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Different effects of acute clenbuterol on vasomotor response in mesenteric arteries from young and old spontaneously hypertensive rats

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Abstract

We analysed the influence of aging on the acute effect of clenbuterol, a β_2-adrenoceptor agonist, on the vasoconstrictor response induced by electrical field stimulation in mesenteric arteries from young and old spontaneously hypertensive rats (SHRs). Clenbuterol increased the contraction elicited by electrical field stimulation in arteries from both groups, and this was prevented by propranolol. N^G-nitro-L-arginine methyl ester (L-NAME) also increased the electrical field stimulation-elicited contractions in arteries from both age groups. However, pretreatment with capsaicin increased the electrical field stimulation-induced contractions in young SHRs, but did not modify it in old SHRs. In segments from young SHRs, the treatment with the calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP-(8–37), induced an increase in the electrical field stimulation-induced vasoconstrictor response that was not modified by the subsequent addition of capsaicin. Addition of clenbuterol to L-NAME-treated segments from both groups further increased the response to electrical field stimulation. In segments from young SHRs, clenbuterol failed to increase the electrical field stimulation-induced response in the capsaicin-treated segments, but the response was increased by the subsequent addition of L-NAME. The addition of L-NAME to the clenbuterol-treated segments from old SHRs did not modify the enhanced electrical field stimulation response. Electrical field stimulation induced a similar tritium release in arteries from young and old SHRs preincubated with [^{3}H]noradrenaline. In arteries from young SHRs, isoproterenol increased this release and the increase was abolished by propranolol. Clenbuterol increased the stimulated tritium overflow and exogenous noradrenaline response only in segments from old SHRs, and both effects were abolished by propranolol. To summarize and conclude, clenbuterol increased the electrical field stimulation-induced contraction in segments from both age groups. In young SHRs, clenbuterol seems to inhibit CGRP release, while in old SHRs, it increases the release of and response to noradrenaline and decreases neuronal nitric oxide (NO) release.

Keywords: Aging; Clenbuterol; β-Adrenoceptor; Hypertension; Nitrergic innervation; Adrenergic innervation; Mesenteric artery, rat

1. Introduction

Vascular tone is regulated by several mechanisms. Depending on the type of vessel, innervation plays a more or less important role (Vanhoutte et al., 1981; Marco et al., 1985; Kawasaki et al., 1988). The perivascular sympathetic innervation of rat mesenteric arteries (Li and Duckles, 1992) releases noradrenaline when electrically stimulated. The released noradrenaline then either induces a contractile response via α-adrenoceptor activation (Marín and Balfagón, 1998; Ferrer et al., 2000; Ferrer and Balfagón, 2001), or activates β-adrenoceptors and relaxes the blood vessels (Vanhoutte et al., 1981). Nitric oxide (NO) relaxes smooth muscle cells (Holzmann, 1982; Ignarro and Kadowitz, 1985) and we have demonstrated that electrical field stimulation also induces NO release from nitrergic nerves in rat mesenteric arteries (Marín and Balfagón, 1998; Ferrer et al., 2000). Sensory innervation involvement has also been reported for the vasomotor response to electrical field stimulation in rat mesenteric artery (Li and Duckles, 1993); this sensory response depends on the essential neurotransmitter, calcitonin gene-related peptide (CGRP) (Kawasaki et al., 1988; Li and Duckles, 1993), which induces relaxation in smooth muscle cells (Wimalawansa, 1996).

β-Adrenoceptors are known to exist in several types of vascular wall cells. Presynaptic β-adrenoceptors and their role in facilitating noradrenaline release in adrenergic nerve endings have been demonstrated in some vessels (Misu and Kubo, 1986; Nedergaard and Abrahmsen, 1990; Encabo et al., 1996). Positive modulation of neuronal NO release by β-

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adrenoceptors has been demonstrated in several rat blood vessels (Moreno et al., 1996; Marín and Balfagón, 1998). Blockade of β-adrenoceptors in rat heart has been shown to increase CGRP release (Chen et al., 1996; Yeh et al., 1998). Postsynaptic β-adrenoceptors induce a smooth muscle vasodilator response (Vanhoutte et al., 1981; Marín and Balfagón, 1998), as well as endothelial NO release (Arribas et al., 1994).

Clenbuterol is a selective β_2-adrenoceptor agonist used to treat asthma as well as to increase muscle mass (Choo et al., 1992). We have demonstrated that, in mesenteric arteries from normotensive rats, clenbuterol does not induce vasodilation and does not modify the noradrenaline release induced by electrical field stimulation. In addition, in mesenteric arteries from Wistar rats, clenbuterol increased the neuronal NO release induced by electrical field stimulation (Marín and Balfagón, 1998), but did not affect electrical field stimulation-induced NO release in Sprague–Dawley rats (Ferrer and Balfagón, 2001). Studies performed in mesenteric arteries from aged Sprague–Dawley rats showed that clenbuterol did not induce vasodilation, although it did increase the release of noradrenaline from adrenergic endings and of NO from nitrergic endings in mesenteric arteries (Ferrer and Balfagón, 2001). To our knowledge, there are no studies analyzing the effect of clenbuterol on sensory innervation in this artery.

Taken together, these earlier results suggest that the activation of the β-adrenoceptor by clenbuterol could alter the vasomotor response induced by electrical field stimulation through different mechanisms, which themselves can vary with age in hypertension.

Therefore, the present work used mesenteric arteries from spontaneously hypertensive rats (SHRs) to analyse whether aging altered the vasomotor response induced by electrical field stimulation when the β-adrenoceptors were activated with clenbuterol, and to identify the mechanism involved.

2. Materials and methods

2.1. Tissue preparation

The mesenteric arteries were isolated from two groups of male SHRs aged either 3–6 months (weighing 200–250 g) or 22–24 months (weighing 400–450 g). The rats were killed by CO_2 inhalation; the first branch of the mesenteric artery was carefully dissected out, cleaned of connective tissues, and placed in Krebs–Henseleit solution (KHS) at 4 °C. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the USA National Institutes of Health (NIH publication no. 85-23, revised in 1985).

2.2. Vascular reactivity

The method used for isometric tension recording has been described elsewhere (Nielsen and Owman, 1971; Marín and Balfagón, 1998; Ferrer et al., 2000). Briefly, two parallel stainless steel pins were introduced through the lumen of the vascular segment: one fixed to the bath wall, and the other connected to a force transducer (Grass FTO3C; Grass, Quincy, MA, USA); this was connected in turn to a Grass model 7D polygraph. For electrical field stimulation experiments, segments were mounted between two platinum electrodes 0.5 cm apart connected to a stimulator (model S44; Grass) modified to supply adequate current strength. The segments were suspended in an organ bath containing 5 ml of KHS at 37 °C continuously bubbled with a 95% O_2–5%CO_2 mixture (pH 7.4). The experiments were performed with endothelium-denuded segments to eliminate the main source of vasoactive substances, including NO, and to avoid any action by different drugs on the endothelial cells that could lead to misinterpretation of results. Endothelium was removed by gently rubbing the luminal surface of segments with a thin wooden stick. The segments were subjected to a tension of $0.5 \times g$ that was readjusted every 15 min during a 90-min equilibration period before drug administration. After this, K^+ was added to KHS up to a 75 mM K^+ concentration to check functional integrity and a reproducible response to K^+ was established in each experiment. The absence of vascular endothelium was proven by the inability of 10 μM acetylcholine to relax segments precontracted with 1 μM noradrenaline. The absence of endothelium was also confirmed at the end of the experiments using a silver nitrate stain according to the method of Caplan et al. (1971).

Frequency–response curves to electrical field stimulation (1, 2, 4, 8, and 16 Hz) or concentration–response curves to noradrenaline (10 nM–10 μM) were performed. The parameters used for electrical field stimulation were 200 mA, 0.3 ms, and 1–16 Hz for 30 s, with an interval of 1 min between each stimulus—the time required to recover basal tone. A rest period of at least 1 h was necessary to avoid desensitization between consecutive curves. Four successive frequency–response curves separated by 1-h intervals produced similar contractile responses. When assessing the effect of 0.1 μM tetrodotoxin and 1 μM phentolamine on the contraction elicited by electrical field stimulation, these substance were added to the bath 20 min in advance.

To determine the participation of neuronal NO in responses induced by electrical field stimulation and/or noradrenaline for segments from both rat groups, 10 μM N^G-nitro-L-arginine methyl ester (L-NAME) was added to the bath 30 min before performing the second frequency–response curve or concentration–response curve for noradrenaline. In a previous work (Marín and Balfagón, 1998), we had found that 10 and 100 μM L-NAME induced similar effects on the response induced by electrical field stimulation in rat mesenteric arteries.

To determine the effect of clenbuterol on the response induced by electrical field stimulation, this drug was added to the bath 30 min before the second frequency–response

stimulation. To find that the modulatory effects on the response to electrical field stimulation were due to 1 μM clenbuterol receptor activation, another set of experiments was performed to obtain a second response curve for electrical field stimulation in the presence of the β-adrenoceptor antagonist receptor, 1 μM propranolol, as was a third curve in the presence of the antagonist plus clenbuterol.

To analyse the possible participation of substances other than NO in the effect of clenbuterol, other experiments were performed with L-NAME added before the second frequency–response curve, and L-NAME plus clenbuterol before a third curve.

We have previously reported the participation of sensory innervation in the electrical field stimulation-induced response in young SHRs. To analyse the possible participation of this innervation in old SHRs as well, 0.5 μM capsaicin, a sensory neurotoxin, was added to the bath 60 min before the second frequency–response curve and concentration–response curve to noradrenaline. To find if sensory innervation was involved in the increased electrical field stimulation response to clenbuterol in segments from young SHRs, experiments were performed, adding capsaicin before the second frequency–response curve, capsaicin plus clenbuterol before the third frequency–response curve, and capsaicin plus clenbuterol plus L-NAME before the fourth curve.

To study if the CGRP was implicated in the response to electrical field stimulation, experiments were performed by adding the CGRP receptor antagonist, 0.5 μM CGRP-(8–37), 30 min before the second frequency–response curve and then the antagonist plus capsaicin before the third frequency–response curve.

To determine if NO was involved in the increased electrical field stimulation response induced by clenbuterol in segments from old SHRs, a frequency–response curve was performed in the presence of clenbuterol plus L-NAME.

The possible effects of clenbuterol, propranolol, and L-NAME on basal tone and on noradrenaline response curves were also tested in segments from both age groups.

The ability of clenbuterol (1 μM) or sodium nitroprusside (10 nM–10 μM) to induce relaxation was analysed in segments from both groups precontracted with noradrenaline. The possible effect of clenbuterol on the sodium nitroprusside response curve was also examined in segments from both age groups.

2.3. Tritium release experiments

Denuded segments of rat mesenteric arteries 4 mm in length were set up in a nylon net and immersed for 30 min in 10 ml of KHS at 37 °C continuously gassed with a 95% O_2–5% CO_2 mixture (stabilisation period). Thereafter, they were incubated for 60 min in 1 ml of bubbled KHS at 37 °C containing (±)[^{3}H]noradrenaline (0.33 μM, 10 μCi/ml, sp. act. of 10 Ci/mmol). Afterwards, the arteries were transferred to a superfusion chamber with two parallel platinum electrodes, 0.5 cm apart, connected to a stimulator (model S44; Grass) for electrical field stimulation. The arteries were superfused at a rate of 2 ml/min with oxygenated KHS at 37 °C for 100 min, during which time the steady state level of basal tritium efflux was reached. Cocaine (10 μM) and normetanephrine (10 μM) were added to the superfusion fluid after the incubation period, and kept there throughout the experiment to block neuronal and extraneuronal uptake of noradrenaline, respectively, in order to improve method sensitivity. Then, two electrical stimulation periods of 60 s (200 mA, 0.3 ms, 4 Hz) were applied to the arteries at 45-min intervals and the superfusate was collected in vials (10 in total) at 30-s intervals. These vials were collected in the following manner: two before stimulation, to determine the basal level of tritium efflux, two during stimulation, and six after the stimulation; the latter were enough to recover the basal level of tritium efflux. Afterwards, Ready-Protein solution (Beckman) was added to the vials and radioactivity was measured in a scintillation counter (Beckman LS 5000 TD).

To evaluate their effects on tritium release, isoproterenol (1 μM), clenbuterol (1 μM), propranolol (1 μM), clenbuterol, or isoproterenol plus propranolol were added 30 min before the second stimulation period.

The stimulation-induced tritium release was calculated by subtracting the basal tritium release from that evoked by electrical stimulation (S_1 and S_2). Thereafter, the ratios of the net tritium release between S_2 and S_1 were calculated to eliminate differences between arteries. The action of the drug on the evoked release was expressed as its effect on these ratios. The amount of radioactivity released was expressed in desintegrations per minute (dpm) per milligram.

2.4. Solutions and drugs

The composition of KHS was as follows (mM): NaCl 115, $CaCl_2$ 2.5, KCl 4.6, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1, Na_2 EDTA 0.03 (to prevent the oxidation of unstable substances). Drugs used were: L-

Table 1
Effect of 0.1 μM tetrodotoxin and 1 μM phentolamine on the frequency–contraction curve for mesenteric artery segments from young and old SHRs

	Frequency (Hz)				
	1	2	4	8	16
Young					
Control	38.7 ± 4.7	42.4 ± 5.3	58.0 ± 6.3	80.2 ± 8.2	114 ± 8
Tetrodotoxin	0*	0*	0*	3.8 ± 2.1*	8.2 ± 3*
Phentolamine	0*	0*	2 ± 1*	7.8 ± 2.1*	25.3 ± 5*
Old					
Control	40.3 ± 4.0	54.3 ± 5.0	70.7 ± 6.8	84.4 ± 7.6	110 ± 7
Tetrodotoxin	0*	0*	0*	2.5 ± 1.8*	7.4 ± 2*
Phentolamine	0*	0*	0*	6.2 ± 3*	31.3 ± 6*

Results (mean ± S.E.M.) are expressed as percentages of the response elicited by 75 mM K^+; $n = 4–6$ animals.

* $P < 0.0001$ vs. control.

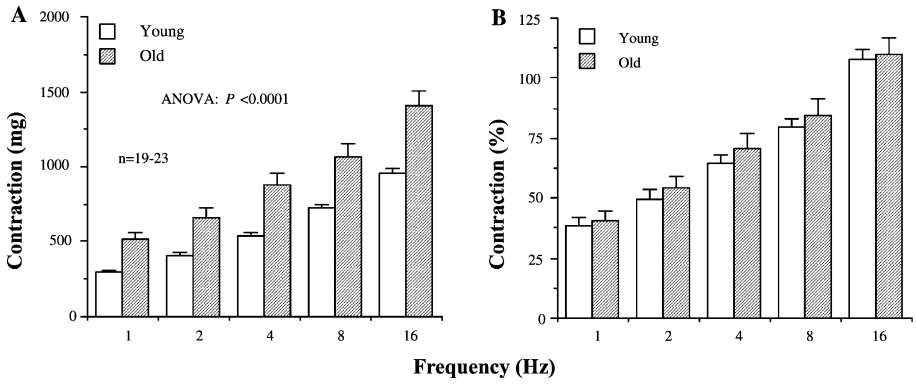

Fig. 1. Effect of age on frequency-dependent contractions of mesenteric artery segments from young and old SHRs. Results (mean ± S.E.M.) are expressed in (A) milligrams and (B) as percentages of the contraction induced by 75 mM K^+ (B) (young, 1035 ± 57 mg; old, 1482 ± 86 mg). *n* = number of animals.

noradrenaline hydrochloride, acetylcholine chloride, normetanephrine, tetrodotoxin, phentolamine, sodium nitroprusside, L-NAME hydrochloride, clenbuterol hydrochloride, propranolol hydrochloride, CGRP, CGRP-(8–37), and isoproterenol (Sigma, St. Louis, MO, USA); cocaine hydrochloride (Depósito de Estupefacientes, Ministerio de Sanidad y Consumo, Madrid, Spain); and (±)[^{3}H]noradrenaline hydrochloride (New England Nuclear, Boston, MA, USA). Stock solutions (10 mM) of drugs were made in distilled water, except for noradrenaline, which was dissolved in a NaCl (0.9%)–ascorbic acid (0.01% wt/vol) solution. These solutions were kept at −20 °C and appropriate dilutions were made in KHS on the day of the experiment.

2.5. Statistical analysis

The responses elicited by electrical field stimulation, K^+, or noradrenaline were expressed in milligrams for comparison between young and old SHRs, and also as a percentage of the contraction induced by 75 mM K^+, to examine the effect of the drugs within each group. Results are given as mean ± S.E.M. 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-measure analysis of variance (ANOVA). To compare the increases induced by some drugs in the electrical field stimulation-induced tritium overflow, an ANOVA followed by the Bonferroni test was used. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. Blood pressure

Systolic blood pressure levels in young and old SHRs were comparable (210 ± 15 and 195 ± 11 mm Hg, $P>0.05$, respectively).

3.2. Vascular reactivity

The contractions induced by electrical field stimulation were practically abolished by 0.1 μM tetrodotoxin and markedly reduced by 1 μM phentolamine in segments from both young and old SHRs (Table 1). The response induced

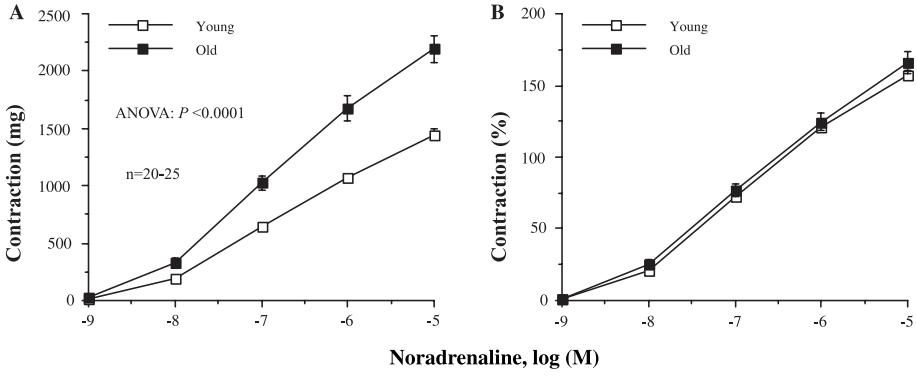

Fig. 2. Effect of age on the concentration–response curves to noradrenaline for mesenteric artery segments from young and old SHRs. Results (mean ± S.E.M.) are expressed in (A) milligrams and (B) as percentages of the contraction induced by 75 mM K^+ (young, 989 ± 22 mg; old, 1361 ± 44 mg). *n* = number of animals.

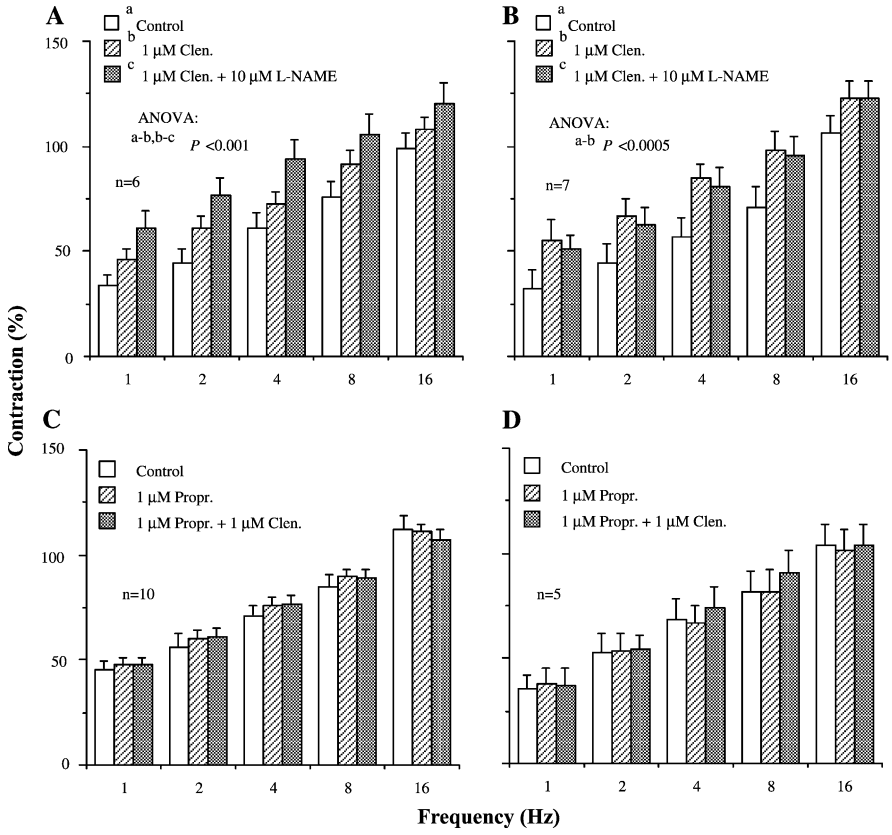

Fig. 3. Effect of clenbuterol (Clen.), Clen. + L-NAME, propranolol (Propr.), or Propr. + Clen. on the frequency–response curves for mesenteric artery segments from young (A,C) and old (B,D) SHRs. Results (mean ± S.E.M.) are expressed as percentages of the contraction induced by 75 mM K^+ (young, 947 ± 47 mg; old, 1332 ± 139 mg). n = number of animals.

by 75 mM K^+ was significantly increased in segments from old SHRs (young rats: 1003 ± 42, n = 43; old rats: 1361 ± 86 mg, n = 44; P < 0.001).

The contractile responses induced by electrical field stimulation or exogenous noradrenaline (10 nM–10 μM) were greater in segments from old rats than from young rats when these responses were expressed as an absolute contraction (milligrams) (Figs. 1A and 2A). However, these responses were similar in mesenteric arteries from both groups when the responses were expressed as a percentage of the contraction induced by 75 mM K^+ (Figs. 1B and 2B).

In segments from both young and old rats, the presence of 1 μM clenbuterol significantly increased the contractile

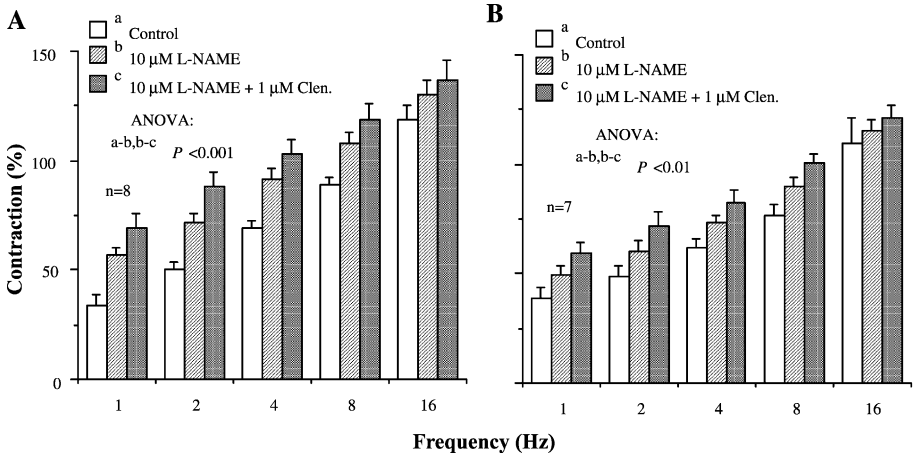

Fig. 4. Effect of L-NAME or L-NAME + Clen. on the frequency–response curves for mesenteric artery segments from young (A) and old (B) SHRs. Results (mean ± S.E.M.) are expressed as percentages of the contraction induced by 75 mM K^+ (young, 1053 ± 87 mg; old, 1482 ± 139 mg). n = number of animals.

Table 2
Differences between the electrical field stimulation-induced contractions in the presence or absence of L-NAME in mesenteric arteries from young and old SHRs

	Frequency (Hz)				
	1	2	4	8	16
Young	22 ± 5	23.2 ± 5	22.3 ± 5	17.6 ± 4.9	10.8 ± 2
Old	7.2 ± 4.0*	9.7 ± 3*	10 ± 2*	10.6 ± 3*	6.2 ± 3

* $P < 0.05$ vs. young SHRs.

response to electrical field stimulation (Fig. 3A and B); the effect of clenbuterol was prevented by preincubation with propranolol (Fig. 3C and D).

The contraction induced by electrical field stimulation was significantly increased by preincubation with 10 μM L-NAME in segments from both groups (Fig. 4A and B). The increase induced by L-NAME was greater in segments from young rats than from old SHRs (Table 2).

In L-NAME-preincubated segments from both young and old SHRs, the addition of clenbuterol further increased the response induced by electrical field stimulation (Fig. 4); this increase was similar to that induced by electrical field stimulation in the presence of clenbuterol alone in segments from young SHRs, but was significantly less in segments from old SHRs (Fig. 4A and B compared to Fig. 3A and B).

In segments from young SHR segments preincubated with clenbuterol, the subsequent addition of L-NAME increased the enhanced response induced by electrical field stimulation to a similar extent to that observed with L-NAME given alone (Fig. 3A). In segments from old SHRs preincubated with clenbuterol, the subsequent addition of L-NAME did not modify the enhanced response induced by electrical field stimulation in the presence of clenbuterol (Fig. 3B).

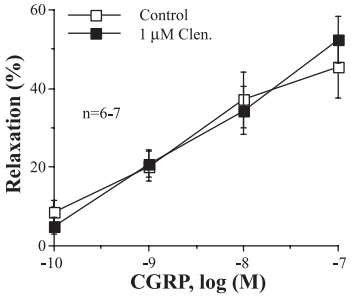

Fig. 6. Effect of clenbuterol (Clen.) on the concentration–response curve to calcitonin-gene related peptide (CGRP) for segments of mesenteric arteries from young SHRs precontracted with noradrenaline (1 μM). Results (mean ± S.E.M.) are expressed as percentages of the contraction induced by 1 μM noradrenaline (control, 1171 ± 74 mg; clenbuterol, 1058 ± 85 mg). n = number of animals.

In segments from young animals, capsaicin increased the contractile response induced by electrical field stimulation, as previously reported (Marín et al., 2000), and subsequent addition of clenbuterol did not enhance this response. The addition of L-NAME to capsaicin-plus-clenbuterol-treated segments increased the response to electrical field stimulation to the same extent as when segments were preincubated with L-NAME alone (Fig. 5A). In segments from old SHRs, capsaicin did not modify the contractile response induced by electrical field stimulation (data not shown). In segments from young animals, CGRP-(8–37) increased the contractile response induced by electrical stimulation, and the subsequent addition of capsaicin did not enhance this response (Fig. 5B).

In segments precontracted with noradrenaline (1 μM and 0.1 μM for segments from young and old animals, respectively), the presence of clenbuterol (1 μM) did not induce any relaxation (data not shown). In segments from young

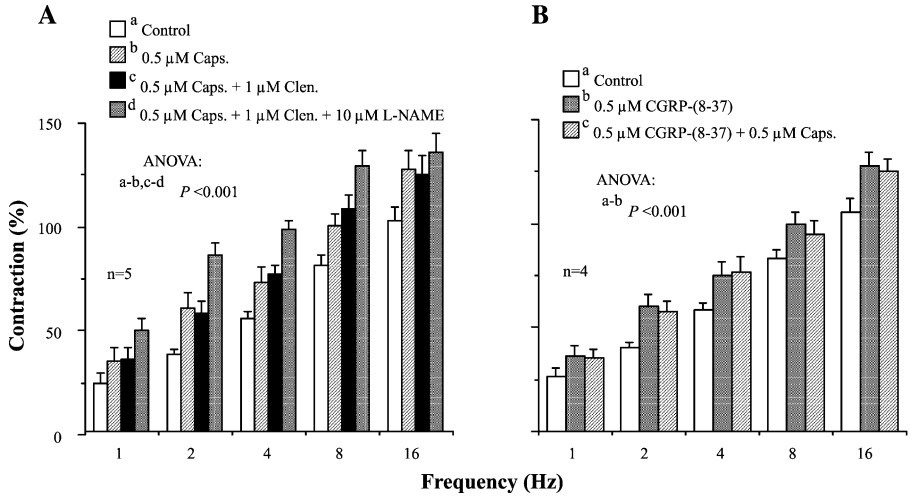

Fig. 5. Effect of capsaicin (Caps.), Caps. + Clen., or Caps. + Clen. + L-NAME (A), and effect of CGRP-(8–37) or CGRP-(8–37) + Caps. (B) on the frequency–response curves for mesenteric artery segments from young SHRs. Results (mean ± S.E.M.) are expressed as percentages of the contraction induced by 75 mM K^+ (895 ± 74 mg). n = number of animals.

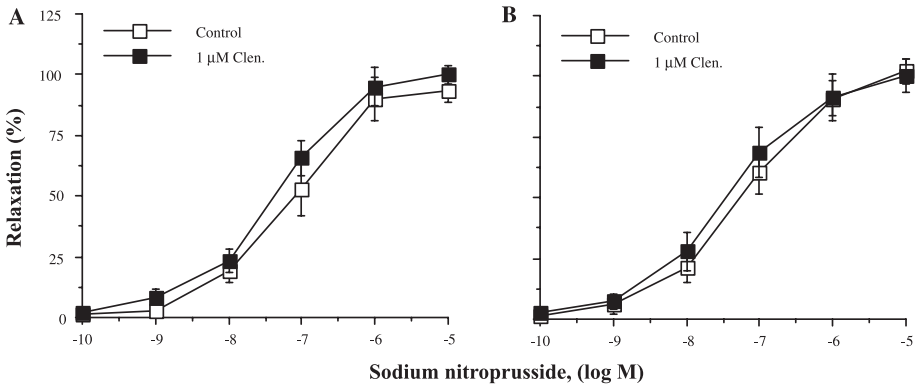

Fig. 7. Effect of clenbuterol on the concentration–response curves to sodium nitroprusside for mesenteric artery segments from young (A) and old (B) SHRs. Results (mean ± S.E.M.) are expressed as percentages of the contraction induced by noradrenaline (young, 1254 ± 65 mg; old, 1160 ± 104 mg). *n* = number of animals.

SHRs precontracted with noradrenaline (1 μM), the exogenous addition of CGRP (0.1 nM–0.1 μM) induced a concentration-dependent vasodilator response that was not modified by clenbuterol (1 μM) (Fig. 6).

In segments precontracted with noradrenaline, the addition of sodium nitroprusside (0.1 nM–10 μM) induced a concentration-dependent vasodilator response that was similar for both age groups, and was not modified by clenbuterol (1 μM) (Fig. 7A and B).

Clenbuterol (1 μM) did not affect basal tone in either group but increased the response to exogenous noradrenaline in old SHRs. This effect of clenbuterol was prevented by propranolol (1 μM). Capsaicin did not affect basal tone or response to exogenous noradrenaline in either group (Fig. 8A and B).

Neither basal tone nor response to exogenous noradrenaline was affected by propranolol in either age group. CGRP-(8–37) did not affect basal tone nor response to exogenous noradrenaline in the young group (data not shown).

3.3. Tritium release experiments

Electrical stimulation induced tritium release; the release obtained in S_2 (young, 1453 ± 110 dpm/mg; old, 1320 ± 97 dpm/mg) was similar to that found in S_1 (young, 1380 ± 120 dpm/mg; old, 1350 ± 123 dpm/mg, $n = 4$; $P > 0.05$). The S_2/S_1 ratio for segments from young animals was not significantly different from the one in old rats (young, 1.1 ± 0.1; old, 1.0 ± 0.02). Tritium release was markedly reduced by 0.1 μM tetrodotoxin (S_2/S_1, young, 0.05 ± 0.02; old, 0.06 ± 0.02, $n = 5$; $P < 0.001$).

For segments from young SHRs, clenbuterol did not alter the stimulated tritium overflow. The presence of 1 μM isoproterenol increased the electrical field stimulation-induced tritium release, and this increase was blocked by previous incubation with propranolol (Fig. 9). However, in segments from old SHRs, the presence of 1 μM clenbuterol increased the electrical field stimulation-induced tritium release, and this increase was blocked by prior incubation

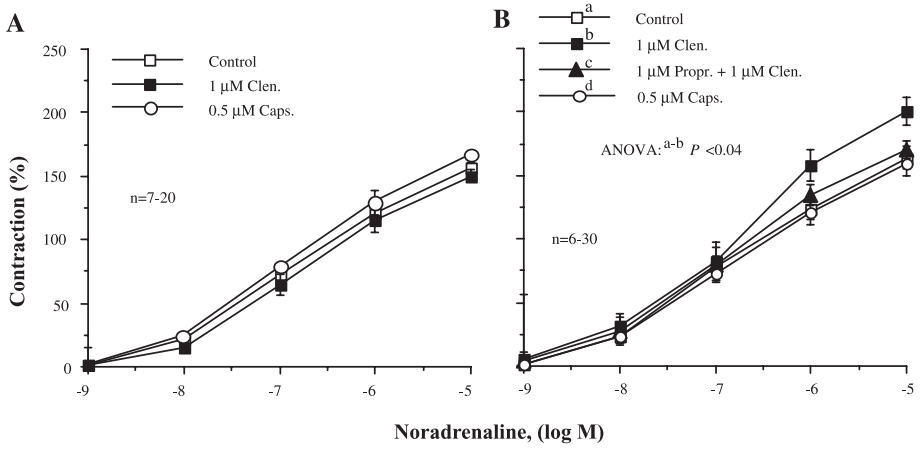

Fig. 8. Effect of clenbuterol (Clen.) or capsaicin (Caps.) on the concentration–response curves to noradrenaline for mesenteric artery segments from young SHRs (A), and effect of Clen., propranolol (Prop.) + Clen., or Caps. on the concentration–contraction curves to noradrenaline for mesenteric artery segments from old SHRs (B). Results (mean ± S.E.M.) are expressed as percentages of a previous tone with 75 mM K^+ (young, 989 ± 22 mg; old, 1361 ± 44 mg). *n* = number of animals.

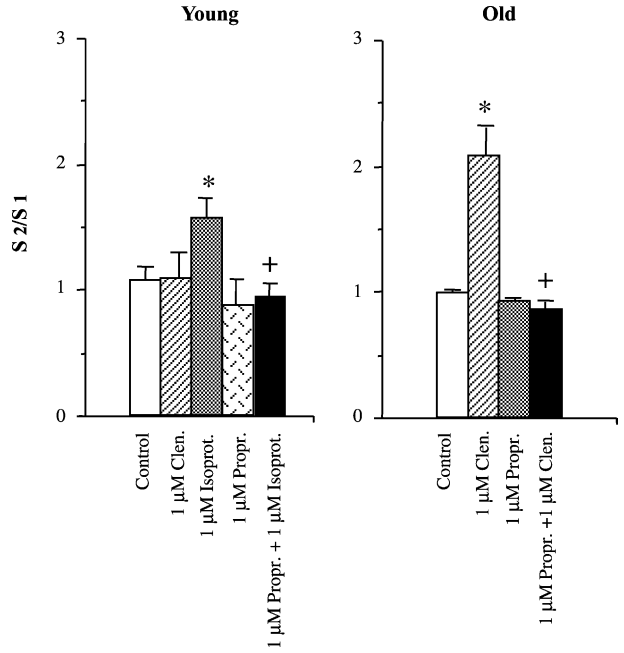

Fig. 9. Effect of clenbuterol (Clen.), isoproterenol (Isoprot.), propranolol (Propr.), or Propr. + Isoprot. on the electrically stimulated tritium release in mesenteric artery segments from young SHRs. Effect of Clen., Propr., or Propr. + Clen. on the electrical stimulated tritium release in mesenteric artery segments from old SHRs. Results (mean ± S.E.M.) are expressed as the ratio S_2/S_1. n = number of animals. *$P<0.05$ vs. control; $^{+}P<0.05$ vs. Clen. or Isoprot.

with propranolol (Fig. 9). Age altered neither basal tritium release (young, 114 ± 7; old, 123 ± 10, $n=4–6$; $P>0.05$) nor tissue tritium content (young, 82,588 ± 6530; old, 93,007 ± 7270; $P>0.05$) in SHRs at the end of the experiments.

4. Discussion

The present results showed that electrical field stimulation induced contractile responses in endothelium-denuded mesenteric segments from young and old SHRs. These responses were mainly mediated by an adrenergic nerve terminal release of noradrenaline-activating α-adrenoceptors in segments from either SHR age group.

Segments from old SHRs also showed an increased response to K^+, in contrast to the results obtained with mesenteric arteries from old normotensive rats (Marín and Rodríguez-Martínez, 1999; Ferrer and Balfagón, 2001) and with aorta from SHRs (Ponte et al., 1996). This difference could be due to the alteration of vessel structure associated with the vascular remodeling that occurs in hypertension (Mulvany, 1990; Arribas et al., 1997). On the other hand, hypertension and aging are associated with stiffness, which involve calcification, large amounts of collagen, and fragmentation and rupture of elastic tissues (Safar et al., 2000). The increase of contraction induced by K^+ could also be associated with changes in intracellular processes.

Segments from old SHRs showed an increased response to electrical field stimulation; this could indicate: (a) an increase in noradrenaline release from sympathetic nerve endings, and/or (b) an age-associated increase in the sensitivity of vascular smooth muscle cells to noradrenaline. The fact that age did not modify electrical field stimulation-induced tritium overflow while noradrenaline responses were increased suggests that the latter caused the increased responses to electrical field stimulation. The vascular remodelling associated with chronic hypertension could also be implicated in the increased response to electrical field stimulation. Various studies concerning noradrenaline release and vasoconstrictor responses in aged and hypertensive animals have reported conflicting results (Borkowski and Porter, 1984; Borkowski et al., 1992; Marín, 1995; Buchholz et al., 1998; Ferrer and Balfagón, 2001; Marín et al., 2000).

Clenbuterol increased the vasoconstrictor response to electrical field stimulation in arteries from both groups of rats. This effect was antagonized by propranolol, suggesting that it was mediated by the activation of β_2-adrenoceptors. Rat mesenteric arteries are rich in sympathetic (Li and Duckles, 1992), sensory (Li and Duckles, 1993), and nitrergic innervation (Marín and Balfagón, 1998), all of which modulate vasomotor tone. Therefore, the increase in vasoconstrictor response induced by electrical field stimulation in the presence of clenbuterol may be associated with alterations in neurotransmitter(s) release and/or the vasomotor response these alterations induce.

Some studies have shown the existence of prejunctional facilitatory β-adrenoceptors in mesenteric arteries from hypertensive, but not from normotensive, rats (Misu and Kubo, 1986; Misu et al., 1987). However, in mesenteric arteries from young SHRs, we found that clenbuterol did not significantly alter either basal or electrical field stimulation-induced tritium overflow. However, other β-adrenoceptors agonists such as isoproterenol had a facilitatory effect on tritium release, which was abolished by propranolol. This suggests the existence of facilitatory presynaptic β-adrenoceptors in this artery (Kubo et al., 1984). The fact that clenbuterol did not increase tritium release suggests the existence of other mechanism(s) that would compensate for the facilitatory effect of β-adrenoceptor activation by this agonist.

Activation of postsynaptic β-adrenoceptors induces a variable vasodilator response that varies with the vascular bed analysed (Encabo et al., 1996; Marín and Balfagón, 1998; Conde et al., 2000; Guimarães and Moura, 2001). In addition, both increased (McGrath, 1982) and decreased (Wilffert et al., 1983) α-adrenoceptor-mediated contractions have been described in the presence of the β-agonists. The results now obtained with mesenteric segments from young SHRs showed that clenbuterol did not induce relaxation and did not modify the response to exogenous noradrenaline, thereby ruling out a possible postsynaptic interaction between clenbuterol and noradre-

naline. They also disprove the participation of adrenergic innervation in the increased contractile response to electrical field stimulation induced by clenbuterol in mesenteric arteries from young SHRs, and suggest the participation of another neurotransmitter(s).

We have recently demonstrated that electrical field stimulation induces the release of neurotransmitters from nitrergic and sensory nerves in mesenteric arteries from young SHRs (Marín et al., 2000). Additionally, we have demonstrated that the β_2-adrenoceptor agonist, clenbuterol, increased neuronal NO release in normotensive rats, so that an effect of clenbuterol on NO release cannot be discounted in SHRs. β-Adrenergic modulation of CGRP release has also been described for rat heart (Chen et al., 1996; Yeh et al., 1998). Consequently, we analysed the possible participation of these innervations in the increased response to electrical field stimulation induced by clenbuterol. The fact that, in segments preincubated with L-NAME, clenbuterol induced an increase similar to that observed with clenbuterol given alone indicates that neuronal NO was not involved in the increased vasoconstrictor response to electrical field stimulation observed in the presence of clenbuterol.

The possible participation of sensory innervation, the main neurotransmitter of which is CGRP (Kawasaki et al., 1988; Ralevic, 2002), was studied using the sensory neurotoxin, capsaicin, and the CGRP receptor antagonist, CGRP-(8–37) (Han et al., 1990). The presence of both drugs increased the vasoconstrictor response to electrical field stimulation. Preincubation with capsaicin abolished the increase induced by clenbuterol. These results indicate that, in mesenteric arteries from young SHRs, clenbuterol increases the contractile response to electrical field stimulation through a mechanism that seems to involve sensory innervation function and CGRP release. Additionally, the fact that clenbuterol did not modify the vasodilator response induced by CGRP in mesenteric arteries from young SHRs seems to confirm that the clenbuterol effect is mediated by a decrease in CGRP release. Moreover, the facts that (1) the addition of L-NAME to arteries preincubated with capsaicin and clenbuterol induced an increase in vasoconstrictor response similar to that observed when L-NAME was given alone, and that (2) the vasodilator response to sodium nitroprusside was not modified in the presence of clenbuterol, reinforce the suggestion that clenbuterol does not modify neuronal NO release or its effect in mesenteric arteries from young SHRs. Together, these results indicate that the increase in vasoconstrictor response to electrical field stimulation observed in the presence of clenbuterol in mesenteric arteries from young SHRs is associated with a decrease in CGRP release.

It has been noted that β-adrenoceptor-mediated responses of the cardiovascular system are usually reduced with aging and hypertension (Borkowski et al., 1992; Arribas et al., 1994; Docherty, 1996; Van der Zypp et al., 2000), although we have recently demonstrated increases in presynaptic β_2-adrenoceptor-mediated noradrenaline and neuronal NO release with aging (Ferrer and Balfagón, 2001). We analysed the possible effect of clenbuterol on adrenergic neurotransmission in segments from old SHRs preincubated with [^{3}H]noradrenaline. The results showed that clenbuterol did not affect the basal tritium release, although it did increase the release induced by electrical field stimulation, in contrast to what was observed in segments from young SHRs. This increase was prevented by propranolol, indicating the involvement of facilitatory presynaptic β_2-adrenoceptors. This mechanism could be related to the increased electrical field stimulation-induced vasoconstrictor response in the presence of clenbuterol, although a postsynaptic effect cannot be discarded. Similar to observations in young SHRs, clenbuterol did not induce a vasodilator response in precontracted segments from old SHRs, either. However, we observed that clenbuterol did increase the response to exogenous noradrenaline in the older rats, through a β-related mechanism. This result may indicate the existence of an interaction between α- and β-adrenoceptors, as has been described (McGrath, 1982; Wilffert et al., 1983). In mesenteric arteries from old normotensive rats, clenbuterol failed to modify the vasoconstrictor response to exogenous noradrenaline (Ferrer and Balfagón, 2001), indicating that this interaction could be due to the association of hypertension and aging.

The participation of nitrergic and sensory innervation in the enhanced electrical field stimulation-induced vasoconstrictor response in the presence of clenbuterol cannot be discarded. However, capsaicin did not affect the vasoconstrictor response induced by either electrical field stimulation or exogenous noradrenaline in segments from old animals. This result indicates that, as reported from studies on aging (Wimalawansa, 1996; Sun et al., 1998; Lu et al., 2001), sensory innervation does not participate in the response induced by electrical field stimulation in segments from old SHRs. On the other hand, the addition of clenbuterol in the presence of L-NAME did not induce a further increase in the electrical field stimulation-induced vasoconstrictor response, suggesting the participation of neuronal NO in the effect induced by clenbuterol. This, together with the fact that the vasodilator response to sodium nitroprusside remained unmodified by the presence of clenbuterol, indicates that clenbuterol abolished neuronal NO release.

In summary, all these results indicate that acute β_2-adrenoceptor activation by clenbuterol increases the vasoconstrictor response to electrical field stimulation in mesenteric segments from both young and old SHRs. However, the mechanisms involved in this effect are different. In young SHRs, clenbuterol does not modify the functioning of nitrergic innervation, it inhibits the vasomotor response of sensory innervation, and it does not modify the release of and response to noradrenaline. In old SHRs, clenbuterol increases the release of, and response to, noradrenaline and seems to abolish the functionality of nitrergic innervation.

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