Involvement of potassium channels in the protective effect of 17β-estradiol on hypercholesterolemic rabbit carotid artery

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Abstract

The involvement of endothelium-derived hyperpolarizing factor (EDHF) in the protective effect of 17β-estradiol was investigated on the phenylephrine-precontracted carotid artery from cholesterol fed rabbits. Animals were fed for 8 weeks as follows: control group, standard chow; (control + estradiol) group, standard chow + 17β-estradiol; standard chow + 1% cholesterol, cholesterol group; or (cholesterol + estradiol) group, 1% cholesterol chow + 17β-estradiol. Relaxations to acetylcholine (ACh) (3 nM–30 μM) were performed with Nω-nitro-L-arginine methyl ester (300 μM) and indomethacin (10 μM). Charybdotoxin (50 nM) or apamin (50 nM), glibenclamide (10 μM) or 4-aminopyridine (1 mM) were used to block, respectively, calcium-activated-K+ channels, adenosine triphosphate (ATP)-sensitive-K+ channels and voltage-dependent K+ channels. In the control group, ACh induced a residual concentration-dependent relaxation. This response was impaired by hypercholesterolemia and restored by 17β-estradiol. In control and cholesterol groups, 4-aminopyridine or glibenclamide did not affect this relaxation, but in (control + estradiol) and (cholesterol + estradiol) groups, glibenclamide suppressed it. In all groups, this persisting relaxation was completely abolished by charybdotoxin alone or with apamin, by hemoglobin (10 μM), a nitric oxide scavenger, or by LY83183 (10 μM), a guanylate cyclase inhibitor. Thus, in the rabbit carotid artery, the protective effect of 17β-estradiol against hypercholesterolemia is probably mediated by a nitric oxide/cyclic GMP pathway which activates calcium-targeted and ATP-dependent K+ channels. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: 17β-Estradiol; Nitric oxide; Hypercholesterolemia; Acetylcholine; Carotid artery; K+ channels

1. Introduction

Several studies, in vitro and in vivo, have reported that endothelium-dependent relaxations are due essentially to release of three vasodilator factors: nitric oxide [1,2], prostacyclin [3] and endothelium-derived hyperpolarizing factor (EDHF) [4–6]. Both nitric oxide and prostacyclin effects have been extensively investigated, while the nature and the site of action of EDHF remain unclear. It was proposed that EDHF induces smooth muscle relaxation by hyperpolarizing the membrane of vascular smooth muscle. The stimulation of this endothelium-dependent hyperpolarization is due to the opening of K+ channels [7,8]. Moreover, the nature of targeted K+ channels is variable, depending upon species, arteries and agonists [5,9,10]. The identity of this factor was also controversial. It may be a product of the cytochrome P450 pathway [11], a cannabinoid [12], K+ [13] or nitric oxide [14–16].

Cardiovascular diseases such as hypercholesterolemia or atherosclerosis are associated with an impairment of endothelium-dependent relaxations [17,18]. It has been reported that this dysfunction is due to an alteration of nitric oxide synthase [19] or an increased degradation of nitric oxide by oxidants [20,21]. The contribution of EDHF under these pathophysiological conditions remains incompletely understood. Some reports have shown a reduction of EDHF-induced relaxations in a model of hypercholesterolemia [22,23].

Impairment of endothelium-dependent vasorelaxation in hypercholesterolemia was prevented by chronic treatment with 17β-estradiol, via either an increase of nitric oxide synthesis on the vessel wall [24], its effect on plasma lipids [25], or the regulation of antioxidant...
enzyme activities [26]. Some reports on a model of rabbit aortic endothelial cells have shown that estrogen enhanced the activity of Ca^{2+}-activated K^+ channels [27]; other studies have demonstrated that acute estrogen administration in the canine coronary circulation induced epicardial coronary vasodilation through opening of adenosine triphosphate (ATP)-sensitive potassium channels and calcium channel antagonism [28]. Thus, the relative contribution of EDHF in this beneficial effect of estrogen has not been clarified.

Our studies are the first to examine the involvement of EDHF in the protective effect of 17β-estradiol in hypercholesterolemic rabbit carotid arteries, and to determine both the nature of EDHF and its target K^+ channels, in these arteries, by using selective blockers.

2. Methods

2.1. Arterial ring preparation

Male New Zealand rabbits (2.5–3 kg) were assigned randomly to four dietary groups for 8 weeks: control group, normal rabbit diet; (control + estradiol) group, normal rabbit diet + 17β-estradiol di-undecylate (intra-muscular (i.m.) injection, 700 μg per week); cholesterol group, standard chow + 1% cholesterol; and (cholesterol + estradiol) group, 1% cholesterol chow + 17β-estradiol di-undecylate (i.m. injection, 700 μg per week). All animals were sacrificed by exsanguination after anesthesia with sodium pentobarbital (30 mg kg^-1 intravenously). The common carotid artery was isolated, placed in ice cold Krebs (NaCl, 118.3 mM; KCl, 4.7 mM; CaCl₂, 2.5 mM; MgSO₄, 1.2 mM; KH₂PO₄, 1.2 mM; glucose, 11.1 mM; and NaHCO₃, 24.9 mM; pH 7.4), cleaned of connective tissue and cut into transverse rings 3 mm long. Special care was taken to avoid damage to the endothelium. Each ring was then suspended vertically in the organ chamber (volume, 25 ml) between two stainless steel hooks, in Krebs solution maintained at 37°C, and gassed with 95% oxygen and 5% carbon dioxide. One of the hooks was fixed to a stand, while the other was attached to an isometric force transducer and the tension recorded on a Gould polygraph model 272. Rings were initially stretched until the resting tension reached 3 g and then allowed to equilibrate for 45 min; during this period, resting tension was continuously monitored and, if necessary, readjusted to 3 g by further stretching.

2.2. Determination of serum cholesterol and triglyceride concentrations

Blood samples were collected in tubes from each animal before excision of the aorta. After centrifugation at 2000 × g for 10 min, the total serum cholesterol and triglyceride concentrations were measured by a standard enzymatic method (cholesterol enzymatic PAP 61225 for cholesterol and 61236 for triglycerides; Bio Merieux, France).

2.3. Experimental protocols

In a first set of experiments, rings were treated for 40 min with the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (300 μM) and the cyclooxygenase inhibitor indomethacin (10 μM). They were then precontracted with phenylephrine (1 μM) and exposed to increasing cumulative concentrations of acetylcholine (3 nM–30 μM). Some carotid artery rings were precontracted with elevated potassium Krebs solution (50 mM), which was made by equimolar replacement of NaCl with KCl. In some experiments, the endothelium was removed by gently rubbing the endothelial surface with a cannula. Removal of the endothelium was confirmed by the absence of a relaxant response to acetylcholine (3 μM).

To determine the nature of targeted K^+ channels, in the presence of L-NAME and indomethacin, either tetraethylammonium (10 mM), charybdotoxin (50 nM) alone or with apamin (50 nM), glibenclamide (10 μM) or 4-aminopyridine (1 mM) were added 40 min before testing the acetylcholine (ACH) effect, to block, respectively, calcium-activated K^+, ATP-sensitive K^+ and voltage-dependent delayed rectifier K^+ channels.

In another set of experiments, in the presence of L-NAME and indomethacin, the rings were preincubated with hemoglobin (10 μM), a nitric oxide scavenger, LY83183 (10 μM), a specific inhibitor of guanylate cyclase or clotrimazole (1 μM) and an inhibitor of cytochrome P450-monoxygenase.

In all experiments, the phenylephrine concentration was adjusted in order to obtain a similar amount of precontraction.

2.4. Statistical analysis

Relaxations were expressed as the percentage of the contraction induced by 1 μM phenylephrine. All results are expressed as mean ± S.E.M. Each concentration–response curve was characterized by determining the pD₂ (the negative log molar concentration of the drug inducing a half-maximal response) and the maximal response (E_max) to drug. The results were analysed using Student’s t-test for paired observations. When more than two groups were compared, statistical significance was assessed by an analysis of variance. If a significant F value was found, Sheffe’s test was used to identify differences among groups. P < 0.05 was considered to be a significant probability.
2.5. Drugs

Phenylephrine, acetylcholine, N\textsuperscript{ω} nitro-l-arginine methyl ester, indomethacin, tetraethylammonium, charybdotoxin, apamin, 4-aminopyridine, hemoglobin, LY83183 and clotrimazole were purchased from Sigma Chemical Co. Glibenclamide was purchased from Research Biochemicals Inc. 17β-Estradiol di-undecylate was purchased from Theramex. Stock solutions of phenylephrine, acetylcholine, L-NAME, charybdotoxin, apamin and 4-aminopyridine were diluted in distilled water. Clotrimazole, LY83183 and indomethacin were dissolved in ethanol. Glibenclamide was diluted in dimethylsulphoxide. The final concentrations of all solvents used (<0.1%) had no effects in preliminary experiments. 17β-Estradiol di-undecylate was emulsioned in vegetable oil.

3. Results

3.1. Serum cholesterol and triglyceride levels

Serum cholesterol and triglyceride levels did not differ between control groups treated or not with 17β-estradiol. Serum total cholesterol levels were markedly elevated in groups fed a high cholesterol diet, whether or not treated with 17β-estradiol. In the (cholesterol + estradiol) group, the serum total cholesterol level was significantly lower than in the cholesterol group. Serum triglyceride levels were also significantly greater in the cholesterol group compared with the control group, but remained practically unchanged in the (cholesterol + estradiol) group (Table 1).

3.2. Effects of acetylcholine

Acetylcholine (3 nM–30 μM) caused a concentration-dependent relaxation in carotid artery precontracted with 1 μM phenylephrine. Neither hypercholesterolemia nor treatment with 17β-estradiol affected significantly this relaxation (Fig. 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (mM)</th>
<th>Triglycerides (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.6 ± 0.3</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>(Control + estradiol)</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>47.9 ± 3.3*</td>
<td>11.3 ± 2.0*</td>
</tr>
<tr>
<td>(Cholesterol + estradiol)</td>
<td>34.9 ± 3.2*</td>
<td>2.6 ± 0.5*</td>
</tr>
</tbody>
</table>

* Each value is the mean ± S.E.M. from eight animals.

* Significantly different versus the control group (P < 0.05).

* Significantly different versus the cholesterol group (P < 0.05).

3.3. L-NAME and indomethacin-insensitive relaxation induced by acetylcholine

In all groups, removal of the endothelium or precontraction of the arteries by elevated external K\textsuperscript{+} (50 mM) completely abolished the L-NAME and indomethacin-insensitive relaxation induced by acetylcholine. Neither L-NAME (300 μM) nor indomethacin (10 μM) modified significantly the level of phenylephrine-induced precontraction.

3.3.1. Control group

In the presence of L-NAME and indomethacin, acetylcholine (3 nM–30 μM) induced a concentration-dependent relaxation of endothelium-intact rings from rabbit carotid arteries. The pD\textsubscript{2} value was 6.34 ± 0.05 and the maximal response (E\textsubscript{m}) reached 30.8 ± 3.5% (n = 8) (Fig. 1).

3.3.2. Control group treated with 17β-estradiol

In the presence of L-NAME and indomethacin, treatment with 17β-estradiol did not modify significantly the acetylcholine response of carotid arteries isolated from the control group. The pD\textsubscript{2} value was 6.74 ± 0.04 (versus 6.34 ± 0.05), and the maximal response (E\textsubscript{m}) was 33.4 ± 2.9% (versus 30.8 ± 3.5%) (n = 8) (Fig. 1).

3.3.3. Cholesterol group

The concentration–response curve to ACh was significantly shifted to the right compared with the control.
group. $E_m$ value was decreased to 12.2 ± 1.7%, while the $pD_2$ value was not significantly modified: 6.36 ± 0.14 (versus 6.34 ± 0.05) ($n = 8$) (Fig. 1).

3.3.4. Cholesterol group treated with 17β-estradiol

The ACh-induced vasorelaxation was similar to the control group: the $pD_2$ value was 6.53 ± 0.06 (versus 6.34 ± 0.05) ($n = 8$) and the $E_m$ was 29.5 ± 3.9% (versus 30.8 ± 3.5%) ($n = 8$) (Fig. 1).

3.4. Effects of tetraethylammonium, glibenclamide, 4-aminopyridine, apamin + charybdotoxin on L-NAME and indomethacin-insensitive relaxation to acetylcholine

3.4.1. Control group

Tetraethylammonium inhibited partially, but significantly, the L-NAME/indomethacin-insensitive relaxation to ACh. The $pD_2$ value was 5.94 ± 0.05 (versus 6.34 ± 0.05) ($n = 8$) and the maximal response was 16.8 ± 4.8% (versus 30.8 ± 3.5%) ($n = 8$). Neither glibenclamide nor 4-aminopyridine affected significantly this residual relaxation to ACh. The $pD_2$ values were, respectively, 6.61 ± 0.04 and 6.30 ± 0.14 (versus 6.34 ± 0.05) ($n = 8$), while the maximal responses were, respectively, 25.9 ± 5.5 and 34.2 ± 6.7% (versus 30.8 ± 3.5%) ($n = 8$). Charybdotoxin, a blocker of large-conductance Ca$^{2+}$-activated potassium channels (BK$_{Ca}$), completely inhibited this response. Addition of apamin, a blocker of small-conductance Ca$^{2+}$-activated potassium channels (SK$_{Ca}$), to charybdotoxin did not significantly modify the response to ACh (Fig. 2).

3.4.2. Control group treated with 17β-estradiol

Like the control group, in the presence of L-NAME and indomethacin, 4-aminopyridine did not modify relaxation to ACh; the $pD_2$ value was 6.46 ± 0.02 (versus 6.34 ± 0.05) ($n = 8$) and the maximal response was 32.1 ± 3.4% (versus 30.8 ± 3.5%) ($n = 8$). Tetraethylammonium reduced the L-NAME/indomethacin-resistant relaxation to ACh; the $pD_2$ value was 6.61 ± 0.04 (versus 6.34 ± 0.05) ($n = 8$) and the maximal response was 22.1 ± 2.2% (versus 30.8 ± 3.5%) ($n = 8$). Charybdotoxin, alone or in the presence of apamin, abolished this relaxation. However, unlike the control group, glibenclamide abolished this relaxation in the control group treated with 17β-estradiol; the $pD_2$ value was 5.94 ± 0.05 (versus 6.34 ± 0.05) ($n = 8$) and the maximal response was 6.2 ± 0.5% (versus 30.8 ± 3.5%) ($n = 8$) (Fig. 3).

3.4.3. Cholesterol group

Neither glibenclamide nor 4-aminopyridine modified significantly the L-NAME/indomethacin-resistant relaxation to ACh. The $pD_2$ values were, respectively, 6.46 ± 0.10 and 6.67 ± 0.07 (versus 6.36 ± 0.14) ($n = 8$). Similarly, the maximal responses were not significantly different: $E_m$ 12.6 ± 0.4 and 11.5 ± 1.4% (versus 12.2 ± 1.7%) ($n = 8$). However, tetraethylammonium reduced partially the L-NAME/indomethacin-resistant relaxation. The $pD_2$ value was 5.90 ± 0.14 (versus 6.36 ± 0.14) ($n = 8$) and the maximal response was 8.1 ± 0.1% (versus 12.2 ± 1.7%) ($n = 8$). Charybdotoxin alone or with apamin completely abolished this relaxation (Fig. 4).

3.4.4. Cholesterol group treated with 17β-estradiol

Pretreatment with 4-aminopyridine did not induce a statistically significant change of the L-NAME/indomethacin-insensitive relaxation to ACh, $pD_2 = 6.14 ± 0.09$ (versus 6.53 ± 0.06%) ($n = 8$). In contrast, both tetraethylammonium, glibenclamide and charybdotoxin alone or with apamin practically abolished this relaxation (Fig. 5).

3.5. Effect of hemoglobin, LY83183 or clotrimazole on L-NAME/indomethacin-insensitive relaxation to acetylcholine

In all groups, in the presence of L-NAME + indomethacin, pretreatment with hemoglobin (10 μM), LY83183 (10 μM) or clotrimazole (1 μM), completely abolished the ACh-induced relaxation (Fig. 6).

Fig. 3. Mean concentration–response curves for acetylcholine in the rabbit carotid artery, precontracted with phenylephrine (1 μM) and isolated from the (control + estradiol) group, in the presence of tetraethylammonium (1 mM), charybdotoxin (50 nM), alone or with apamin (50 nM), 4-aminopyridine (1 mM) or glibenclamide (10 μM). Arteries were preincubated with L-NAME (300 μM) and indomethacin (10 μM). Points are the mean from eight separate experiments, vertical lines show standard error of the mean.

4. Discussion.

In our data, 8-week feeding of rabbits with 1% cholesterol induced an increase of the plasma cholesterol concentrations. This hypercholesterolemia was associated with a liver injury: the livers were pale yellow, had a finely nodular surface and were characterized by a severe fibrosis. It has been clearly established that the hypercholesterolemic diet caused marked alterations in the plasma lipoproteins, particularly an increase of low-density lipoprotein (LDL) and a decrease of high-density lipoprotein. The liver being the main organ of LDL catabolism and having receptors for the persistent excess LDL, this liver injury developed concomitant with arterial injury. In our preliminary studies (data not shown), we observed atheromatous plaques and modifications of vascular reactivity of many rabbit arteries. These plaques were more important in the thoracic aorta than in the abdominal aorta, but were absent in the carotid, middle cerebral and saphenous arteries. Additionally, these arteries presented modifications of responsiveness to vasodilators but not to vasoconstrictors. Indeed, hypercholesterolemia did not modify the response to acetylcholine in the carotid artery, but reduced it by 28% in the saphenous artery, by 30% in the middle cerebral artery, by 33% in the abdominal aorta (not shown) and by 50% in the thoracic aorta [26]. In contrast, vasodilatory responses of all these arteries to an endothelium-independent agonist (sodium nitroprusside) were not altered by high-cholesterol diet for 8 weeks. Contractile responses to phenylephrine (receptor-dependent vasoconstrictor) or high K+ solution (voltage-dependent vasoconstrictor) were also not significantly modified in 8-week dietary cholesterol rabbit arteries compared with control arteries. These results suggest that the severity of effects of hypercholesterolemia depended on the type of the artery.

Furthermore, several data have shown a protective effect of chronic treatment with 17β-estradiol in hypercholesterolemic blood vessels. This effect was attributable to its favorable effects on lipid profile [25] or its direct vascular effects by a regulation of the production and the release of NO [18] or prostacyclin [29]. In contrast, little is known about the effects of estrogen on the production of EDHF. In our data, treatment by 17β-estradiol for 8 weeks attenuated the serum cholesterol level in hypercholesterolemic rabbits (Table 1), confirming the beneficial effect of estrogen on the plasma lipids. The new finding of this study is the observation of an additional mechanism for the protective effect of chronic treatment by 17β-estradiol against hypercholesterolemia. In our experiments, we observed a L-NAME/indomethacin-resistant relaxation to acetylcholine in carotid arteries isolated from control rabbits. This relaxation was suppressed by removal of the endothelium, by elevated external K+ or by addition of charybdotoxin, suggesting the presence of an EDHF-

Fig. 4. Mean concentration–response curves for acetylcholine in the rabbit carotid artery, precontracted with phenylephrine (1 μM) and isolated from the cholesterol group in the presence of tetraethylammonium (1 mM), charybdotoxin (50 nM), alone or with apamin (50 nM), 4-aminopyridine (1 mM) or glibenclamide (10 μM). Arteries were preincubated with L-NAME (300 μM) and indomethacin (10 μM). Points are the mean from eight separate experiments, vertical lines show standard error of the mean.
dependent relaxation induced by acetylcholine on the same artery. Furthermore, in our study, on rabbits fed high cholesterol, this L-NAME/indomethacin-insensitive relaxation to ACh was significantly reduced, suggesting that this response may be impaired by hypercholesterolemia. The work of Urakami-Harasawa et al. [23] supports this hypothesis, and shows that aging and hypercholesterolemia significantly reduced EDHF-mediated relaxation evoked by ACh and bradykinin in large human arteries. Similarly, Cowan and Steffen [22] showed that treatment of the rabbit abdominal aorta with lysophosphatidylcholine, which plays an important role in promoting the atherogenic process, inhibited the relaxation mediated by EDHF. Furthermore, in the rabbit carotid artery, Najibi et al. [31] demonstrated that hypercholesterolemia for 10 weeks did not affect the relaxations to ACh and proved that this normal relaxation occurred, despite the absence of NO-mediated increased cGMP, by activation of charybdotoxin-sensitive potassium channels. Our experiments confirmed these data, since no significant modification of the relaxation responses to ACh was observed in the cholesterol group, compared with the control group (Fig. 1). Nevertheless, in the presence of L-NAME and indomethacin, the residual relaxation to ACh was reduced in hypercholesterolemic arteries. This response was abolished by a NO scavenger such as hemoglobin or an inhibitor of soluble guanylate cyclase such as LY83183 (Fig. 5), suggesting a reduction of the NO–cGMP pathway by hypercholesterolemia. In the

![Diagram](image_url)

Fig. 5. Mean concentration–response curves for acetylcholine in the rabbit carotid artery, precontracted with phenylephrine (1 μM) and isolated from the (cholesterol + estradiol) group, in the presence of tetraethylammonium (1 mM), charybdotoxin (50 nM), alone or with apamin (50 nM), 4-aminopyridine (1 mM) or glibenclamide (10 μM). Arteries were preincubated with L-NAME (300 μM) and indomethacin (10 μM). Points are the mean from eight separate experiments, vertical lines show standard error of the mean.

![Diagram](image_url)

Fig. 6. Effect of hemoglobin (10 μM), LY83183 (10 μM) or clotrimazole (1 μM) on the maximal response to acetylcholine, in the rabbit carotid artery, isolated from control, (control + estradiol), cholesterol and (cholesterol + estradiol) groups. The arteries were treated with L-NAME (300 μM) and indomethacin (10 μM), and precontracted with phenylephrine (1 μM). Points are the mean from eight separate experiments, vertical lines show standard error of the mean.
presence of L-NAME and indomethacin, the treatment by 17β-estradiol during 8 weeks allowed relaxation to ACh, which was, otherwise impaired by the high-cholesterol diet. This vasorelaxation was abolished in the presence of potassium chloride. This is a novel finding that supports the hypothesis that chronic estrogen treatment protects hypercholesterolemic rabbit carotid arteries by an EDHF-mediated mechanism. The estrogen treatment probably increases the release of EDHF from the endothelium of hypercholesterolemic rabbit carotid arteries.

The chemical identity of EDHF remains elusive. In our study, in all groups, hemoglobin, abolished this EDHF-mediated response, suggesting that nitric oxide mediated this remaining relaxation. Previous studies [14,15] reported that NO induced hyperpolarization. In the rabbit isolated carotid artery, Cohen et al. [16] have demonstrated that high concentrations of L-NAME (30 μM) did not fully block endothelium-relaxation and hyperpolarization, and proved that NO, rather than a different factor, was the mediator of ACh-induced endothelium-dependent relaxation and hyperpolarization in this artery. In recent data, in rat superior mesenteric artery, Simonsen et al. [32] confirmed these results, and showed that NO mediated the L-NORG-resistant relaxations to ACh. Thus, in our experiments, it is likely that NO mediated the indomethacin/L-NAME-insensitive relaxation to ACh. In addition, LY83183, a specific inhibitor of guanylate cyclase, completely blocked this residual relaxation to ACh in the presence of NO synthase and cyclooxygenase inhibitors, suggesting that activation of guanylate cyclase may be one mechanism by which NO evoked this residual relaxation to ACh. Therefore, the protective effect of estrogen on hypercholesterolemic carotid artery is mediated by a NO–cGMP pathway. These results agree with several studies [24,33,34], in which estradiol can increase the production of endothelium-derived nitric oxide. However, in all groups, clotrimazole, an inhibitor of cytochrome P450-monoxygenase, abolished the L-NAME/indomethacin-insensitive relaxing response to ACh in the carotid artery. This result could suggest that this relaxation involves a cytochrome P450-derived arachidonic acid metabolite. Some studies [29,35] have proposed that EDHF may be a cytochrome P450 product in rabbit carotid artery. Nevertheless, the site of action of clotrimazole is controversial. Rittenhouse et al. [36] have shown that clotrimazole is a nonspecific cytochrome P450 inhibitor and could be a potent direct inhibitor of calcium-dependent potassium channels. Other authors proved that this compound inhibits not only EDHF-mediated relaxations, but also response to potassium channel openers [37] and endothelium-derived NO [38]. Thus, in our study, clotrimazole probably inhibited the activation of potassium channels by nitric oxide.

Furthermore, to investigate the implication of K+ channels in the protective effect of 17β-estradiol on hypercholesterolemic carotid artery, different K+ channels blockers were used. In the carotid artery isolated from control and hypercholesterolemic rabbits, charybdothoxin alone or with apamin, abolished the L-NAME/indomethacin-resistant relaxation to acetylcholine, while neither 4-aminopyridine nor glibenclamide affected this response. This suggests that only Ca2+-activated K+ channels are involved in the EDHF-mediated relaxation in these groups. These results agree with recent work on the carotid artery isolated from control rabbits [29] and from hypercholesterolemic rabbits [30], which showed that charybdothoxin completely inhibited the EDHF-mediated relaxations to ACh. In control and hypercholesterolemic rabbits treated with 17β-estradiol, charybdothoxin alone or with apamin or glibenclamide suppressed this indomethacin- and L-NAME-resistant endothelium-dependent relaxation to ACh, suggesting that at least two kinds of K+ channels are involved in the effect of 17β-estradiol: Ca2+-dependent and ATP-sensitive potassium channels. Our results are reminiscent of those by Rusko et al. [27], which showed that the acute effect of 17β-estradiol was to markedly enhance the activity of the Ca2+-activated K+ channels on endothelial cells of rabbit aorta. White et al. [39] also found that 17β-estradiol relaxes porcine coronary arteries by opening Kcath channels via cGMP-dependent phosphorylation. In accordance, Node et al. [40] have also demonstrated that 17β-estradiol reduces myocardial infarct size and the incidence of ischemia in the canine heart, by a NO pathway and the opening of Kcath channels. There is, thus, strong evidence to support the activation of Kcath channels by estrogen. Nevertheless, an interesting finding in the present study is that the specific KATP antagonist, glibenclamide, also abolished the relaxation to ACh in the control and cholesterol groups treated with estrogen, suggesting that ATP-sensitive potassium channels may be other candidates involved in the estrogenic action on carotid arteries. In previous work, Sudhir et al. [28] showed that acute estrogen administration on canine epicardial coronary arteries induced a vasodilatory response. This effect was reduced by pretreatment with glibenclamide, suggesting that opening of ATP-sensitive potassium channels may be a mechanism of this vasodilator effect. Additionally, our data indicates that chronic treatment by 17β-estradiol on hypercholesterolemic rabbit carotid arteries activates both the ATP-sensitive and Ca2+-activated potassium channels, since either glibenclamide or charybdothoxin abolish the restored relaxation to ACh.

In conclusion, in the rabbit carotid artery, 8 weeks of high-cholesterol diet impaired the EDHF-mediated relaxation to ACh. Chronic treatment with 17β-estradiol prevented this impairment. This estrogenic protective
effect appears to be mediated by release of nitric oxide, which activates calcium- and ATP-dependent potassium channels via a cyclic GMP pathway. Further studies are required to show whether EDHF is involved in this beneficial effect of 17β-estradiol in other arteries.

Acknowledgements

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References