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 thickening, 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 lesions 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).
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 extracellular matrix and calcium, together with monocyte infiltration and smooth muscle proliferation and migration particularly occurring 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**

Acetylcholine chloride, phenylephrine hydrochloride, 5-hydroxytryptamine creatinine sulphate, nitroglycerin solution (1 %), potassium chloride and indomethacin sodium were purchased from Merck, Darmstad, Germany; sodium pentabarbitral 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°C, continuously oxygenated with 95%O2-5%CO2 (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 pD2 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 (Figure 1a, Figure 1c).

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

**Vascular 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 nifedipine (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 pD2 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 $E_{\text{max}}$ 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 significantly decreased $E_{\text{max}}$ 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 ($E_{\text{max}}$ 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 acetylcholine in either sham or collared arteries (Table 4).

The maximal acetylcholine relaxations ($E_{\text{max}}$ 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 $E_{\text{max}}$ values of nitroglycerine (Table 5). Nifedipine treatment also significantly increased $E_{\text{max}}$ 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 dihydropyridine 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 insufficient treatment period of the drug. This possibility seems to be unlikely due to the effectiveness 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 releasing 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, metabolites of nitric oxide, in collared arteries (Sözmen et al., 2000). Therefore the ineffectiveness of nifedipine on the formation of intimal thickening in collar model may be attribute 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 hypertension 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 ineffectiveness 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 dihydropyridine 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 acetylcholine 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 differences between the initial phenylephrine-induced contractions observed in sham or collared arteries. However, it was demonstrated that increased maximum relaxation response to acetylcholine 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 acetylcholine, the collaring decreased the sensitivity to acetylcholine, 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 acetylcholine 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 acetylcholine-induced relaxation responses in the collar model.

Nifedipine treatment affected neither maximum contractile response nor the sensitivity to acetylcholine, 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 accompanied 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 yakın yerleştirilmesi intimal kalınlamaya 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, yakının oluşturduğu vasküler reaktivite değişiklikleri üzerinde de herhangi bir etki oluşturmuştur.

Acknowledgements

This study was supported by Ege University Research Found (Project No:95 ECZ 002). I wish to thank Prof. Levent Üstünes for his kind support and scientific contributions, and Prof. Ashlı Özer for her support, and also Prof. Necmettin Özdemir and Prof. Yıldız Erhan for offered facilities and scientific support in evaluation of transverse sections used in morphometric analysis.
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 $E_{\text{max}}$ values for 120 mM potassium chloride-induced contractions.

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<th>Placebo $(n=7)$</th>
<th>Nifedipine $(n=9)$</th>
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<tr>
<td><strong>KCl, Maximum Contraction, $E_{\text{max}}$ (g)</strong></td>
<td></td>
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<tr>
<td>Sham</td>
<td>5.91 ± 0.69</td>
<td>1.05 ± 0.11 +++</td>
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<tr>
<td>Collared</td>
<td>0.90 ± 0.29 *</td>
<td>0.27 ± 0.06 **</td>
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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 pD₂ and Eₘₐₓ values for 5-HT-induced contractions.

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<tr>
<td><strong>5-HT, pD₂</strong></td>
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<tr>
<td>Sham</td>
<td>6.95 ± 0.08</td>
<td>7.12 ± 0.07</td>
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<tr>
<td>Collared</td>
<td>7.22 ± 0.09 *</td>
<td>7.08 ± 0.09</td>
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<td>Collar</td>
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<tr>
<td>Nifedipine</td>
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<td><strong>Interaction:</strong></td>
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<tr>
<td><em>Nifedipine by Collar</em></td>
<td><em>P &lt; 0.05</em></td>
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5-HT, Maximum Contraction, Eₘₐₓ (g)

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<th>Placebo (n=7)</th>
<th>Nifedipine (n=9)</th>
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<tbody>
<tr>
<td>Sham</td>
<td>5.29 ± 0.46</td>
<td>3.03 ± 0.36 ++</td>
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<tr>
<td>Collared</td>
<td>1.60 ± 0.27 *</td>
<td>0.78 ± 0.17 ** +</td>
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<td><strong>Significance of factors in analysis of variance:</strong></td>
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<tr>
<td>Collar</td>
<td><em>P &lt; 0.001</em></td>
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<td>Nifedipine</td>
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<td><em>Nifedipine by Collar</em></td>
<td><em>P &lt; 0.05</em></td>
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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 pD₂ and Eₘₐₓ values for phenylephrine-induced contractions.

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<td><strong>Phenylephrine, pD₂</strong></td>
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<tr>
<td>Sham</td>
<td>6.29 ± 0.07</td>
<td>6.21 ± 0.08</td>
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<tr>
<td>Collared</td>
<td>5.69 ± 0.09</td>
<td>5.88 ± 0.08</td>
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Significance of factors in analysis of variance:

Collar | P < 0.001
Nifedipine | n.s.

**Interaction:**

Nifedipine by Collar | n.s.

**Phenylephrine, Maximum Contraction, Eₘₐₓ (g)**

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<tr>
<td>Sham</td>
<td>7.82 ± 0.54</td>
<td>3.98 ± 0.33</td>
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<tr>
<td>Collared</td>
<td>2.52 ± 0.50 **</td>
<td>1.71 ± 0.42 **</td>
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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 $E_{\text{max}}$ values for acetylcholine-induced relaxations.

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<td><strong>Acetylcholine, $pD_2$</strong></td>
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<tr>
<td>Sham</td>
<td>6.83 ± 0.18</td>
<td>6.72 ± 0.19</td>
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<tr>
<td>Collared</td>
<td>6.41 ± 0.26</td>
<td>6.68 ± 0.14</td>
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Significance of factors in analysis of variance:

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<tr>
<td>Collar</td>
<td>$P &lt; 0.05$</td>
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<td>Nifedipine</td>
<td>n.s.</td>
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Interaction:

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Nifedipine by Collar</strong></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Acetylcholine, Maximum Relaxation, $E_{\text{max}}$ (%)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Sham</td>
<td>86.2 ± 3.45</td>
</tr>
<tr>
<td>Collared</td>
<td>98.0 ± 8.58</td>
</tr>
</tbody>
</table>

Significance of factors in analysis of variance:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Collar</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Interaction:

<p>| | |</p>
<table>
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<tr>
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</thead>
<tbody>
<tr>
<td><strong>Nifedipine by Collar</strong></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Placebo ($n=8$)</th>
<th>Nifedipine ($n=6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nitroglycerine, pD$_2$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7.57 ± 0.09</td>
<td>7.69 ± 0.15</td>
</tr>
<tr>
<td>Collared</td>
<td>7.36 ± 0.17</td>
<td>7.67 ± 0.13</td>
</tr>
</tbody>
</table>

Significance of factors in analysis of variance:

Collar  
Nifedipine  
Interaction:

*Nifedipine by Collar*  

**Nitroglycerine, Maximum Relaxation, $E_{max}$ (%)**

<table>
<thead>
<tr>
<th></th>
<th>Placebo ($n=8$)</th>
<th>Nifedipine ($n=6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>77.1 ± 5.24</td>
<td>95.2 ± 2.74 *</td>
</tr>
<tr>
<td>Collared</td>
<td>106.5 ± 4.07 *</td>
<td>95.8 ± 2.98</td>
</tr>
</tbody>
</table>

Significance of factors in analysis of variance:

Collar  
Nifedipine  
Interaction:

*Nifedipine by Collar*  

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).
References


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