Dexamethasone Decreases Contraction to Electrical Field Stimulation in Mesenteric Arteries from Spontaneously Hypertensive Rats through Decreases in Thromboxane A₂ Release

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ABSTRACT

Glucocorticoids play a role in the control of vascular smooth muscle tone through the alteration of vasoconstrictor and vasodilator factor production. We studied the effect of dexamethasone on vasoconstriction induced by electrical field stimulation (EFS) in rat mesenteric arteries (MAs) and the role of hypertension in this effect. Endothelium-denuded MAs were obtained from Wistar-Kyoto rats and spontaneously hypertensive rats (SHRs). EFS response was analyzed by isometric tension recordings and cyclooxygenase (COX-1 and COX-2) expression by Western blot. Noradrenaline (NA) release was evaluated in segments incubated with [³H]NA. Dexamethasone (0.1 and 1 μM; 2–8 h) reduced vasoconstriction to EFS (200 mA, 0.3 ms, 1–16 Hz), in a dose- and time-dependent manner only in SHRs. However, the EFS-induced release of [³H]NA was increased in SHR arteries preincubated with dexamethasone (1 μM; 6 h). The thromboxane A₂ (TxA₂) synthase inhibitor fur-
control of vascular smooth muscle tone by acting on both endothelial and vascular smooth muscle cells. In endothelial cells, glucocorticoids suppress the production of vasodilators such as prostacyclin and nitric oxide (Zingarelli et al., 1994; Jun et al., 1999). In vascular smooth muscle cells, glucocorticoids enhance agonist-mediated pharmacomechanical coupling at multiple levels and increase responses to vasoconstrictor agents, like noradrenaline (NA) (Yang and Zhang, 2004). Meanwhile, dexamethasone regulates neuronal CGRP expression and release (Supowit et al., 1995), and we have recently demonstrated that glucocorticoid receptor activation increases the vasodilator response to CGRP in mesenteric arteries (MAs) from SHRs (Balfagón et al., 2004; Márquez-Rodas et al., 2006).

Vascular tone is also regulated by COX-derived prostanooids produced in the vascular wall. Several authors have demonstrated the production of contractile prostanoids in response to vasoconstrictor agents, including α-adrenergic agonists (Taberner et al., 1999; Xavier et al., 2003; Alvarez et al., 2005). These mediators may be synthesized by the constitutive and inducible isoforms of cyclooxygenase (COX-1 and COX-2, respectively). COX-2 is not normally expressed but can be induced in some pathological situations such as hypertension (Alvarez et al., 2005). Some authors have suggested that contractile COX-2 products contribute to the increased vasoconstrictor responses observed in hypertension (Alvarez et al., 2007). Because glucocorticoids inhibit the COX-2 expression in the vascular wall, it seems possible that inhibition of COX-2 derivates would be implicated in the vascular effects of dexamethasone.

The results mentioned above led us to hypothesize that dexamethasone could alter the vasomotor response induced by EFS to different extents and through different mechanisms in normo- and hypertension. Therefore, the goal of this work was to study the effect of dexamethasone on the EFS-induced vasoconstrictor response in MAs from normotensive and hypertensive rats, as well as the role of COX-2 in that dexamethasone effect.

**Materials and Methods**

**Animals and Tissue Preparation.** Six-month-old male Wistar-Kyoto (WKY) rats and SHRs were used. After sacrifice by CO2 inhalation, the superior mesenteric artery was carefully dissected out, cleaned of connective tissue, and placed in Krebs-Henseleit solution (KHS) at 4°C. The investigation conforms to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996).

**Vascular Reactivity.** The method used for isometric tension recording has been described elsewhere (Balfagón et al., 2004). Experiments were performed in endothelium-denuded segments to eliminate the main source of vasoactive substances and so avoid any action by different drugs on the endothelial cells that could lead to misinterpretation of results. The segments were subjected to a ten- sion of 0.5g that was readjusted every 15 min during a 90-min equilibration period. After that, the vessels were exposed to 75 mM KHS at 37°C and continuously gassed with a 95% O2-5% CO2 atmosphere. This allowed the vessels to stabilize before the first frequency-response curve and maintain it until the end of the experiments. The participation of sensory innervation was studied by adding the sensory neurotoxin capsaicin (0.5 μM) to the bath 60 min before the first frequency-response curve and maintaining it until the end of the experiments.

The possible role of adrenergic mechanisms in the effect of dexamethasone on the EFS-induced vasoconstrictor response was studied by evaluating the response to exogenous NA (10 nM–10 μM) and the release of NA (see tritium release experiments below) from adrenergic endings in MAs from SHRs. Concentration-response curves to NA were performed in the presence of dexamethasone (1 μM) for 6 h.

To analyze a possible role of COX derived in vasoconstriction induced by EFS, frequency-response curve to EFS was performed in the presence of the COX-1/2 inhibitor indomethacin. To analyze the role of COX-2-derived TXA2 in vasoconstriction induced by both EFS and exogenous NA, frequency-response curve to EFS and concentration-dependent curves to NA were performed in the presence of the selective COX-2 inhibitor NS-398 (10 μM), the TXA2 synthase inhibitor furegrelate (10 μM), or the TXA2 receptor antagonist SQ 29548 (1 μM). EFS and exogenous NA responses were also performed in the presence of dexamethasone (1 μM) plus NS-398 (10 μM), furegrelate (10 μM), or SQ 29548 (1 μM).

**Tritium Release Experiments.** Denuded mesenteric segments from SHRs were set up in a nylon net and immersed for 30 min in 10 ml of KHS at 37°C and continuously gassed with a 95% O2-5% CO2 mixture (stabilization period). Thereafter, they were incubated for 60 min in 1 ml of bubbled KHS at 37°C containing (−)/[3H]noradrenaline (0.33 μM, 10 μCi/ml, specific activity of 10 Ci/mmol). Afterward, the arteries were transferred to a superfusion chamber with two parallel platinum electrodes, 0.5 cm apart, connected to a stimulator (model S44; Grass Instruments, Quincy, MA) for EFS. The arteries were superfused at a rate of 2 ml/min with oxygenated KHS at 37°C for 100 min, after which time the steady-state level of basal tritium efflux was reached. Two electrical stimulation periods of 60 s (200 mA, 0.3 ms, 4 Hz) were applied to the arteries as described above for vascular reactivity in the absence and in the presence of dexamethasone. The superfusate was collected in vials (10 in all) at 30-s intervals. These vials were collected in the following manner: three before stimulation, to determine the basal level of tritium efflux, two during stimulation, and five after the stimulation; the latter time was enough to recover the basal level of tritium efflux. Afterward, Ready-Protein solution (PerkinElmer, Turku, Finland) was added to the vials, and radioactivity was measured in a scintillation counter (Beckman LS 5000 TD).

The possible role of β-adrenergic receptors in the dexamethasone effect was analyzed in another set of experiments in which β-antagonist propranolol (1 μM) was added to the bath 30 min before the second stimulation period in the presence and absence of dexamethasone. The stimulated tritium release was calculated by subtracting
the basal tritium release ($B_1$ and $B_2$) from that evoked by EFS ($S_1$ and $S_2$). Thereafter, the ratios for net tritium release between $S_i/S_1$ were calculated to eliminate differences between arteries. The effect of different drugs on the evoked tritium release was expressed as their effect on these ratios. The amount of radioactivity released was expressed in disintegrations per minute per milligram wet tissue.

**Western Blot.** Superior mesenteric arteries from control WKY rats and SHRs were incubated 6 h in dexamethasone (1 μM) and then homogenized in a buffer composed of sodium vanadate (1 mM), 1% SDS, and Tris-HCl (0.01 M), pH 7.4. Homogenates containing 20 μg of protein were electrophoretically separated on a 10% SDS-polyacrylamide gel and transferred to a polyvinylidene membrane. The membrane was blocked for 2 h at room temperature in a Tris-buffered saline solution (100 mM, 0.9% NaCl, 0.1% SDS, and 0.01% Tween 20 with 5% nonfat powdered milk before being incubated overnight at 4°C with mouse monoclonal antibody for COX-1 (1:1000; Cayman Chemical, Ann Arbor, MI) and with rabbit polyclonal antibody for COX-2 (1:200; Cayman Chemical). After washing, the membrane was incubated with a 1:1000 dilution of anti-rabbit IgG antibody conjugated to horseradish peroxidase (Amersham International, Little Chalfont, UK). The membrane was thoroughly washed, and the immunocomplexes were detected using an enhanced horseradish peroxidase/luminol chemiluminescence system (ECL plus; Amersham International). The same membrane was used to determine α-actin expression using a monoclonal anti-α-actin antibody (1:3000 dilution; Sigma-Aldrich, Madrid, Spain).

**Measurement of Thromboxane A2 Release.** To measure the release of the TxA2 metabolite, thromboxane B2, we used the TxB2 immunoassay (Cayman Chemical). Endothelium-denuded segments of mesenteric arteries were preincubated for 30 min in 5 ml of KHS containing 20 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 11.1 mM glucose, and 0.03 mM NaN3 EDTA.

**Data Analysis and Statistics.** The responses elicited by EFS or NA were expressed as a percentage of the contraction induced by 75 mM KCl. All results are expressed as mean ± S.E.M. The number (n) of rats is indicated in the figure legends. Statistical analysis was done by comparing the curve obtained in the presence of the different substances with the previous or control curve by means of repeated-measures analysis of variance followed by Bonferroni's test. For Western blot, TxA2 release, and tritium release experiments, statistical analyses were done using Student's t test. Data analysis was carried out with the Prism 3.0 program (GraphPad Software, Inc., San Diego, CA). p Values less than 0.05 were considered significant.

### Results

Five consecutive EFS curves were performed at 2-h intervals in the same segments of MAs from WKY rats, and SHRs induced similar contractions (data not shown). In segments from SHRs, incubation (2–6 h) with dexamethasone (1 μM) reduced the contraction induced by EFS in a time-dependent manner (Fig. 1A), whereas this response remained unmodified in WKY rats (data not shown). The low dexamethasone concentration (0.1 μM) required an 8-h incubation to affect the response to EFS in SHRs (Fig. 1B). Because dexamethasone had no observable effect on the response to EFS in

![Fig. 1](image-url)
propranolol to the bath 30 min before treatment with the drug. The vasoconstrictor response induced by EFS was reduced by indomethacin (10 μM, data not shown), furegrelate (10 μM), NS-398 (10 μM), or SQ 29548 (1 μM) (Fig. 3). No additional effect was observed when these drugs were administered simultaneously with dexamethasone (1 μM, 2–6 h) (Fig. 3).

The vasoconstrictor response to exogenous NA was significantly reduced in segments preincubated for 6 h with 1 μM dexamethasone. Preincubation with indomethacin (data not shown), furegrelate, NS-398, or SQ 29548 also reduced the vasoconstrictor response to NA, and no additional effect was observed when these drugs were simultaneously administered with dexamethasone (Fig. 4, A and B).

**Tritium Release.** Two consecutive EFS in mesenteric segments from SHRs incubated with [3H]NA induced a similar tritium overflow (S1 = 408 ± 69 dpm/mg, S2 = 340 ± 57 dpm/mg; n = 4). The addition of dexamethasone (1 μM) at the end of the first EFS period and maintained until the end of the experiment increased the tritium overflow at 6 h (S1 = 344 ± 45 dpm/mg, S2 = 454 ± 67 dpm/mg; n = 4). The β-antagonist propranolol (1 μM) added to the bath 30 min before S2 did not modify the tritium overflow in the control situation (S1 = 486 ± 33 dpm/mg, S2 = 437 ± 57 dpm/mg; n = 4). In segments pretreated with dexamethasone, adding propranolol to the bath 30 min before S2 abolished the dexamethasone effect (S1 = 476 ± 74 dpm/mg, S2 = 381 ± 57 dpm/mg; n = 4) (Fig. 5).

**Thromboxane A2 Release.** EFS produced an increment in the TxB2 release in endothelium-denuded mesenteric arteries from SHRs, and this increase was inhibited by pretreatment with the α-adrenergic antagonist phentolamine (1 μM) (Fig. 6). In addition, basal and EFS-stimulated TxB2 levels were decreased by a 6-h preincubation with 1 μM dexamethasone (Fig. 6).

**Discussion**

It has been reported that glucocorticoids play an important role in the control of vascular smooth muscle tone by modifying vasoconstrictor responses to different vasoactive agents and by altering vascular prostaglandin and/or nitric oxide production (Ullian, 1999; Yang and Zhang, 2004). However, the involvement of glucocorticoids in the neural vasomotor response has not yet been studied. We and others (Kawasaki et al., 1988; Ralevic et al., 1995; Wimalawansa, 1996; Marín et al., 2000) have previously demonstrated that EFS induces contractile responses in endothelium-denuded mesenteric arteries from WKY rats and SHRs, as the integrated result of the release of different neurotransmitters, mainly vasoconstrictor NA from adrenergic nerve terminals. The first objective of the present study was to analyze whether dexamethasone was able to modify the vasoconstrictor response induced by EFS and whether hypertension also influenced this effect. Since previous reports have shown that actions of dexamethasone are dose- and time-dependent (Laan et al., 1999), two doses (1 and 0.1 mM) of dexamethasone and different incubation periods were studied. These doses were selected because local glucocorticoids formed in the vascular tissue can locally reach a higher concentration than in plasma, playing an autocrine and/or paracrine role within the vascular wall in pathophysiology conditions (Walker, 2007). The dexamethasone had no effect on EFS-induced vasoconstriction in segments from WKY rats at any dose or incubation time. However, in segments from SHRs, it decreased the EFS-induced vasoconstriction in a dose- and time-dependent manner. These results indicate that dexamethasone only modulates the EFS-induced vasmotor response in hypertensive conditions, suggesting that it acts on a specific mechanism that is associated to hypertension.

**COX Expression.** In endothelium-denuded segments from SHRs, 6-h preincubation with dexamethasone (1 μM) did not affect COX-1 protein expression, but it decreased COX-2 protein expression (Fig. 7).

**Fig. 2.** Effect of dexamethasone (Dex) (1 μM, 2–6 h) and Dex (1 μM) + L-NAME (100 μM) (A) and effect of capsaicin (Caps 0.5 μM) and Dex (1 μM) + Caps (0.5 μM) (B) on the frequency-response curve in mesenteric artery segments from SHRs. Results (means ± S.E.M.) are expressed as a percentage of tone induced by 75 mM KCl. n, number of animals = 6. *p < 0.05 versus control.
Therefore, the dexamethasone effect observed in SHRs could be due to increased vasodilator neurotransmitter release or decreased vasoconstrictor neurotransmitter release as well as alterations in vasomotor response. We have previously demonstrated in MAs from SHRs that EFS induces NO release from nitrergic nerves, and this release negatively modulates the vasomotor response in the MA (Marín et al., 2000). Because glucocorticoids are able to increase (Limbourg and Liao, 2003) or decrease NO release in different vessels (Yang and Zhang, 2004), we analyzed the possible participa-

**Fig. 4.** Effect of NS-398 (10 μM), SQ 29548 (1 μM), and furegrelate (Fure; 10 μM) on the frequency-response curve in mesenteric artery segments from SHRs untreated (A) or treated (B) with Dex. Results (means ± S.E.M.) are expressed as a percentage of tone induced by 75 mM KCl. n, number of animals used is indicated in figure; *, p < 0.05 versus control.

**Fig. 3.** Effect of NS-398 (10 μM), SQ 29548 (1 μM), and furegrelate (Fure; 10 μM) on the frequency-response curve in mesenteric artery segments from SHRs either untreated (A, C, and D) or treated (1 μM, 6 h) (B, D, and F) with Dex. Results (means ± S.E.M.) are expressed as a percentage of tone induced by 75 mM KCl. n, number of animals used is indicated in figure; *, p < 0.05 versus control.
...oratory effect on EFS-induced vasoconstriction, suggesting the presence of L-NAME, dexamethasone maintained the inhibition of this innervation in the effect of dexamethasone. However, although dexamethasone increases NA release, the effect of dexamethasone, excluding the participation of sensory innervation in the dexamethasone effect.

Once we had discounted the participation of nitricergic and sensory innervation, we analyzed possible changes in adrenergic innervation induced by dexamethasone. Because NA is the main adrenergic neurotransmitter to be released by EFS in rat MA, the effect of dexamethasone in SHRs could be due to a decrease in NA release and/or alteration in the sensitivity of vascular smooth muscle cells to NA. It is known that during electrical field stimulation, ATP and neuropeptide Y are coreleased with NA, which in rat mesenteric arteries induces vasoconstriction (Donoso et al., 1997), so it is still possible that a slight participation of these neurotransmitters in the EFS-induced vasomotor response could explain the effect of dexamethasone. However, in the frequency range used in the current work, the direct participation of these neurotransmitters is very scarce since the vasoconstrictor response to EFS was practically abolished in the presence of the α-adrenoceptor antagonist phentolamine (Marín et al., 2000). Taking these earlier observations into account, we focused our investigation on NA release and the vasoconstrictor response to exogenous NA. The results obtained in mesenteric arteries from SHRs indicate that dexamethasone had two opposite effects on the vascular adrenergic mechanism; it increased NA release but decreased the vasoconstrictor response to NA. The increase in NA release contrasts with earlier reports that glucocorticoids did not alter sympathetic activity (Sudhir et al., 1989; Whitworth et al., 1995). However, despite the many studies demonstrating the involvement of several complex mechanisms in the enhancement of catecholamine-stimulated vascular contractions by glucocorticoids, other studies have and still others have, like us, found a reduction in vasoconstriction (Ullian, 1999; Yang and Zhang, 2004).

Presynaptic β₂-adrenoceptor modulates noradrenaline release from adrenergic endings by increasing NA release. In addition, it has been demonstrated that dexamethasone upregulates the number and function of adrenoceptors (Lenders et al., 1995; Kalavantavanich and Schramm, 2000). The results presented here show that the β-antagonist propranolol abolished the effect of dexamethasone on NA release. This result led us to speculate that dexamethasone increased NA release by increasing presynaptic β₂-adrenoceptor function. However, although dexamethasone increases NA release, this effect cannot compensate for the decreased vasoconstrictor response to NA in mesenteric arteries from SHRs. Our results also suggest that other neuronal-independent mechanisms, probably associated with the NA vasoconstrictor response, are involved in the effects of dexamethasone in the EFS-induced vasoconstriction.

Vasoconstrictor response to α-adrenergic agonist is mediated in part by the release of COX derivatives (Tabernero et al., 1999; Xavier et al., 2003; Alvarez et al., 2005). Recently, we have reported that vasoconstrictor response to phentolamine...
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In summary, the results presented here demonstrate that dexamethasone decreased the vasoconstrictor response to EFS in mesenteric arteries from SHRs but not from WKY rats. In SHRs, dexamethasone increased NA release, and this response is mediated by an increased function of β-presynaptic adrenoceptors. Dexamethasone reduces vasoconstrictor response to NA and EFS in MAs from SHRs by decreasing COX-2 expression and consequently TxA2 release in response to α-adrenergic activation. The undetectable COX-2 expression in MAs from normotensive animals explains the non-effect of dexamethasone in these animals. In summary, in MAs from SHRs, a part of increased β-adrenoceptor-mediated NA release, dexamethasone decreases the vasoconstrictor response to EFS by an inhibition of COX-2 expression that decreases smooth muscle-derived TxA2 release by α-adrenoceptor activation.

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References


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