ELSEVIER

European Journal of Pharmacology 294 (1995) 753–761

ejp

Dexamethasone influences intimal thickening and vascular reactivity in the rabbit collared carotid artery

Dominica J.M. Van Put, Cor E. Van Hove, Guido R.Y. De Meyer, Floris Wuyts, Arnold G. Herman, Hidde Bult *

Division of Pharmacology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk, Belgium

Received 5 July 1995; revised 25 September 1995; accepted 29 September 1995

Abstract

Intimal thickening predisposes to atherosclerosis and is often associated with alterations of the vascular reactivity of the artery. We investigated whether dexamethasone inhibited the intimal thickening and reactivity changes induced by a silicone collar placed around the left rabbit carotid artery for 2 weeks. The sham-operated, right artery served as control. Dexamethasone (1 mg/kg/day), given in the drinking water ($n = 10$) or by a subcutaneous minipump ($n = 10$), abolished intimal thickening compared to that of both placebo groups ($n = 10$). Both dexamethasone and the collar suppressed the isometric force development of isolated segments elicited by KCl in organ chamber experiments. The collar raised the sensitivity to serotonin (5-hydroxytryptamine, 5-HT) and the maximum force development (E_{max}) after normalization for the KCl responses. Dexamethasone exerted complex effects on 5-HT contractions in sham arteries: the curves often became biphasic, and sensitivity and E_{max} of the first phase were depressed by dexamethasone. In contrast, dexamethasone raised the hypersensitivity of collared arteries to 5-HT even further. Collar and dexamethasone did not influence endothelium-dependent relaxations elicited by acetylcholine or the calcium ionophore A-23187. It is concluded that dexamethasone interfered with neo-intima formation in the collar model, presumably by inhibition of smooth muscle cell migration and/or proliferation, without restoring contractile behaviour. Therefore, the collar-induced alterations in the reactivity of the smooth muscle cells in the media appear to be unrelated to the process of intimal thickening.

Keywords: Intimal thickening; Vascular reactivity; Glucocorticoid; Nitric oxide (NO); Endothelium; Carotid artery, rabbit

1. Introduction

Intimal thickening is an essential prerequisite for the development of atherosclerosis. These early stages are generally associated with changes in vascular reactivity, which may be clinically important, since they predispose the myocardium to ischemic episodes, even if atherosclerotic lesions are angiographically not detectable (Vrints et al., 1992a, b). Hyperreactivity to serotonin (5-hydroxytryptamine, 5-HT) and decreased vasodilator responses to agonists operating via receptors on endothelial cells are most frequently seen, as demonstrated by studies in humans (Vrints et al., 1992b), hypercholesterolemic primates (Heistad et al., 1984) and rabbits (Jayakody et al., 1987; Verbeuren et al., 1986), and after perivascular manipulation of rabbit carotid arteries (De Meyer et al., 1990, 1991; Dusting et al., 1990). The latter model described by Booth et al. (1989) was used in the present study.

Dexamethasone concentration dependently attenuates intimal thickening in several models of atherosclerosis and intimal proliferation (Asia et al., 1993; Colburn et al., 1992; Hagihara et al., 1991; Prescott et al., 1989), but its effects have never been documented in the collar model. Moreover, the literature on the effects of glucocorticoids on vascular reactivity is relatively scant and controversial. Therefore, the aims of our study were first to investigate whether treatment with dexamethasone, a drug known to be active in other models, reduced intimal thickening in the col-

* Corresponding author. Tel.: 00 32 3 820.27.38; fax: 00 32 3 820.25.67.

SSDI 0014-2999(95)00635-4

lared carotid artery of the rabbit and second, whether this suppression influenced the changes in vascular reactivity associated with intimal proliferation.

2. Materials and methods

2.1. Materials

The Krebs-Ringer solution contained: 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 mM. In the depolarizing potassium chloride solution the concentrations of KCl and NaCl were 60.6 mM and 62.4 mM, respectively. Dexamethasone sodium phosphate (USP XXI) was obtained from AKZO Brussels, Belgium; sodium pentobarbital from Sanofi, Libourne, France; heparin Leo from Therabel Pharma, Brussels, Belgium; Alzet osmotic pumps 2ML2 from Charles River France, Cléon, France; sodium indomethacin from Merck, Sharp and Dohme, Brussels, Belgium; serotonin creatinine sulphate monohydrate from Janssen Chimica, Geel, Belgium; A-23187 from Sigma, Deisenhofen, Germany; acetylcholine from Sterop, Brussels, Belgium; phenylephrine chloride from Winthrop, Brussels, Belgium. Silicone (Silastic E RTV Dow Corning) and silicone glue (Silastic 732 RTV Dow Corning) were gifts from Compagnie Commerciale de Matières Premières, Antwerp, Belgium.

Drugs used in the organ chambers were diluted in distilled water. A-23187 was dissolved in dimethyl sulphoxide before dilution. Ascorbic acid 0.01% was added to the serotonin and phenylephrine solutions.

2.2. Study design and surgical procedure

Male New Zealand white rabbits (2.3–3.5 kg, $n = 40$) were randomized into 4 groups: group 1 received tap water (control group for the oral treatment), group 2 dexamethasone 1 mg/kg/day in the drinking water, group 3 received saline via an osmotic minipump (control group for the parenteral administration) and group 4 dexamethasone 1 mg/kg/day, delivered subcutaneously by an osmotic minipump. They were fed on standard laboratory chow throughout the study. After acclimatization for at least one week, they were anaesthetized with sodium pentobarbital (30 mg/kg i.v.) and both carotid arteries were exposed surgically. Around the left carotid artery a non-occlusive biologically inert, soft, flexible, silicone collar (2.2 cm in length) was placed and closed with silicone glue as described (Booth et al., 1989). The contralateral artery was used as a control. It was manipulated identically (sham-operated) but was not enclosed by a collar. Simultaneously, osmotic minipumps were implanted under the dorsal skin, slightly posterior to the scapulae in groups 3 and 4. After 14 days the rabbits were anticoagulated with heparin (150 U/kg i.v.), anaesthetized, and both carotid arteries were dissected and placed in cold Krebs-Ringer solution.

2.3. Administration of dexamethasone

The oral treatments were started 24 h before collar implantation, and simultaneously groups 3 and 4 were given a bolus of either saline (NaCl 0.9%) or dexamethasone (1 mg/kg) intravenously. In addition, all oral and parenteral groups received either a bolus of saline or dexamethasone (1 mg/kg) intravenously 0.5 h before anaesthesia. The osmotic pumps (2 ml) were filled with either saline (group 3) or dexamethasone (25 mg/ml in saline, group 4) according to the Alzet osmotic pumps Technical Information Manual.

From the day before surgery until the end of the experiment the rabbits received 150 ml tap water with (group 2) or without (groups 1, 3, 4) dexamethasone at 8 a.m. Care was taken that it was completely consumed. Thereafter, the rabbits had free access to drinking water until 6 p.m., when all drinking bottles were removed.

2.4. Vascular reactivity

After careful removal of the collar and loose connective tissue, single rings (3 mm length) were cut from the sham-operated and the central region of the collar-treated carotid arteries. The rings were suspended in organ chambers filled with 25 ml Krebs-Ringer solution maintained at 37°C and continuously gassed with 95% O_2-5% CO_2. Tension was measured isometrically with a Statham UC2 force transducer (Gould, Cleveland, OH, USA) connected to a data acquisition system (Moise 3, EMKA technologies, Paris, France). The preparations were gradually stretched to a force of 8 g, which had been determined in previous experiments to bring both collar and sham segments to the optimum length-tension relationship (De Meyer et al., 1990, 1991). The segments were then allowed to equilibrate for 45 min. Subsequently the rings were contracted with a depolarizing potassium chloride solution (60 mM, supra-maximal concentration). After wash out of the KCl solution, the bath solutions always contained 3×10^{-6} M indomethacin to prevent possible interference due to the release of prostanoids, since collaring augments prostacyclin-mediated relaxations (De Meyer et al., 1991). A cumulative concentration-response curve was made for serotonin (10^{-8}–10^{-5} M). Subsequently, cumulative concentration-response curves were made for acetylcholine (3×10^{-9}–3×10^{-6} M) and A-23187 (10^{-9}–10^{-7} M) after contraction with phenylephrine (3.5×10^{-7} M, the EC_{50} concentration). Between concentration-response curves

segments were allowed to equilibrate for 30 min, during which the bath solution was exchanged 3 times. At the end of the experiment the length of each segment was measured.

In a second experiment the relaxations elicited by acetylcholine were studied in segments of non-manipulated carotid arteries from 4 untreated rabbits.

2.5. Histological examination

Two segments, adjacent to those used for vascular reactivity, were cut from each carotid artery and placed in methacarn fixative (methanol 60%; 1,1,1-trichloroethane 30%; glacial acetic acid 10%). The tissues were dehydrated in a graded series of isopropanol (70 to 100%) followed by toluol. After tissues were embedded in paraffin, transverse sections were cut and stained with sirius haematoxylin. The cross-sectional area of intima and media was measured using a digitizing tablet (Sigmascan, Jandel Scientific, Erkrath, Germany) and a microscope with low power magnification. Intimal thickening was calculated as a percentage of the intimal area with respect to the medial area, and expressed as intima/media ratio (%). Immunohistochemical staining of proliferating cell nuclear antigen was carried out as described earlier (Kockx et al., 1992), in order to count the number of proliferating cells. Proliferating cell nuclear antigen-positive nuclei were counted in the entire media of 8 sections at 100 × magnification, and for each artery the average of these 8 values was determined.

2.6. Statistical analysis

The raw data (g contraction) were fitted to a logistic function (Nakashima et al., 1982) using Minuit (James and Roos, 1974) to estimate pD_2 values. The pD_2 is the negative logarithm of the molar concentration (EC_{50}) producing half of the maximal response (E_{max}) of the segment to that agonist. Statistical evaluation was performed by analysis of variance (ANOVA) with treatment (dexamethasone or placebo) and administration route (oral or parenteral) as between-rabbit factors, and collar (present or absent) as within-rabbit factor. If the variances of the samples were unequal, logarithmically transformed values were analysed. If there were interactions between the factors, paired or unpaired Student's t-tests were performed. Non-parametric tests (Mann-Whitney test and Wilcoxon matched-pairs signed-rank test) were used to compare the proliferating cell nuclear antigen counts. Differences were considered to be significant at $P < 0.05$. Results are shown as means ± S.E.M., n refers to the number of rabbits.

3. Results

3.1. Survival and body weight

Three rabbits died shortly after collar implantation, one of which received parenteral placebo (group 3) and two which received parenteral dexamethasone (group 4), and thus data for 37 animals could be analysed. The mean body weight (2.86 ± 0.05 kg, $n = 40$) was not different among the 4 groups at the start of the experiment. In both control groups body weight increased (respectively 135 ± 60 g, $n = 10$ and 175 ± 27 g, $n = 9$) in the 2 weeks after collaring. When dexamethasone was given orally, the growth tended to be smaller (increase 56 ± 27 g, $n = 10$), but the difference was statistically not significant. When dexamethasone was given parenterally, the rabbits lost weight (decrease −278 ± 41 g, $n = 8$) and were significantly different from all other groups.

3.2. Histology

In sham-operated arteries the intima/media ratio was less than 3% (Fig. 1). Because of the intimal thickening, this ratio increased to about 10% in collared segments. The intimal thickening was almost completely inhibited by both dexamethasone treatments and the ratio of collared segments was not different from that of the sham-treated segments

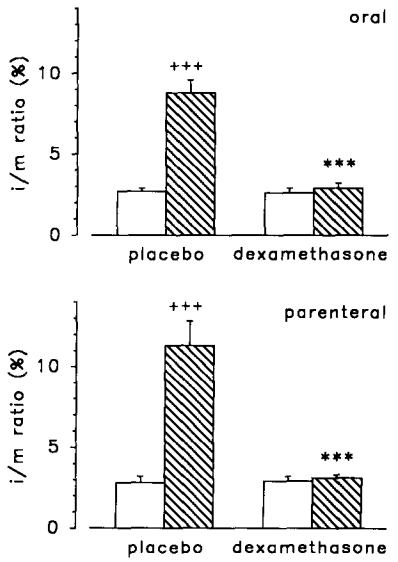

Fig. 1. Effect of dexamethasone on collar-induced intimal thickening. Upper panel: oral drug treatment. Lower panel: parenteral drug treatment. Sham-operated arteries, open columns; collared arteries, hatched columns. Results are means (± S.E.M., vertical bars) of the intima/media (i/m) ratio (%, $n = 8$–10). $^{***}P < 0.001$, different from placebo. $^{+++}P < 0.001$, different from sham.

Table 1
The number of proliferating cell nuclear antigen-positive nuclei in the media: effects of dexamethasone and collar

	Placebo ($n = 19$)	Dexamethasone ($n = 18$)
Sham	3 ± 1	0.4 ± 0.3 ***
Collar	17 ± 4 +++	12 ± 2 +++

Means ± S.E.M. of the total number of proliferating cell nuclear antigen-positive nuclei in the media. For each artery the average count of 8 cross-sections was used. Different from placebo groups: *** $P < 0.001$ (Mann-Whitney U-test); different from sham-operated arteries: +++ $P < 0.001$ (Wilcoxon matched-pairs signed-rank test).

(dexamethasone-sham, placebo-sham). The cross-sectional area of the media was not influenced by the collar or the two dexamethasone treatments (data not shown).

The number of proliferating cell nuclear antigen-positive nuclei in the media of sham operated arteries was low (about 0.3% of the nuclei in a cross-section of the media) and was significantly increased by collaring (about 1.7% of the nuclei in the media, Table 1). Dexamethasone reduced the number of proliferating cell nuclear antigen-positive nuclei in the media of the sham-operated arteries; in collared arteries the decrease was statistically not significant.

3.3. Vascular reactivity

The length of the rings from collared (3.00 ± 0.04 mm, $n = 37$) or sham-operated (3.07 ± 0.03 mm, $n = 37$) arteries was statistically not different, nor were there any differences in length among the 4 treatment groups (data not shown). The administration route was also without a statistically significant effect on the reactivity to constricting and dilating agents. Therefore, the data of oral and parenteral treatments were pooled.

3.3.1. Contractions

Collaring as well as dexamethasone treatment reduced the contraction in response to 60 mM KCl to the same extent, and their effects were additive (Table 2).

In segments of placebo-treated rabbits the serotonin

Table 2
Effect of dexamethasone and collaring on the contractile response (gram) of carotid arteries to 60 mM KCl

	Placebo ($n = 19$)	Dexamethasone ($n = 18$)
Sham	8.0 ± 0.4	6.3 ± 0.5 *
Collar	6.2 ± 0.5 +++	5.1 ± 0.5 +++,*

Values are shown as means ± standard error of the mean. Significance of factors in analysis of variance: * dexamethasone: $F(1,35) = 6.74$, $P = 0.014$; +++ collar $F(1,35) = 15.57$, $P < 0.001$; interaction dexamethasone by collar, $F(1,35) = 0.59$, not significant, $P = 0.448$.

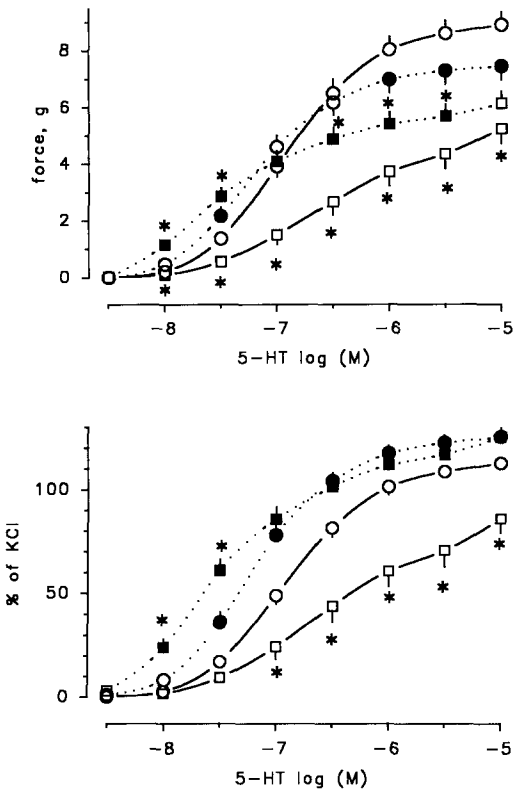

Fig. 2. Contractile responses to serotonin (5-HT) in sham-operated (solid lines) and collared (dotted lines) carotid arteries from placebo- (○) and dexamethasone- (□) treated rabbits. Top panel: raw data; bottom panel: contractions expressed as percentages of the response to 60 mM KCl. Results given as means ± S.E.M. (vertical bars), $n = 18$–19. Filled symbols, the responses of collared arteries were different from those of sham-operated arteries ($P < 0.05$, interaction in ANOVA, followed by paired Student's t-test); * responses of vessels from dexamethasone-treated rabbits different from those from placebo-treated rabbits ($P < 0.05$, unpaired Student's t-test).

concentration-response curves were monophasic (Fig. 2) and attained a maximum (difference between 3×10^{-6} and 10^{-5} M less than 6%). In contrast, only in 10 (out of the 36) dexamethasone-treated segments was the serotonin curve monophasic. In the remaining segments contractions often appeared to attain a plateau between 10^{-6} and 3×10^{-6} M, but force increased further (more than 6% difference between 3×10^{-6} and 10^{-5} M). A (second) maximum was not attained. This tendency for biphasic curves is visible from the mean values (Fig. 2). Both the treatment with dexamethasone and the collar decreased the first E_{max} (at 3×10^{-6} M serotonin), but the effects were clearly not additive and the combination did not lead to a further decrease (Fig. 2, top). After normalization for the responses to KCl, the E_{max} of collared segments was slightly but significantly above the E_{max} of the sham vessels (Fig. 2, bottom). Dexamethasone and collaring had opposite effects on the sensitivity to serotonin: the corticoid decreased, whereas the collar increased the

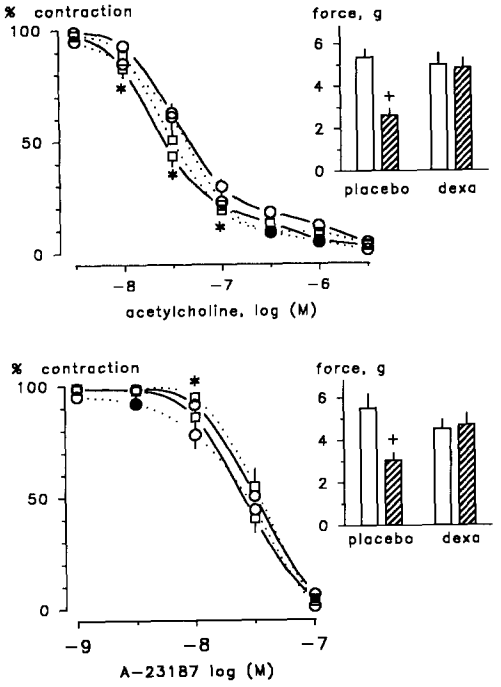

Fig. 3. Relaxations in response to acetylcholine (top panel) and A-23187 (bottom panel) in 0.35 μM phenylephrine contracted sham-operated (solid lines) and collared (dotted lines) carotid arteries from placebo- (○) and dexamethasone- (□, dexa) treated rabbits. Filled symbols, collared arteries different from sham-operated arteries ($P < 0.05$, paired Student's t-test); * responses of arteries from dexamethasone-treated animals different from those of placebo-treated animals ($P < 0.05$, unpaired Student's t-test). Inserts: initial contractions elicited by 3.5×10^{-7} M phenylephrine of sham-operated (open columns) and collared arteries (hatched columns). $^{+}$Different from all other groups ($P < 0.05$, interaction in ANOVA, followed by paired and unpaired Student's t-tests). Results given as means ± S.E.M. (vertical bars), $n = 18$–19.

sensitivity. Moreover, their combination led to a surprising further rise in sensitivity, and the difference in sensitivity between sham and collared segments was almost 10-fold (Table 3, Fig. 2). When the pD_2 analysis was limited to the first contraction phase by neglecting the upper serotonin concentrations (10^{-5}, or 3×10^{-6} and 10^{-5} M), identical conclusions were reached (Table 3), although the pD_2 of 3 sham vessels from dexamethasone-treated rabbits could not be estimated after omission of two concentrations (3×10^{-5} and 10^{-5} M). These simulations proved that the logistic model was sufficiently robust to allow fitting and a reliable estimation of the first pD_2, even when the dose-response curves appear to be incomplete. Therefore, it is concluded that treatment with dexamethasone lowered both the sensitivity and the first E_{max} in sham-operated vessels, whereas it raised sensitivity in collared arteries without influencing E_{max}.

3.3.2. Relaxations

Relaxations were studied after the segments were constricted with 0.35 μM phenylephrine. The initial contraction was not influenced by dexamethasone or the combination of collar and dexamethasone, but was reduced by the collar alone (Fig. 3). Acetylcholine induced concentration-dependent relaxations. Neither E_{max} (Fig. 3) nor pD_2 were influenced by the collar, dexamethasone, or their combination. Although the relaxations of sham segments from dexamethasone-treated rabbits were somewhat more pronounced than those of segments from placebo rabbits (Fig. 3), the tendency to an increased pD_2 for acetylcholine (Table 3) was statistically not significant (ANOVA, dexamethasone $F(1,35) = 3.29$, $P = 0.060$). The calcium ionophore A-23187 induced concentration-dependent relaxations. E_{max} and pD_2 of A-23187 were not influenced by the collar or dexamethasone (Fig. 3, Table 3), but the collar caused a slight desensitization in dexamethasone-treated rabbits.

To rule out that differences among the initial phenylephrine-induced contractions influenced the characteristics of the relaxation response, normal carotid arteries were constricted with 0.1, 0.35 and 1 μM phenylephrine (Fig. 4). This led to a 5-fold difference among the initial contractions, but the acetylcholine-induced relaxations were very similar after normalization (Fig. 4). Indeed, neither the E_{max} (Fig. 4) nor the pD_2 of acetylcholine (7.66 ± 0.16, 7.54 ± 0.20

Table 3
Summary of pD_2 values of serotonin, acetylcholine and A-23187

	Placebo ($n = 19$)		Dexamethasone ($n = 18$)	
	Sham	Collar	Sham	Collar
5-HT				
10^{-9} to 10^{-5} M	6.90 ± 0.05	7.20 ± 0.06 $^{+++}$	6.53 ± 0.11 **	7.46 ± 0.10 $^{+++,*}$
10^{-9} to 3×10^{-6} M	6.91 ± 0.05	7.23 ± 0.06 $^{+++}$	6.60 ± 0.09 **	7.54 ± 0.10 $^{+++,**}$
10^{-9} to 10^{-6} M	6.95 ± 0.05	7.25 ± 0.06 $^{+++}$	6.70 ± 0.10 *,†	7.57 ± 0.10 $^{+++,*}$
Acetylcholine	7.32 ± 0.06	7.35 ± 0.06	7.55 ± 0.05	7.40 ± 0.05
A-23187	7.47 ± 0.05	7.58 ± 0.07	7.63 ± 0.06	7.45 ± 0.04 $^{+}$

Results given as means ± S.E.M.; † $n = 15$. Dexamethasone group different from placebo group: * $P < 0.05$; ** $P < 0.01$; collared arteries different from sham-operated arteries: $^{+}$ $P < 0.05$; $^{+++}$ $P < 0.001$ (interactions in ANOVA, followed by unpaired and paired Student's t-tests).

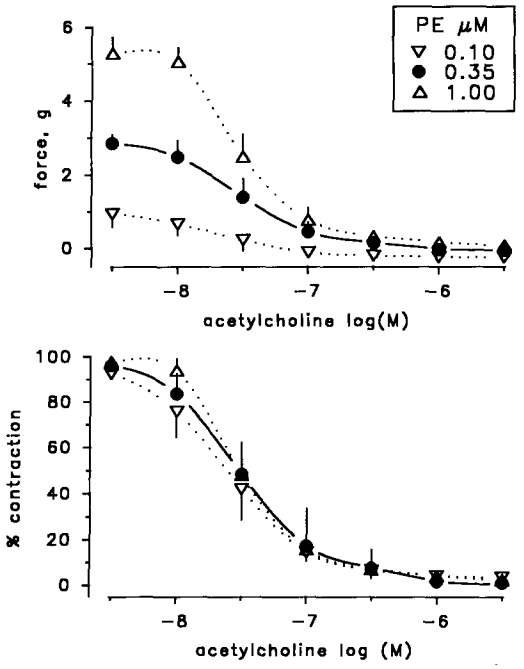

Fig. 4. Relaxations in response to acetylcholine in rabbit carotid arteries contracted with the EC_{50} (0.35 μM, ○), a lower (0.1 μM, ▽) or a higher (1 μM, △) concentration of phenylephrine (PE). Top panel: raw data; bottom panel: contraction as a percentage of the initial contraction. Results given as means ± S.E.M. (vertical bars), $n = 4$.

and 7.51 ± 0.11, at 0.1, 0.3 and 1 μM phenylephrine respectively, $n = 4$) was influenced by the 3 different contraction levels.

4. Discussion

4.1. Inhibition of intimal proliferation

Placing a collar around the rabbit carotid artery elicits a 20-fold stimulation of the proliferation of medial smooth muscle cells within 12 h. The mitotic rate is still elevated on day 14, but less pronouncedly (4-fold increase, Kockx et al., 1992), as confirmed by the present proliferating cell nuclear antigen data. Within 3 days smooth muscle cells begin to migrate to the intima. The newly arrived smooth muscle cells maintain a high proliferation rate in the intima and deposit matrix components (Kockx et al., 1992). The sham operation stimulates smooth muscle cell proliferation in the media as well, presumably as a result of slight medial injury, but the peak is smaller and returns to baseline within 14 days. Furthermore, it is not followed by smooth muscle cell migration and does not lead to intimal thickening (Kockx et al., 1992, 1993).

The intimal thickening resulting from migration, proliferation and matrix deposition was almost abolished by dexamethasone. This proves that the collar model is susceptible to pharmacological modification and confirms the activity of glucocorticoids in models of atherosclerosis and intimal thickening (Asia et al., 1993; Colburn et al., 1992; Hagihara et al., 1991; Prescott et al., 1989). The proliferating cell nuclear antigen data further suggested that dexamethasone suppressed the smooth muscle cell proliferation induced in the sham-operated artery, which is in accordance with in vitro smooth muscle cell culture studies (Berk et al., 1991; Gordon et al., 1987; Voisard et al., 1994). It should be noted, however, that the proliferating cell nuclear antigen data failed to document a statistically significant anti-proliferative effect of the glucocorticoid in the media of collared arteries. This could be due to the late time point, as the average proliferation rate was only 3-fold above the sham level and was rather variable, as it had returned to baseline in several segments.

Since collar-induced smooth muscle cell proliferation and migration are preceded by a transient influx of polymorphonuclear leukocytes from the lumen into the media (Kockx et al., 1992), the inhibition of intimal thickening by dexamethasone could be associated with suppression of leukocyte number and/or activation in the media, as suggested previously for intimal thickening in rat femoral arteries (Prescott et al., 1989). However, others demonstrated that polymorphonuclear leukocytes have no effect on smooth muscle cell migration to the intima in the rabbit carotid artery (Kling et al., 1992). Therefore, repression or stimulation of the transcription of cytokines or other genes with glucocorticoid receptor regulatory elements (Beato, 1989; Gustafsson et al., 1987) could contribute to the inhibition of smooth muscle cell migration and proliferation (Berk et al., 1991; Gordon et al., 1987; Voisard et al., 1994) by dexamethasone. Since dexamethasone abolished intimal thickening, it must have prevented smooth muscle cell migration. However, further studies at earlier time points are required to document whether the glucocorticoid inhibited smooth muscle cell migration directly, or via a reduction of the proliferation of the medial smooth muscle cells.

4.2. Contractile responses

Collaring suppressed force development in response to a supramaximal concentration of KCl, confirming previous results (De Meyer et al., 1994). The reduced force development cannot be explained by NO released by the inducible nitric oxide synthase. Functional studies failed to give indications for the induction of inducible nitric oxide synthase by the collar (De Meyer et al., 1994), as opposed to balloon denudation of rat (Joly et al., 1992) or rabbit (Bosmans et al., 1994) carotid arteries and rabbit atherosclerotic aortas (Verbeuren et al., 1993). Moreover, inducible nitric

oxide synthase expression is suppressed by glucocorticoids (Radomski et al., 1990), whereas dexamethasone failed to reverse the collar effect. The decreased overall contractile capacity could be due to collar-induced injury of the media, leading to the transition of smooth muscle cells from a contractile to a synthetic phenotype (Manderson et al., 1989). Although the cross-sectional area of the media (this study, Kockx et al., 1992) and the number of smooth muscle cell nuclei in the media remain unaltered (Kockx et al., 1992), the media of collared arteries indeed contains fewer smooth muscle cells with a contractile phenotype (Beesley et al., 1992).

Dexamethasone slightly decreased the force development in response to the supra-maximal concentration of KCl in sham-operated arteries, indicating that the glucocorticoid interfered with the overall contractile capacity of medial smooth muscle cells as well. Measurement of the cross-sectional area did not point to a diminished thickness of the media as a possible explanation. The decline was unexpected, as the KCl-elicited responses of naive rabbit carotid arteries were not affected by one week of dexamethasone treatment (Sessa and Nasjletti, 1990; Sessa et al., 1990). The longer duration of the treatment or a delayed replacement of smooth muscle cells injured during the sham operation, as suggested by the smaller number of proliferating cell nuclear antigen-positive nuclei in the media, could possibly explain this discrepancy.

The finding that dexamethasone abolished intimal thickening without restoring K^+ contractions further indicates that the defective contractile behaviour can occur independently of intimal thickening.

In view of the diminished capacity to develop force, it was not surprising that collaring reduced the E_{max} to serotonin (De Meyer et al., 1994). After normalization to KCl, the E_{max} even appeared to be slightly enhanced, confirming the results of others (Dusting et al., 1990). Moreover, collaring enhanced the sensitivity to serotonin. The leftward shift of the serotonin curve is consistently found in this model (De Meyer et al., 1990, 1994; Dusting et al., 1990; Sobey et al., 1991), in models of atherosclerosis (Henry and Yokoyama, 1980; Heistad et al., 1984; Verbeuren et al., 1986; Yokoyama et al., 1983) and in early and advanced coronary atherosclerosis in humans (Vrints et al., 1992a). Different mechanisms have been proposed to explain this hypersensitivity (De Meyer et al., 1990; Dusting et al., 1990), including alterations of the 5-HT receptors on the smooth muscle cells, dysfunction of endothelial 5-HT_1 receptors, resulting in a diminished NO production, or decreased uptake of 5-HT by the endothelium.

Dexamethasone exerted complex effects on the reactivity of sham-operated arteries to serotonin. The responses often became biphasic, and both E_{max} and pD_2 of the first phase decreased profoundly, whereas E_{max} and pD_2 of the second phase could not be estimated. The depression of the first E_{max} did not disappear after normalization for the contractility in response to KCl. Very similar reductions of E_{max} and pD_2 occur with agonists of thromboxane A_2 receptors and are explained by a decrease in receptor number after dexamethasone treatment (Sessa and Nasjletti, 1990; Sessa et al., 1990). In rabbit blood vessels, biphasic contractions in response to serotonin may occur at concentrations above 10^{-5} M, due to activation of postsynsaptic α-adrenoceptors under conditions of either serotonergic receptor blockade or tachyphylaxis (Purdy et al., 1987; Cain and Nicholson, 1989). Therefore it is conceivable that the depression of the first phase unmasked this second, α-adrenoceptor-mediated contractile phase in response to serotonin, particularly since the affinity, number and/or functional coupling of α-adrenoceptors are known to be elevated after glucocorticoid treatment (Chan et al., 1991; Schömig et al., 1976).

Despite the attenuated responses in sham-operated segments, and in spite of the absence of intimal thickening, the collar-induced supersensitivity to serotonin was not normalized, but was even augmented by dexamethasone. This would be compatible with inhibition of inducible nitric oxide synthase expression by the glucocorticoid. However, this explanation is improbable, in view of the lack of functional evidence for inducible nitric oxide synthase expression in collared arteries (De Meyer et al., 1994). The contribution of prostanoids can be ruled out as well, since indomethacin was present in the organ chamber. As the supersensitivity to serotonin precedes intimal thickening (De Meyer et al., 1990; Dusting et al., 1990), Dusting et al. suggested that it resulted from the early migration of inflammatory cells. The finding that dexamethasone inhibits this migration (Yarwood et al., 1993) while further enhancing the sensitivity of collared arteries (the present study) does not lend support to this hypothesis. Although it is unclear whether collaring modifies the subtype, number, affinity and/or functional coupling of serotonin receptors, the results obtained with dexamethasone rather indicate that these changes in vascular reactivity can occur independently of intimal thickening in this model.

As expected from the decreased KCl response, the collar attenuated the contractions evoked by 0.35 μM (EC_{50}) of phenylephrine as well, but in contrast to KCl and 5-HT, these contractions were restored by dexamethasone. The latter observation is in line with the usually observed increased responsiveness to catecholamines (Chan et al., 1991; Davies, 1989; Kalsner, 1969a, b), possibly due to elevation of the number, affinity and/or functional coupling of α_1-adrenoceptors (Chan et al., 1991; Schömig et al., 1976) after glucocorticoid treatment. However, full concentration-response curves are needed to confirm this finding.

4.3. Relaxations

The responses to the calcium ionophore A-23187 were not significantly influenced by the collar, confirming previous results (De Meyer et al., 1991). However, in the current set of experiments no impairment of the acetylcholine-induced relaxations could be detected, in contrast with previous observations reported by us (De Meyer et al., 1991, 1992) and others (Arthur and Dusting, 1992). This discrepancy cannot be attributed to the different initial contractions, as these were smaller in collared segments in previous studies as well (De Meyer et al., 1991), and we showed that even a wider range of initial contractions was without effect on the pD_2 or E_{max} of endothelium-dependent relaxations in untreated rabbit carotid arteries. In accordance with Milner et al. (1988), the endothelium-dependent relaxations were not affected by the prolonged dexamethasone treatment, indicating that neither endothelial muscarinic receptors nor the activity of the constitutive NO synthase is influenced by glucocorticoids.

In conclusion, we demonstrated that dexamethasone abolished collar-induced neo-intima formation. However, this did not prevent the development of changes in vascular reactivity to KCl and serotonin to any extent. This indicates that these collar-induced alterations of the contractile behaviour of the smooth muscle cells in the media are not directly related to the process of intimal thickening.

Acknowledgements

The authors wish to thank F. Jordaens and Ms R. Van den Bossche for their technical assistance, and Ms L. Van den Eynde for typing the manuscript. G.R.Y.D.M. is Senior Research Assistant and D.J.M.V.P. is Research Assistant of the National Fund for Scientific Research, Belgium. This study was supported by the F.G.W.O. (Grant 3.0068.94) and by the Belgian Programme on Interuniversity Poles of Attraction by the Belgian State, Prime Minister's Office, Science Policy Programming.

References

Arthur, J.F. and G.J. Dusting, 1992, Selective endothelial dysfunction in early atheroma-like lesions in the rabbit, Coron. Artery Dis. 3, 623.

Asia, K., C. Funaki, T. Hayashi, K. Yamada, M. Naito, M. Kuzuya, F. Yoshida, N. Yoshimine and F. Kuzuya, 1993, Dexamethasone-induced suppression of aortic atherosclerosis in cholesterol-fed rabbits. Possible mechanisms, Arterioscler. Thromb. 13, 892.

Beato, M., 1989, Gene regulation by steroid hormones, Cell 56, 335.

Beesley, J.E., A.C. Honey and J.F. Martin, 1992, Ultrastructural assessment of lesion development in the collared rabbit carotid artery model, Cells Materials 2, 201.

Berk, B.C., J.B. Gordon and R.W. Alexander, 1991, Pharmacologic roles of heparin and glucocorticoids to prevent restenosis after coronary angioplasty, J. Am. Coll. Cardiol. 17, 111B.

Booth, R.F.G., J.F. Martin, A.C. Honey, D.G. Hassall, J.E. Beesley and S. Moncada, 1989, Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation, Atherosclerosis 76, 257.

Bosmans, J.M., H. Bult, C.J.M. Vrints, M.M. Kockx, M. Claeys, J.P. Snoeck and A.G. Herman, 1994, Balloon angioplasty leads to induction of vascular NO-synthase, in: The Biology of Nitric Oxide. 3. Physiological and clinical aspects, eds. S. Moncada, M. Feelisch, R. Busse and E.A. Higgs (Portland Press, London) p. 34.

Cain, C.R. and C.D. Nicholson, 1989, Comparison of the effects of cromakalim, a potassium conductance enhancer, and nimodipine, a calcium antagonist, on 5-hydroxytryptamine responses in a variety of vascular smooth muscle preparations, Naunyn-Schmied. Arch. Pharmacol. 340, 293.

Chan, M.Y., S. Dai, J.H. He and C.W. Ogle, 1991, In-vivo and in-vitro studies on the effects of chronic dexamethasone treatment on cardiovascular responses to sympathetic stimulation, Arch. Int. Phys. Biochem. Biophys. 99, 323.

Colburn, M.D., W.S. Moore, H.A. Gelabert and W.J. Quinones-Baldrich, 1992, Dose responsive suppression of myointimal hyperplasia by dexamethasone, J. Vasc. Surg. 15, 510.

Davies, A.O., 1989, Steroid hormone-induced regulation of adrenergic receptors, in: Anti-Inflammatory Steroid Action, eds. R.P. Schleimer, H.N. Claman and A. Oronsky (Academic Press, San Diego) p. 97.

De Meyer, G.R.Y., H. Bult, J.F. Martin, A.-E. Van Hoydonck and A.G. Herman, 1990, The effect of a developing neo-intima on serotonergic and adrenergic contractions, Eur. J. Pharmacol. 187, 519.

De Meyer, G.R.Y., H. Bult, A.-E. Van Hoydonck, F.H. Jordaens, N. Buyssens and A.G. Herman, 1991, Neointima formation impairs endothelial muscarinic receptors while enhancing prostacyclin-mediated responses in the rabbit carotid artery, Circ. Res. 68, 1669.

De Meyer, G.R.Y., H. Bult and A.G. Herman, 1992, Selective muscarinic alterations of nitric oxide-mediated relaxations by neointima, J. Cardiovasc. Pharmacol. 20, S205.

De Meyer, G.R.Y., H. Bult, L. Üstünes, M.M. Kockx, F.H. Jordaens, L.L. Zonnekeyn and A.G. Herman, 1994, Vasoconstrictor responses after neo-intima formation and endothelial removal in the rabbit carotid artery, Br. J. Pharmacol. 112, 471.

Dusting, G.J., A. Curcio, P.J. Harris, B. Lima, M. Zambetis and J.F. Martin, 1990, Supersensitivity to vasoconstrictor action of serotonin precedes the development of atheroma-like lesions in the rabbit, J. Cardiovasc. Pharmacol. 16, 667.

Gordon, J.B., B.C. Berk, M.A. Bettman, A.P. Selwyn, H. Renke and R.W. Alexander, 1987, Vascular smooth muscle proliferation following balloon injury is synergistically inhibited by low molecular weight heparin and hydrocortisone, Circulation 76, VI-213.

Gustafsson, J.-A., J. Carlstedt-Duke, L. Poellinger, S. Okret, A.C. Wikstrom, M. Bronnegard, M. Gillner, Y. Dong, K. Fuxe, A. Cintra, A. Harfstrand and L. Agnati, 1987, Biochemistry, molecular biology, and the physiology of the glucocorticoid receptor, Endocr. Rev. 8, 185.

Hagihara, H., A. Nomoto, S. Mutoh, I. Yamaguchi and T. Ono, 1991, Role of inflammatory responses in initiation of atherosclerosis: effects of anti-inflammatory drugs on cuff-induced leukocyte accumulation and intimal thickening of rabbit carotid artery, Atherosclerosis 91, 107.

Heistad, D.D., M.L. Armstrong, M.L. Marcus, D.J. Piegors and A.L. Mark, 1984, Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys, Circ. Res. 54, 711.

Henry, P.D. and M. Yokoyama, 1980, Supersensitivity of atherosclerotic rabbit aorta to ergonovine, J. Clin. Invest. 66, 306.

James, F. and M. Roos, 1974, Minuit: a system for minimizing a function of n parameters and computing the parameter errors and correlations. Cern Computer Centre Program Library, D506-D516, pp. A100(1)-G301, Geneva, Switzerland.

Jayakody, L., M. Senaratne, A. Thomson and T. Kappagoda, 1987, Endothelium-dependent relaxation in experimental atherosclerosis in the rabbit, Circ. Res. 60, 251.

Joly, G.A., V.B. Schini and P.M. Vanhoutte, 1992, Balloon injury and interleukin-1β induce nitric oxide synthase activity in rat carotid arteries, Circ. Res. 71, 331.

Kalsner, S., 1969a, Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta, Circ. Res. 24, 383.

Kalsner, S., 1969b, Steroid potentiation of responses to sympathomimetic amines in aortic strips, Br. J. Pharmacol. 36, 582.

Kling, D., J. Fingerle and M.J. Harlan, 1992, Inhibition of leukocyte extravasation with a monoclonal antibody to CD18 during formation of experimental intimal thickening in rabbit carotid arteries, Arterioscler. Thromb. 12, 997.

Kockx, M.M., G.R.Y. De Meyer, W.A. Jacob, H. Bult and A.G. Herman, 1992, Triphasic sequence of neointimal formation in the cuffed carotid artery of the rabbit, Arterioscler. Thromb. 12, 1447.

Kockx, M.M., G.R.Y. De Meyer, L.J. Andries, H. Bult, W.A. Jacob and A.G. Herman, 1993, The endothelium during cuff-induced neointima formation in the rabbit carotid artery, Arterioscler. Thromb. 13, 1874.

Manderson, J.A., P.R.L. Mosse, J.A. Safstrom, S.B. Young and G.R. Campbell, 1989, Balloon catheter injury to rabbit carotid artery. I. Changes in smooth muscle phenotype, Arterioscler. Thromb. 9, 289.

Milner, P.G., N.R. Izzo Jr., J. Saye, A.L. Loeb, R.A. Johns and M.J. Peach, 1988, Endothelium-dependent relaxation is independent of arachidonic acid release, J. Clin. Invest. 81, 1795.

Nakashima, A., J.A. Angus and C.I. Johnston, 1982, Comparison of angiotensin converting enzyme inhibitors captopril and MK421-diacid in guinea pig atria, Eur. J. Pharmacol. 81, 487.

Prescott, M.F., C. McBride and M. Court, 1989, Development of intimal lesions after leukocyte migration into the vascular wall, Am. J. Pathol. 135, 835.

Purdy, R.E., D.L. Murray and G.L. Stupecky, 1987, Receptors for 5-hydroxytryptamine in rabbit blood vessels: activation of alpha adrenoceptors in rabbit thoracic aorta, J. Pharmacol. Exp. Ther. 240, 535.

Radomski, M.W., R.M. Palmer and S. Moncada, 1990, Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells, Proc. Natl. Acad. Sci. USA 87, 10043.

Schömig, A., B. Lüth, R. Dietz and F. Gross, 1976, Changes in vascular smooth muscle sensitivity to vasoconstrictor agents induced by corticosteroids, adrenalectomy and differing salt intake in rats, Clin. Sci. Mol. Med. 51, 61s.

Sessa, W.C. and A. Nasjletti, 1990, Dexamethasone selectively attenuates prostanoid-induced vasoconstrictor responses in vitro, Circ. Res. 66, 383.

Sessa, W.C., P.V. Halushka, A. Okwu and A. Nasjletti, 1990, Characterization of the vascular thromboxane A_2/prostaglandin endoperoxide receptor in rabbit aorta, Circ. Res. 67, 1562.

Sobey, C.G., G.J. Dusting and O.L. Woodman, 1991, Enhanced vasoconstriction by serotonin in rabbit carotid arteries with atheroma-like lesions in vivo, Clin. Exp. Pharmacol. Physiol. 18, 367.

Verbeuren, T.J., F.H. Jordaens, L.L. Zonnekeyn, C.E. Van Hove, M.C. Coene and A.G. Herman, 1986, Effect of hypercholesterolemia on vascular reactivity in the rabbit. 1. Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits, Circ. Res. 58, 552.

Verbeuren, T.J., E. Bonhomme, M. Laubie and S. Simonet, 1993, Evidence for induction of non-endothelial NO-synthase in aortas of cholesterol-fed rabbits, J. Cardiovasc. Pharmacol. 21, 841.

Voisard, R., U. Seitzer, R. Baur, P.C. Dartsch, H. Osterhues, M. Höher and V. Hombach, 1994, Corticoid agents inhibit proliferation of smooth muscle cells from human atherosclerotic arteries in vitro, Int. J. Cardiol. 43, 257.

Vrints, C.J.M., H. Bult, J. Bosmans, A.G. Herman and J.P. Snoeck, 1992a, Paradoxical vasoconstriction as result of acetylcholine and serotonin in diseased human coronary arteries, Eur. Heart J. 13, 824.

Vrints, C.J.M., H. Bult, E. Hitter, A.G. Herman and J.P. Snoeck, 1992b, Impaired endothelium-dependent cholinergic coronary vasodilation in patients with angina and normal coronary arteriograms, J. Am. Coll. Cardiol. 19, 21.

Yarwood, H., S. Nourshargh, S. Brain and T.J. Williams, 1993, Effect of dexamethasone on neutrophil accumulation and oedema formation in rabbit skin: an investigation of site of action, Br. J. Pharmacol. 108, 959.

Yokoyama, M., H. Akito, T. Mizutani, H. Fukuzaki and Y. Watanabe, 1983, Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hyperlipidemia, Circ. Res. 53, 63.