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Vasoconstrictor responses after neo-intima formation and endothelial removal in the rabbit carotid artery

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1 The present study examined the responses of the rabbit carotid artery to five vasoconstrictors after neo-intima formation induced by perivascular collar treatment and evaluated the role of constitutive and inducible nitric oxide (NO) synthase and endothelial cells (ECs).

2 Ring segments of the rabbit carotid artery were mounted in organ chambers for isometric tension recording. Neo-intima-bearing vessels developed less force (E_{max}) in response to KCl, the thromboxane-mimetic U-46619 and 5-hydroxytryptamine (5-HT), but not to angiotensin I and II.

3 The collar-treatment increased the sensitivity to 5-HT, and decreased the sensitivity to angiotensin II. The sensitivity to U-46619 and angiotensin I remained unchanged.

4 Mechanical removal of ECs and inhibition of NO biosynthesis by N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (L-NOARG) increased the sensitivity to 5-HT in sham and collar-treated segments to the same extent. The effects of collar-treatment and endothelial removal or treatment with inhibitors of NO biosynthesis were additive. Inhibition of NO biosynthesis failed to augment sensitivity to 5-HT after endothelial denudation. L-NOARG increased the force development to KCl in sham and collar-treated segments to the same extent. However, L-NMMA and L-NOARG failed to augment the contractile responses of neo-intima-bearing vessels to 5-HT and KCl after endothelial removal.

5 The responses to angiotensin I were not altered, either by the neo-intima or by endothelial removal. In arteries with a neo-intima the sensitivity to angiotensin II was decreased. Removal of the endothelium or incubation with L-NOARG counteracted this rightward shift and increased E_{max}.

6 Our results demonstrate that contractions to 5-HT, angiotensin II and KCl are modulated by NO in both sham and neo-intima-bearing vessels. Inhibition of NO biosynthesis and collar treatment resulted in additive effects on the EC_{50} values, suggesting that the 5-HT and angiotension (AT) receptors on the smooth muscle cells are also modified by the formation of a neo-intima. Furthermore, the reduced contractile responses of segments with a neo-intima are not due to NO formed by an inducible NO synthase in those vessels.

Keywords: Nitric oxide; inducible nitric oxide synthase (iNOS); endothelium; neo-intima; 5-HT; angiotensin; U-46619; potassium chloride; silicone collar

Introduction

Patients with atherosclerosis are prone to the development of vasospasm (Maseri *et al.*, 1978). In animals, cholesterol feeding produces augmented responses to vasoconstrictor stimuli (Heistad *et al.*, 1984; Verbeuren *et al.*, 1986). However, very little is known about the vascular responsiveness in early atherosclerosis. Recently, a new model of early atherosclerosis has been developed (Booth *et al.*, 1989). By positioning a non-occlusive, soft silicone collar around the rabbit carotid artery, a neo-intima develops within seven days via smooth muscle cell infiltration into the subendothelium (Kockx *et al.*, 1992). A neo-intima can be considered as a site of predilection for atherosclerosis (Wilens, 1951; Stary *et al.*, 1992). Previously, it has been demonstrated that the nitric oxide synthase: guanosine 3':5'-cyclic monophosphate (cyclic GMP) system remains intact in neo-intima-bearing vessels, but that muscarinic endothelium-dependent relaxation becomes selectively impaired (De Meyer *et al.*, 1991; 1992; Arthur & Dusting, 1992).

Furthermore, during the formation of a neo-intima the sensitivity to 5-HT but not to noradrenaline, is increased even without a cholesterol-rich diet (De Meyer *et al.*, 1990). Thus, the presence of a neo-intima can selectively alter contractile responses. On the other hand, several lines of evidence suggest that endothelial cells can modulate the responses to vasoconstricting agents (for review, see Vanhoutte *et al.*, 1986). In view of this, we studied the contribution of the endothelial cells to the altered 5-HT-responses in vessels with a neo-intima. In addition, another platelet-derived constrictor, thromboxane A_2 (via its stable analogue, U-46619) and angiotensin I and II were investigated. A depolarizing potassium chloride solution was used to evaluate non-receptor mediated force development by the various tissues. Furthermore, the hypothesis that reduced contractile responses of segments with a neo-intima might be due to NO formed by an inducible NO synthase in those vessels was also tested.

Methods

Induction of a neo-intima and preparation of segments

Male New Zealand white rabbits (2.5–3.0 kg) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.) and both carotid arteries were exposed surgically. A non-occlusive silicone collar of 2.2 cm length was placed 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 the surrounding connective tissue and the

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vagus nerve and received a similar stretch to the contralateral collared artery. The rabbits were fed a standard laboratory chow. Seven days later they were given heparin (150 u kg^{-1}, i.v.) as an anticoagulant and anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). The sham and collar-treated carotid artery were dissected and immediately placed in a gassed (95% O_2:5% CO_2) physiological salt solution. The vessels were carefully cleaned of loose connective tissue. Subsequently, two rings (3 mm long) were cut from the right (sham-treated) and left (central collar-treated region) carotid artery. One segment was manipulated very carefully in order to preserve the endothelium; the second segment was denuded of endothelial cells by rubbing the tip of a pair of forceps against the intimal or neo-intimal surface. In another series of experiments a collar was positioned around both the left and right carotid artery for 14 days. In this case the segment proximal to the collar was used as sham.

The rings were mounted in organ chambers filled with 25 ml physiological salt solution, maintained at 37°C and continuously gassed with 95% O_2:5% CO_2. Tension was measured isometrically with a Statham UC_2 force transducer. After an equilibration period of 15 min, the segments were gradually stretched and placed at the optimal point of their length tension relationship (7.2 g ± 0.2 g for 26 control segments and 6.9 g ± 0.2 g for 26 collar-treated segments) using 50 mM KCl. The rings were then allowed to equilibrate for 45 min at their optimal length. The bath medium was changed every 15 min during equilibration.

Experimental protocol

Cumulative (0.5 log unit) concentration-response curves were made to 5-HT (10^{-10} to 10^{-5} M), U-46619 (10^{-8} to 10^{-6} M), angiotensin I (10^{-8} to 10^{-5} M) and angiotensin II (10^{-11} to 10^{-6} M). Concentration-response curves to the various agonists were randomized. Maximum contractile force to a single concentration of 120 mM KCl (Tesfamariam *et al.*, 1989) was also determined at the end of each experiment. Between agonists the bath medium was exchanged three times and tissues were allowed to equilibrate for 30 min. The efficacy of the endothelial removal was evaluated in each segment with acetylcholine (Furchgott & Zawadzki, 1980). In a second experiment we examined the effects of N^G-nitro-L-arginine (L-NOARG, 2×10^{-5} M, Moore *et al.*, 1990) on the contractions induced by 5-HT and angiotensin II in endothelium-intact sham-treated and collar-treated arteries. In a third experiment the effect of N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M) on 5-HT-induced contractions in rubbed and unrubbed sham-treated and collar-treated arteries was investigated after 14 days of collar treatment. L-NOARG and L-NMMA were added to the organ chambers 40 min prior to experimentation. To inhibit prostaglandin biosynthesis, indomethacin (3×10^{-6} M) was present in the physiological salt solution in the second and third series of experiments.

Materials

Acetylcholine chloride, angiotensin I (acetate salt, human) and angiotensin II (acetate salt, human) were obtained from Sigma, St. Louis, MO, U.S.A. 5-Hydroxytryptamine creatinine sulphate monohydrate and L-NOARG (N^G-nitroamidino-L-2,5-diaminopentanoic acid) from Janssen Chimica, Beerse, Belgium. U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) and L-NMMA were gifts from Dr S. Moncada, Wellcome Research Laboratories, Beckenham, Kent, UK. Indomethacin sodium was obtained from Merck, Sharp and Dohme, München, Germany. All drugs were made up in distilled water each day before experimentation. Sodium pentobarbitone was obtained from Psyphac, Brussels, Belgium and heparin Leo from Therabel Pharma, Brussels, Belgium. Silicone (Silastic 732, Dow Corning) was kindly provided by CCMP, Antwerp, Belgium. The physiological salt solution contained (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, CaEDTA 0.025 and glucose 11.1. Potassium 50 mM and 120 mM were prepared by equimolar replacement of sodium in the salt solution.

Data analysis

The concentration-response curves were analysed as increase in tension (g) from resting and the maximum response (E_{max}) was determined. The negative logarithm of the concentration of agonist that produced a contraction halfway between the minimal and maximal contraction obtained with that agonist ($-\log EC_{50}$) was calculated for each segment, using linear regression analysis (Tallarida & Murray, 1981). The data were analysed by analysis of variance (ANOVA) (Sokal & Rohlf, 1981) with collar (two levels, present or not) as within rabbit factor and endothelial cells (two levels, with or without), L-NOARG (two levels, with or without) or L-NMMA (two levels, with or without) as within artery factor. Only when the interaction between two factors in ANOVA was statistically significant was the ANOVA test supplemented by an additional statistical analysis (Student's *t* test). The SPSS/PC^+ package (SPSS, Chicago, IL, U.S.A.) was applied for these purposes. A probability of error less than 0.05 was selected as the criterion for statistical significance. All data are given as the mean ± s.e.mean. The number of carotid arteries reported (*n*) equals the number of rabbits used.

Results

Maximum contractile force to KCl (120 mM)

The arteries which had been surrounded by the collar developed significantly less tension (E_{max}) in response to KCl than did the sham-treated vessels. Mechanical removal of the endothelial cells did not influence E_{max} (Table 1).

Influence of collar and ECs on responses to 5-HT

As indicated by the decrease of the EC_{50} values, removal of the endothelial cells from sham-treated arteries marginally enhanced the sensitivity to 5-HT (1.7 fold, Table 2). This effect was also observed after collar-treatment (1.9 fold). However, collar-treatment by itself also induced an increased sensitivity to 5-HT as compared to the sham vessels (4.7 fold and 5.2 fold in arteries with ECs and without ECs respectively). The tendency to a diminished maximum contractile force development in response to 5-HT in collared arteries was statistically not significant either in vessels with ECs or in vessels without ECs (Table 1).

Influence of collar and ECs on responses to U-46619

The sensitivity to U-46619 remained unchanged after collar-treatment or endothelial removal (Table 2). However, the maximum contractile force developed by this agent was slightly (i.e. 1.5 g or 20%) decreased in the segments surrounded by the collar (Table 1).

Influence of collar and ECs on responses to angiotensin I and II

The reactivity (EC_{50} and E_{max} values) in response to angiotensin I was not altered, either by the collar-treatment, or by the removal of the endothelial cells (Tables 1 and 2).

The carotid arteries were about 30 times more sensitive to angiotensin II than to angiotensin I. Analysis of the EC_{50} values showed that the effects of neo-intima and endothelial removal were not additive. Indeed, removal of the endothelial cells increased the potency of angiotensin II but this was only seen in segments with collar-induced neo-intima formation and not in sham-treated segments (Table 2). The sensitivity

Table 1 Maximum contractile force (E_{max}) to 5-hydroxytryptamine (5-HT), U-46619, angiotensin I, angiotensin II and KCl in rings from rabbit carotid arteries without (sham) and with a silicone collar in position for 7 days and influence of the removal of the endothelial cells

E_{max} (g)	*5-HT* ($n = 9$)	*U-46619* ($n = 8$)	*Angiotensin I* ($n = 5$)	*Angiotensin II* ($n = 8$)	*KCl* 120 mM ($n = 9$)
Sham (contralateral)					
+ ECs	6.7 ± 0.5	7.0 ± 0.8	3.9 ± 0.9	2.9 ± 0.4	8.2 ± 0.8
− ECs	6.8 ± 0.5	6.9 ± 0.8	3.5 ± 0.5	2.7 ± 0.5	7.8 ± 0.7
Collar					
+ ECs	6.2 ± 0.6	5.6 ± 0.8	4.0 ± 0.8	2.8 ± 0.4	4.7 ± 0.6
− ECs	5.5 ± 0.7	5.4 ± 0.7	4.3 ± 0.4	4.1 ± 0.6	4.3 ± 0.7
Significance of factors in ANOVA:					
Collar	NS	$P = 0.025$	NS	$P = 0.036$	$P < 0.001$
ECs	NS	NS	NS	NS	NS
Collar by ECs	NS	NS	NS	NS	NS

Values are shown as means ± s.e.mean. n represents the number of animals.
NS: not significant. ECs: endothelial cells.

Table 2 EC_{50} values of 5-hydroxytryptamine (5-HT), U-46619, angiotensin I and angiotensin II in rings from rabbit carotid arteries without (sham) and with a silicone collar in position for 7 days and influence of the removal of the endothelial cells

EC_{50} (− log M)	*5-HT* ($n = 9$)	*U-46619* ($n = 8$)	*Angiotensin I* ($n = 5$)	*Angiotensin II* ($n = 8$)
Sham (contralateral)				
+ ECs	7.10 ± 0.06	7.10 ± 0.08	7.00 ± 0.09	8.44 ± 0.08
− ECs	7.34 ± 0.08	6.91 ± 0.11	6.83 ± 0.06	8.43 ± 0.07
Collar				
+ ECs	7.77 ± 0.12	7.14 ± 0.13	6.62 ± 0.27	8.06 ± 0.12*
− ECs	8.06 ± 0.05	7.19 ± 0.07	6.74 ± 0.07	8.56 ± 0.17
Significance of factors in ANOVA:				
Collar	$P < 0.001$	NS	NS	NS
ECs	$P = 0.002$	NS	NS	$P = 0.012$
Collar by ECs	NS	NS	NS	$P < 0.001$

Values are shown as means ± s.e.mean. n represents the number of animals.
NS: not significant. ECs: endothelial cells. *Significantly different from corresponding sham (Student's two-tailed paired t test).

Table 3 Maximum contractile force (E_{max}) to 5-hydroxytryptamine (5-HT) and angiotensin II in rings from rabbit carotid arteries without (sham) and with a silicone collar in position for 7 days and influence of N^G-nitro-L-arginine (L-NOARG, 2×10^{-5} M)

E_{max} (g)	*5-HT* ($n = 6$)	*Angiotensin II* ($n = 7$)
Sham (contralateral)		
− L-NOARG	8.5 ± 1.2	3.0 ± 1.0
+ L-NOARG	9.5 ± 0.7	4.0 ± 1.1
Collar		
− L-NOARG	3.8 ± 0.6	1.5 ± 0.7
+ L-NOARG	3.9 ± 0.8	3.1 ± 0.8
Significance of factors in ANOVA:		
Collar	$P = 0.013$	NS
L-NOARG	NS	$P = 0.014$
Collar by L-NOARG	NS	NS

Values are shown as means ± s.e.mean. n represents the number of animals. NS: not significant.

Table 4 EC_{50} values of 5-hydroxytryptamine (5-HT) and angiotensin II in rings from rabbit carotid arteries without (sham) and with a silicone collar in position for 7 days and influence of N^G-nitro-L-arginine (L-NOARG, 2×10^{-5} M)

EC_{50} (− log M)	*5-HT* ($n = 6$)	*Angiotensin II* ($n = 7$)
Sham (contralateral)		
− L-NOARG	6.85 ± 0.07	9.01 ± 0.19
+ L-NOARG	7.06 ± 0.10	9.28 ± 0.18
Collar		
− L-NOARG	7.06 ± 0.04	8.40 ± 0.18
+ L-NOARG	7.44 ± 0.08	8.81 ± 0.19
Significance of factors in ANOVA:		
Collar	$P = 0.001$	$P = 0.012$
L-NOARG	$P = 0.026$	$P = 0.007$
Collar by L-NOARG	NS	NS

Values are shown as means ± s.e.mean. n represents the number of animals. NS: not significant.

to angiotensin II decreased in the endothelium intact collar-surrounded segments ($P < 0.05$, Student's t test). Mechanical removal of the endothelial cells of these rings restored the EC_{50} to the sham values (Table 2).

Influence of L-NOARG on the contractions induced by 5-HT, angiotensin II and KCl

In contrast to the first series of experiments, the diminished E_{max} of 5-HT in collar-treated arteries was statistically significant (Table 3). L-NOARG (2×10^{-5} M) decreased the EC_{50} of 5-HT, both in sham and collar-treated segments. The effects of the collar and L-NOARG were additive, as indicated by the absence of interaction (Table 4). The concentration-response curve for angiotensin II shifted to the right in collar-treated segments, confirming the first series of experiments. L-NOARG induced a leftward shift of the angiotensin II curve and enhanced its E_{max} both in control and collar-treated vessels (Table 3). L-NOARG increased the force development to KCl in sham and collar-treated seg-

ments to the same extent. However, L-NOARG failed to augment contractile responses to KCl after endothelial denudation (Table 5).

Influence of L-NMMA on responses to 5-HT in sham and collar-treated arteries with or without ECs

The maximum contractile force developed (E_{max}) in response to 5-HT was significantly increased after L-NMMA treatment both in sham- and collar-treated segments with ECs (Table 6). This increase in E_{max} occurred to the same extent in sham and collar-treated rings. E_{max} was also augmented in the rubbed sham and collar-treated segments after L-NMMA incubation. However, in the rubbed collar-treated segments this was to a smaller extent (Table 6).

The increased sensitivity of the collared segments to 5-HT persisted after L-NMMA incubation (Table 7). L-NMMA incubation increased the sensitivity to 5-HT both in sham and in collar-treated segments to the same extent (1.6 fold). On the other hand, the sensitivity to 5-HT in endothelium denuded sham- and endothelium denuded collar-treated segments was not significantly influenced by L-NMMA.

Table 5 Maximum contractile force (E_{max}) to KCl in rings from rabbit carotid arteries without (sham) or with a silicone collar in position for 14 days and influence of the endothelial cells and N^G-nitro-L-arginine (L-NOARG, 2×10^{-5} M)

E_{max} (g)	*KCl + ECs* ($n = 13$)	*KCl − ECs* ($n = 13$)
Sham (proximal)		
− L-NOARG	6.5 ± 0.6	7.3 ± 0.4
+ L-NOARG	8.3 ± 0.4	7.7 ± 0.5
Collar		
− L-NOARG	4.5 ± 0.6	5.6 ± 0.7
+ L-NOARG	6.1 ± 0.6	6.0 ± 0.6

Significance of factors in ANOVA:

Collar: $P = 0.001$; ECs: NS; L-NOARG: $P = 0.039$; Collar by ECs: NS; Collar by L-NOARG: NS; ECs by L-NOARG: $P = 0.005$; Collar by ECs, by L-NOARG: NS

Values are shown as means ± s.e.mean. n represents the number of animals. NS: not significant. ECs: endothelial cells.

Table 6 Maximum contractile force (E_{max}) to 5-hydroxytryptamine (5-HT) in rings from rabbit carotid arteries without (sham) or with a silicone collar in position for 14 days and influence of the endothelial cells and N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M)

E_{max} (g)	*5-HT + ECs* ($n = 13$)	*5-HT − ECs* ($n = 13$)
Sham (proximal)		
− L-NMMA	6.9 ± 0.5	7.0 ± 0.6
+ L-NMMA	8.0 ± 0.3	8.3 ± 0.5
Collar		
− L-NMMA	5.6 ± 0.6	6.9 ± 0.7
+ L-NMMA	6.6 ± 0.4	7.3 ± 0.7

Significance of factors in ANOVA:

Collar: NS; ECs: NS; L-NMMA: $P = 0.025$; Collar by ECs: NS; Collar by L-NMMA: NS; L-NMMA by ECs: NS; Collar by ECs, by L-NMMA: NS

Values are shown as means ± s.e.mean. n represents the number of animals. NS: not significant. ECs: endothelial cells.

Table 7 EC_{50} values of 5-hydroxytryptamine (5-HT) in rings from rabbit carotid arteries without (sham) or with a silicone collar in position for 14 days and influence of the endothelial cells and N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} M)

EC_{50} (− log M)	*5-HT + ECs* ($n = 13$)	*5-HT − ECs* ($n = 13$)
Sham (proximal)		
− L-NMMA	6.78 ± 0.06	6.89 ± 0.06
+ L-NMMA	6.98 ± 0.06	6.95 ± 0.06
Collar		
− L-NMMA	6.92 ± 0.06	7.09 ± 0.07
+ L-NMMA	7.11 ± 0.11	7.05 ± 0.07

Significance of factors in ANOVA:

Collar: $P = 0.009$; L-NMMA: $P = 0.004$; ECs: NS; Collar by ECs: NS; Collar by L-NMMA: NS; ECs by L-NMMA: $P = 0.016$; Collar by ECs, by L-NMMA: NS

Values are shown as means ± s.e.mean. n represents the number of animals. NS: not significant. ECs: endothelial cells.

Discussion

5-HT and KCl

The present studies confirmed that the collar-induced neo-intima formation is associated with an increased sensitivity to 5-HT (De Meyer *et al.*, 1990). They also indicated that the EC_{50} for 5-HT decreased after mechanical removal of the endothelial cells. Moreover, both effects were additive, as indicated by the absence of interaction (Table 2). L-NOARG and L-NMMA, which block NO biosynthesis (Moore *et al.*, 1990), shifted the 5-HT curve of normal and collared segments with ECs to the left, indicating that NO modulates 5-HT-induced contractions. However, since L-NOARG and L-NMMA decreased the EC_{50} of 5-HT in normal and collar-treated segments to a similar extent, and since the effects of neo-intima formation and L-NOARG or L-NMMA were additive, the increased sensitivity to 5-HT in collar-treated rings cannot be explained by an impaired release of NO. Furthermore, there was an interaction between the presence of ECs and the efficacy of L-NOARG in sham and collared rings. The EC_{50} shift due to L-NOARG was not significant after endothelial removal from normal or collared segments. Therefore, the endothelial cells appear to be the source of NO which modulates the 5-HT responses both in normal and in collared segments. Since indomethacin, a cyclo-oxygenase inhibitor was used throughout the organ chamber experiments with L-NOARG and L-NMMA, it is unlikely that the increased sensitivity to 5-HT after collar treatment is due to an impaired release of prostacyclin or other vasodilator prostaglandins. Hence, these results suggest that the 5-HT receptors on the smooth muscle cells (SMCs) are modified by the collar treatment. Alternatively, the uptake of 5-HT by the endothelium could be compromised, facilitating the access of the amine to the vascular SMCs. This assumption implies a selective alteration of the 5-HT-carrier, since the response to noradrenaline, another amine metabolized by the ECs, was unaltered (De Meyer *et al.*, 1990).

In accordance with the previous experiments, the force development to KCl was always reduced, whereas the reduction of the E_{max} of 5-HT was decreased in the second series of experiments. This discrepancy may be explained by the biological variability in the extent of neo-intima formation. Indeed, previous experiments have shown that the E_{max} of 5-HT decreases significantly as the neo-intima becomes thicker. The cells in the neo-intima are mainly smooth muscle cells, orientated in a longitudinal direction, thus perpen-

dicular to their original position in the media (De Meyer *et al.*, 1991; Kockx *et al.*, 1992). This could further explain the decreased maximum contractile force developed by KCl, U-46619, and 5-HT (in the second experiment). A change of the smooth muscle phenotype from a contractile to a synthetic stage may be another explanation. This phenomenon is observed in smooth muscle cells either adjacent to or within atheromatous plaques (Mosse *et al.*, 1985; Kocher & Gabbiani, 1986). Also during the development of neo-intimal thickening induced by injury with an inflated balloon catheter an altered phenotype is expressed (Manderson *et al.*, 1989a). Under the latter circumstances the maximum contractile force developed by KCl was also decreased (Manderson *et al.*, 1989b).

Joly *et al.* (1992) have shown that balloon denudation leads to expression of an inducible NO synthase. This inducible NO synthase (NOS2) produces NO continuously, in contrast to the calcium-dependent constitutive enzyme (NOS1) in ECs (Moncada *et al.*, 1991). A continuous NO supply could possibly explain the hyporeactivity of the SMCs. Since inflammatory components may be involved in the formation of a neo-intima, we tested the hypothesis that the reduced contractile responses of segments with a neo-intima was due to NO formed by NOS2 in those vessels.

Since after 14 days of collar treatment, L-NMMA as well as L-NOARG increased the force development to 5-HT and KCl in sham- and collar-treated segments to the same extent, the possibility of a continuous release of NO in collar-treated rings seems to be unlikely. This was further confirmed by the observation that L-NMMA and L-NOARG did not augment contractile responses of the neo-intima bearing vessels to 5-HT and KCl after endothelial denudation. Therefore, these results fail to support the assumption that the diminished force development in collared segments was due to the expression of an inducible NOS2 in either media or neo-intima.

Angiotensin

The presence of an intact endothelium may (Oshiro *et al.*, 1985; Bullock *et al.*, 1986; Gruetter *et al.*, 1988) or may not (Saye *et al.*, 1984; D'Orleans-Juste *et al.*, 1985; Gruetter *et al.*, 1988) inhibit vasoconstrictor responses to angiotensin II, depending upon the species and (or) vascular bed from which the vessels are isolated. In the present study endothelial denudation of the control arteries did not alter the reactivity (EC_{50}, E_{max}) to angiotensin II. However, in the arteries with a neo-intima and with an intact endothelium, the sensitivity to this potent vasoconstrictor was decreased. Removal of the endothelial cells of the neo-intima-bearing vessels increased the maximum contractile force and restored the EC_{50} to its value in the sham-treated arteries. This was also indicated by the significant interaction between collar-treatment and endothelial denudation.

Several explanations are conceivable for the decreased sensitivity to angiotensin II. First, an altered sensitivity of the angiotensin II receptors on the smooth muscle cells of neo-intima-bearing vessels. Second, the endothelial cells may produce more vasodilator substances after neo-intima formation. Evidence exists that angiotensin II can stimulate the release of dilator prostaglandins (Desjardins-Giasson *et al.*, 1982) and that angiotensin II-induced endothelium-dependent relaxations of canine renal arteries may be mediated by release of cyclo-oxygenase products from the endothelium (Toda, 1984). Another possible mediator of the decreased sensitivity to angiotensin II is endothelium-derived NO. Third, the endothelial cells of neo-intima-bearing vessels may have an enhanced capacity for degrading angiotensin II.

To elucidate some of these possibilities we performed a second series of experiments and investigated the effects of L-NOARG on the contractions elicited by angiotensin II. L-NOARG evoked a sensitization and slightly enhanced the contractions to angiotensin II. Hence, in the rabbit carotid artery, angiotensin II-induced contractions are modulated by NO. These effects seemed to be more pronounced in neo-intima-bearing vessels. However, the effects of the collar and L-NOARG treatment were additive, suggesting that AT receptors on the SMCs are modified as well by the formation of a neo-intima. The responsiveness to angiotensin I had not been changed by endothelial denudation. Although there was a tendency to a decreased sensitivity to angiotensin I after denudation of ECs, (a finding that would be compatible with angiotensin II formation by angiotensin converting enzyme) this was not statistically significant. The latter is in agreement with Saye *et al.* (1984), who demonstrated that conversion of angiotensin I to angiotensin II in endothelial-disrupted rabbit aortic rings was the same as in intact rings, 5 min after the addition of angiotensin I. As angiotensin I is converted to angiotensin II at the luminal side of the endothelium and then immediately diluted into the organ chamber before it can react with the underlying smooth muscle cells, it was not very likely that significant effects of the endothelial denudation would become apparent in the present experimental set-up. Indeed, neither removal of the endothelial cells, nor the development of a neo-intima affected the responses to angiotensin I.

In conclusion, our results indicate that the presence of a neo-intima diminishes the reactivity to KCl and slightly to U-46619, but not to angiotensin I. NO modulates the contractions to 5-HT and angiotensin II both in sham and neo-intima-bearing vessels. However, the 5-HT and AT receptors on the SMCs seem to be modified as well by the formation of a neo-intima. Furthermore, the reduced contractile responses of segments with a neo-intima are not due to NO formed by an inducible NO synthase in those vessels.

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