for a further 2 weeks.

Morphometry

After 14 days treatment period (placebo or nifedipine), the rabbits (n=20) were given heparin (150 units/kg i.v.) as an anticoagulant and were killed with an overdose of sodium pentobarbital. Then, both carotid arteries were isolated and dissected. Two segments, each one 4 mm long, were cut from both the collared and sham-operated arteries, one for morphometry and the other for organ-bath experiments. One pair of segment from both arteries was immediately placed in formalin fixative solution (0.4 %) for 24 hours, dehydrated in a graded series of isopropyl alcohol (60 to 100 %), then toluol, before being embedded in paraffin. Transverse sections were cut and stained with sirius red haematoxylin. Two transverse sections from each artery were randomly chosen and their video images recorded at x4 magnification by using a video-camera (JVC Color Video Camera, Head Model No. TK-890E, Japan) connected to a light microscope (Olympus BH-2, Japan). The image of each segments from the video recorder (Sony Video Cassette Recorder SL-C6E) were captured via a video-card (Video Blaster SE, Creative Labs. Inc., U.S.A.). The intimal and medial cross-sectional areas were traced by use of a software package (Corel Draw, Version 4.00.A5, Corel Corporation 1993, U.S.A.) and measured by AutoCAD (release 12-cl,1993, Autodesk, Inc., U.S.A.) in both sections as previously reported (Üstünes *et. al.*, 1996). The index defined as intimal/medial area ratio was also calculated for each section. All morphometric measurements and calculations were performed for two randomly chosen sections from both sham and collared segments and the means were determined.

Organ chamber experiments

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 rings were suspended in organ chambers filled with physiological salt solution (Krebs) at $37^{O}C$, continuously oxygenated with $95\%O_2$-$5\%CO_2$ (De Meyer *et al.*, 1991). In order to inhibit endogen cyclooxygenase activity, indomethacin ($3x10^{-6}M$) was added into Krebs solution. Isometric contractile force development was measured by means of a Grass FT3 force transducer and recorded (IOSlab version 3.23 MS8, EMKA Technologies, Paris, France) by means of a personal computer (IBM PS/1). After 15-min equilibration, tissues were gradually stretched to a tension of 7 g, a previously determined optimum resting tension based upon the length-tension relationship, for both sham and collared arteries (Ustunes *et al.*, 1996). The arterial rings were then left to equilibrate for further 45 min along with changing the bath solution every 15 min. At the end of the equilibration period the following concentration-

response curves were constructed for each preparation; acetylcholine (10^{-9}-10^{-4} M) and nitroglycerine (10^{-9}-$3x10^{-5}$ M) after precontraction by $3.5x10^{-7}$ M phenylephrine; phenylephrine (10^{-9}-10^{-4} M); 5-hydroxytryptamine (5-HT, serotonin) (10^{-9}-$3x10^{-5}$ M); potassium chloride (KCl) (120 mM, in depolarizing Krebs). Each agonist was washed out by changing the bath solution three times in a 30 min time period before addition of the next agonist.

Statistical methods

All data are given means ± S.E.M. n indicates the number of animals. Statistical analyses were performed for drug treatments (two levels, nifedipine or placebo, between rabbit factor) and collar (two levels, present or not, within rabbit factor) with a factorial analysis of variance (ANOVA). If there were interactions between the factors in ANOVA, Wilcoxon signed ranks test (sham vs. collared arteries, paired data) and Mann-Whitney U-test (placebo vs. nifedipine, unpaired data) were used. Significance was accepted at $p=0.05$. Means of pD_2 and E_{max} values were compared. Means of all morphometric data from each segment assessment were compared.

Results

Survival and body weight

Only one rabbit from each group died during the treatment period. Nifedipine did not appear to cause any side effects. The body weight of the animals in two groups was not changed by the treatment period (data not shown).

Morphometry

The intimal cross-sectional area and the ratio of intimal area to medial area (index) were significantly increased in collared arteries as compared to those in sham-operated arteries in placebo group (Figure 1a, Figure 1c). Nifedipine treatment did not significantly alter the intimal area and index (Figure1a, Figure 1c).

Neither collar placement nor nifedipine treatment significantly affected medial cross-sectional area (Figure 1b).

Vascuar Reactivity Changes

Contractions

The maximum contractile responses (E_{max}) induced by 120 mM KCl were significantly decreased in collared arteries. As indicated with the presence of an interaction between collar and nifedipin (Table 1, $p<0.001$), treatment with nifedipine significantly attenuated contractile responses to KCl in sham arteries, while it was without effect in the collared arteries (Table 1). 5-HT induced concentration-dependent contractions in sham and collared arteries. As indicated by higher pD_2 values in collared arteries, collar placement significantly increased sensitivity to

5-HT (Table 2). Nifedipine treatment did not affect this hypersensitivity to 5-HT in collared arteries (Table 2).
With regard to E_{max} values, collar placement significantly diminished the maximum contractile force development to 5-HT (Table 2). As indicated with the presence of interaction between collar and nifedipine (Table 2, $p<0.05$), nifedipine treatment more significanly decreased E_{max} values to 5-HT in sham arteries than did in collared arteries (Table 2).
Phenylephrine induced concentration-dependent contractions in sham and collared arteries. Collar placement significantly decreased the sensitivity to phenylephrine (Table 3). Nifedipine treatment did not affect pD_2 values of phenylephrine in either sham or collared arteries (Table 3).
In terms of maximum contractile force development (E_{max} values) to phenylephrine, maximum contractile responses to phenylephrine significantly decreased by collar placing (Table 3).
Treatment with nifedipine attenuated maximum contractions in sham arteries but not in collared arteries, as indicated by interaction between collar and nifedipine treatment (Table 3, $p<0.001$).

Relaxations

Acetylcholine induced concentration-dependent relaxations in both sham and collared arteries with endothelium, precontracted with $3.5x10^{-7}$ M phenylephrine.
Collar placement significantly diminished sensitivity to acetylcholine (Table 5). Nifedipine treatment did not altered the sensitivity to acethylcholine in either sham or collared arteries (Table 4).
The maximal acetylcholine relaxations (E_{max} values) were significantly increased by collar placement, but not affected by nifedipine treatment (Table 4).
Nitroglycerine induced concentration-dependent relaxations in both sham and collared arteries precontracted with $3.5x10^{-7}$ M phenylephrine.
Neither collar placement nor nifedipine treatment significantly influenced the pD_2 values for nitroglycerine-induced relaxations.
However, collaring significantly increased E_{max} values of nitroglycerine (Table 5). Nifedipine treatment also significantly increased E_{max} values in sham arteries but not in collared arteries, as indicated by the presence of interaction between collar and nifedipine treatment (Table 5, $p<0.01$).

Discussion

Nifedipine and intimal hickening

The present study demonstrates that nifedipine (40 mg/kg/day, p.o.), a dihiydropyridine derivative CCB, did not prevent the collar-induced intimal thickening in rabbits. There is a conflicting data on the effects of different CCBs on the formation of intimal thickening (Dol *et al.*, 1995, Hirata *et al.*, 2000, Yamashita *et al.*, 2001, Kerry *et al.*, 2005). This discrepancy is consistent with our previous findings from the studies on collar-induced intimal thickening with different CCBs during the last decade (Ustunes *et al.*, 1996, Kerry *et al.*, 1999, Kerry *et al.*, 2005). Indeed, nicardipine, a dihydropyridine class of CCB has been shown to inhibit intimal thickening (Kerry *et al.*, 1999), but CD-832 (Kerry *et al.*, 2005) and pranidipine, the other dihydropyridine derivatives were found ineffective (unpublished data). These results

demonstrates the heterogenous effects of CCBs even from the same class on the collar-induced intimal thickening.

In the present study, the failure of nifedipine to inhibit intimal thickening in the collar model may not be resulted from an inadequate dosage since nifedipine produced CCB effect as observed by significantly decreased blood pressure at used dosage (data not shown). On the other hand, ineffectiveness of nifedipine may suggest insufficent treatment period of the drug. This possibility seems to be unlikely due to the effectivenes of nicardipine on intimal thickening, another dihidropyridine derivative CCB, in this model along with the same period of the treatment (Kerry *et al.*, 1999).

In addition, the effects of nifedipine on the development of intimal thickening are changeable in different experimental models. Nifedipine was found to be beneficial in hypercholesterolemic atherosclerosis (Henry *et al.*, 1981) and in balloon catheterization-induced intimal thickening (Hirata *et al.*, 2000), but it was shown to be ineffective in vein graft intimal thickening (Norman *et al.*, 1993).

On the other hand, the mechanisms responsible for the collar-induced intimal thickening in rabbits are still debated. De Meyer *et al.* (1997 b) suggested that obstruction of transmural fluid transport by the collar might cause to retention of toxic metabolites and cytokines in collared arterial wall, and might trigger smooth muscle cell proliferation and migration. Similarly, our recent findings demonstrated that endothelin-1 (ET-1), a strong mitogen, plays a direct role in the formation of collar-induced intimal thickening as indicated by increased ET-1 levels in the collared artery segments (Reel B *et al.*, 2005). Indeed, it was known that ET-1 stimulates relasing of cytokines which provokes smooth muscle cell proliferation and migration (Dashwood *et al.*, 2000). In this respect, the lack of effect of nifedipine to inhibit intimal thickening may be resulted from inefficiency of nifedipine on ET-1 induced mitogenesis. Kawanabe *et al.* (2002) reported that nifedipine has a minor role in ET-1 mediated mitogenesis in rabbit internal carotid artery and vascular smooth muscle cells. Besides, nifedipine was found ineffective to inhibit the production of cytokines such as tumor necrosis factor-α (TNF-α) and interleukines (IL-1, -2), and/or adhesion molecules (Cominacini *et al.*, 1999, Matsumori *et al.*, 2000).

Furthermore, It is also known that specific cytokines, including interleukins-1 and –2, stimulate inducible nitric oxide synthase (iNOS) gene expression (Liu *et al.*, 1998), suggesting the possible involvement of nitric oxide in the development of collar-induced intimal thickening. Indeed, the production of nitric oxide by iNOS was shown in modified smooth muscle cells of the developing intima in the collar model (Arthur *et al.*, 1997). Similarly, Sözmen et al. notified the increased total nitrite/nitrate levels, metabolytes of nitric oxide, in collared arteries (Sözmen *et al.*, 2000). Therefore the ineffectivenes of nifedipine on the formation of intimal thickening in collar model may be atribute to the lack of inhibitory effects on cytokine-stimulated iNOS expression (Cominacini *et al.*, 1999, Matsumori *et al.*, 2000).

In addition, recent studies demonstrated that CCBs increased the expression and activities of gelatinase derivative matrix metalloproteinases (MMP-2 and -9) in vascular diseases such as hypertention and aneurysma (Boyle *et al.*, 1998, Zervoudaki *et al.*, 2004). Similarly, nifedipine have been shown to increase the expression of MMP-2 in cultured rat cardiac fibroblasts (Yue *et.al.*, 2004). It was known that the expression and the activities of MMPs increased during the period of the intimal proliferation (Zalsman *et al.*, 1996). Our previous findings pointed out to enhanced levels of gelatinases in collared arteries (Reel B *et al.*, 2004). Therefore, although, we did not examine the effect of nifedipine on MMP levels in the current study, the ineffectivenes of nifedipine on the intimal thickening in this model, might be resulted from activation of MMPs by the drug. The point of view requires investigation in detail.

Nifedipine and vascular reactivity

In the present study, the collar placement induced vascular reactivity changes as previous findings. Maximum force developments in response to KCl, 5-HT and phenylephrine were decreased in collared arteries consistent with previous results (De Meyer *et al.*, 1994, Ustunes *et al.*, 1996, Kerry *et al.*, 2005, Reel *et al.*, 2005). The possible mechanisms of collar-induced impairment in responses to contractile agents were discussed in some detail in earlier reports (Manderson *et al.*, 1989, De Meyer *et al.*, 1991, Beesley *et al.*, 1992, Kockx *et al.*, 1992). The suggestion that the collar may cause mechanically damage on medial layer would be unlikely, due to existence of no mechanical damage on the medial layer according to histological examinations.

Nifedipine treatment did not significantly affect the contractile responses to KCl and phenylephrine in collared arteries. Earlier studies with nicardipine and CD-832, two dihydropridine derivative calcium channel blockers have also shown to be ineffective on responses to contractile agents in the collar model (Kerry *et al.*, 1999, Kerry *et al.*, 2005). However, 5-HT-induced contractions were diminished by nifedipine treatment. Similarly Pfaffendorf et al demonstrated that nifedipine depressed the maximum effect of 5-HT on the rat isolated coronary small artery preparations without influencing the sensitivity (Pfaffendorf *et al.*, 1993).

Collar caused significantly leftward shift in 5-HT but rightward shift in phenylephrine-induced concentration-response curves. These thypical effects of collaring were discussed earlier (Ustunes *et al.*, 1996, Gerts *et al.*, 1999, Kerry *et al.*, 1999, Reel *et al.*, 2005,). As stated in these studies, it is accepted that 5-HT_{1B} receptor subtype is responsible for the development of hypersensitivity to 5-HT in collared arteries. On the other hand decreased sensitivity to phenylephrine is presumably due to diminished responsiveness of α_1-adrenoreceptors. Nifedipine treatment did not alter these characteristic effects. This result suggests that nifedipine treatment did not affect receptor sensitivity to 5-HT and phenylephrine.

In accordance with the findings of previous studies, maximum relaxation response to acethylcholine mediated by the release of nitric oxide was significantly increased in collared arteries (Kerry *et al.*, 1999, Kerry *et al.*, 2005). This result may suggest the differencences between the initial phenylephrine-induced contractions observed in sham or collared arteries. However, it was demonstrated that increased maximum relaxation response to acethylcholine in collared arteries compared to sham operated arteries can not be attributed to diminished levels of precontractions to phenylephrine in collared arteries in previous studies (De Meyer *et al.*, 1991, Van Put *et al.*, 1995).

With regard to the sensitivity to acethylcholine, the collaring decreased the sensitivity to acethylcholine, consistent with earlier results (De Meyer *et al.*, 1991, De Meyer *et al.*, 1992, Kerry *et al.*, 2005, Reel *et al.*, 2005). In contrast to these observations, sensitivity to acethylcholine was not affected by collaring in some of the studies (Van put *et al.*, 1995 and Kerry *et al.*, 1999). Currently, we are unable to explain this discrepancy in acethylcholine-induced relaxation responses in the collar model.

Nifedipine treatment affected neither maximum contractile response nor the sensitivity to acethylcholine, suggesting that nifedipine does not interact with muscarinic receptors.

Furthermore, on the contrary to most of previous experiments, relaxation responses to nitroglycerine mediated by cGMP were significantly increased in collared arteries (De Meyer *et al.*, 1995, Kerry *et al.*, 1999, Yasa *et al.*, 1999). However, the sensitivity to nitroglycerine did not affected by collaring, in consistent with earlier results (Kerry *et al.*, 1999, Yasa *et al.*, 1999).

Treatment with nifedipine did not alter the sensitivity and relaxation responses to nitroglycerine in collared arteries, but enhanced relaxations to nitroglycerine in sham arteries. This finding suggests that nifedipine does not interact cGMP-mediated responses in collared arteries.
In conclusion, the combined data of previous and present studies demonstrated that nifedipine did not prevent collar-induced intimal thickening and did not affect accompained vascular reactivity changes in this model. This result may suggest the heterogenous effects of CCBs on intimal thickening in the collar model.

Özet

Tavşan karotid arteri çevresine yumuşak ve sıkıştırıcı olmayan silikon bir yakanın yerleştirilmesi intimal kalınlaşmaya yol açar. Bu çalışmada dihidropiridin türevi bir kalsiyum kanal blokörü olan nifedipin ile tedavinin (40 mg/kg/gün, p.o.) yaka modelinde intimal kalınlaşma gelişimine ve ona eşlik eden vasküler reaktivite değişikliklerine olan etkisini araştırılmıştır. Nifedipin tedavisi bu modelde yaka ile oluşan intimal kalınlaşmayı inhibe etmediği gibi, yakanın oluşturduğu vasküler reaktivite değişiklikleri üzerinde de herhangi bir etki oluşturmamıştır.

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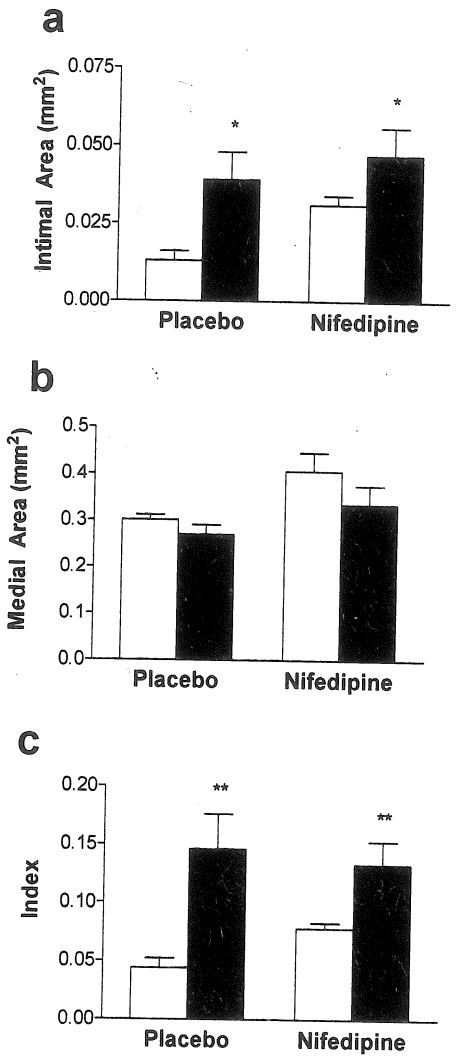

Figure 1: Effect of nifedipine on collar-induced intimal thickening in rabbit carotid artery. Sham-operated arteries (open columns) and collared arteries (black columns) were isolated from rabbits receiving only the vehicle (placebo group, n=6) or 3-week treatment with nifedipine (n=9). Cross-sectional areas of the intima (a), media (b) and intima/media ratio; index (c). Shown are means ± S.E.M. *$P<0.01$, **$P<0.001$ ANOVA (sham-operated vs. collared).

Table 1: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on E_{max} values for 120 mM potassium chloride-induced contractions.

	Placebo *(n=7)*	**Nifedipine** *(n=9)*
KCl, Maximum Contraction, Emax (g)		
Sham	5.91 ± 0.69	1.05 ± 0.11 $^{+++}$
Collared	0.90 ± 0.29 *	0.27 ± 0.06 **
Significance of factors in analysis of variance:		
Collar	*P*<0.001	
Nifedipine	*P*<0.001	
Interaction:		
Nifedipine by Collar	*P*<0.001	

Shown are means±S.E.M. n represents the number of animals in each group. **P*<0.05, ***P*<0.01, sham vs. collared (Wilcoxon signed-rank test). $^{+++}$*P*<0.001, placebo vs. nifedipine (Mann-whitney U-test).

Table 2: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on pD_2 and E_{max} values for 5-HT-induced contractions.

	Placebo *(n=7)*	**Nifedipine** *(n=9)*
5-HT, pD_2		
Sham	6.95 ± 0.08	7.12 ± 0.07
Collared	7.22 ± 0.09 *	7.08 ± 0.09
Significance of factors in analysis of variance:		
Collar	*n.s.*	
Nifedipine	n.s.	
Interaction:		
Nifedipine by Collar	$P < 0.05$	
5-HT, Maximum Contraction, E_{max} (g)		
Sham	5.29 ± 0.46	$3.03 \pm 0.36^{++}$
Collared	1.60 ± 0.27 *	$0.78 \pm 0.17^{**+}$
Significance of factors in analysis of variance:		
Collar	$P < 0.001$	
Nifedipine	$P < 0.001$	
Interaction:		
Nifedipine by Collar	$P < 0.05$	

Shown are means±S.E.M. n represents the number of animals in each group. n.s. Not significant. *P<0.05, **P<0.01, sham vs. collared (Wilcoxon signed-rank test). ^{+}P<0.05, ^{++}P<0.01, placebo vs. nifedipine (Mann-whitney U-test).

Table 3: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on pD_2 and E_{max} values for phenylephrine-induced contractions.

	Placebo *(n=8)*	**Nifedipine** *(n=9)*
Phenylephrine, pD_2		
Sham	6.29 ± 0.07	6.21 ± 0.08
Collared	5.69 ± 0.09	5.88 ± 0.08
Significance of factors in analysis of variance:		
Collar	$P < 0.001$	
Nifedipine	n.s.	
Interaction:		
Nifedipine by Collar	n.s.	
Phenylephrine, Maximum Contraction, E_{max} (g)		
Sham	7.82 ± 0.54	3.98 ± 0.33 $^{+++}$
Collared	2.52 ± 0.50 **	1.71 ± 0.42 **
Significance of factors in analysis of variance:		
Collar	$P < 0.001$	
Nifedipine	$P = 0.001$	
Interaction:		
Nifedipine by Collar	$P < 0.001$	

Shown are means±S.E.M. n represents the number of animals in each group. n.s. Not significant. **$P<0.01$, sham vs. collared (Wilcoxon signed-rank test). $^{+++}P<0.001$, placebo vs. nifedipine (Mann-whitney U-test).

Table 4: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on pD_2 and E_{max} values for acethylcholine-induced relaxations.

	Placebo *(n=7)*	**Nifedipine** *(n=7)*
Acethylcholine, pD_2		
Sham	6.83 ± 0.18	6.72 ± 0.19
Collared	6.41 ± 0.26	6.68 ± 0.14
Significance of factors in analysis of variance:		
Collar	$P < 0.05$	
Nifedipine	n.s.	
Interaction:		
Nifedipine by Collar	n.s.	
Acethylcholine, Maximum Relaxation, E_{max} (%)		
Sham	86.2 ± 3.45	85.4 ± 4.95
Collared	98.0 ± 8.58	107.7 ± 4.79
Significance of factors in analysis of variance:		
Collar	$P < 0.05$	
Nifedipine	n.s.	
Interaction:		
Nifedipine by Collar	n.s.	

Shown are means±S.E.M. n represents the number of animals in each group. n.s. Not significant. $P<0.05$, sham vs. collared (ANOVA).

Table 5: Effects of collar and nifedipine (40 mg/kg/day, p.o.) on pD_2 and E_{max} values for nitroglycerine-induced relaxations.

	Placebo *(n=8)*	**Nifedipine** *(n=6)*
Nitroglycerine, pD_2		
Sham	7.57 ± 0.09	7.69 ± 0.15
Collared	7.36 ± 0.17	7.67 ± 0.13
Significance of factors in analysis of variance:		
Collar	*n.s.*	
Nifedipine	n.s.	
Interaction:		
Nifedipine by Collar	*n.s.*	
Nitroglycerine, Maximum Relaxation, E_{max} (%)		
Sham	77.1 ± 5.24	95.2 ± 2.74 $^{+}$
Collared	106.5 ± 4.07 *	95.8 ± 2.98
Significance of factors in analysis of variance:		
Collar	$P < 0.01$	
Nifedipine	*n.s.*	
Interaction:		
Nifedipine by Collar	$P < 0.01$	

Shown are means±S.E.M. n represents the number of animals in each group. n.s. Not significant. *P<0.05, sham vs. collared (Wilcoxon signed-rank test). ^{+}P<0.05, placebo vs. nifedipine (Mann-whitney U-test).

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