ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7): 595 - 602
http://www.chinaphar.com

Elcatonin-mediated contractile and relaxant responses in SHR femoral artery

R96 A

FUJIMOTO Seigo[1], FUJIMOTO S Keiko[2] (*Department of Pharmacology and [2]Institute of Natural Sciences, Nagoya City University Medical School, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan*)

KEY WORDS acetylcholine; β-adrenergic receptors; cholinergic receptors; elcatonin; vascular endothelium; femoral artery; nitric oxide; norepinephrine; inbred SHR rat

ABSTRACT

AIM: To study the effect of repeated systemic injections of elcatonin (a synthetic analog of eel calcitonin) on the responses of rat femoral artery preparation to vasoactive drugs and to determine subtypes of muscarinic cholinoceptors involved in acetylcholine (ACh)-induced vasorelaxation in elcatonin-treated rats. **METHODS**: Spontaneously hypertensive rats (SHR) were treated sc with elcatonin, 0.5 and 5 U/kg, 3 times a week for 2 weeks. Responses to vasoactive drugs were determined in helically cut strips of femoral arteries of these rats. Schild plot data for muscarinic cholinoceptor antagonists were obtained on these vascular strips, using ACh as an agonist. **RESULTS**: Elcatonin did not alter systemic blood pressure and contractile responses of the femoral artery to KCl, norepinephrine, 5-hydroxytryptamine, and prostaglandin $F_{2\alpha}$. Elcatonin attenuated isoproterenol-induced relaxation, increased ACh- and ATP-induced relaxations, and did not change relaxant responses to sodium nitroprusside and cromakalim in the femoral artery. Nitro *L*-arginine in the combination with tetraethylammonium (or charybdotoxin) completely abolished the relaxant response to ACh in the control but not in the elcatonin-treated arteries. The muscarinic cholinoceptor subtype involved in the ACh-induced relaxation was M_3 in the elcatonin-treated as well as control SHR. **CONCLUSION**: Elcatonin decreases β-adrenoceptor-mediated relaxation and increases M_3 cholinoceptor-mediated relaxation in the SHR femoral artery. Although the ACh-induced relaxation is explained by stimulated releases of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) in the SHR artery, a NO- and EDHF-independent mechanism in addition to NO and EDHF is responsible for the response to ACh in the femoral artery from the elcatonin-treated SHR.

[1] Correspondence to Dr FUJIMOTO Seigo.
Phn 81-52-853-8150. Fax 81-52-842-0863.
Received 2001-02-09 Accepted 2001-04-22

INTRODUCTION

The primary physiological function of calcitonin, a peptide hormone secreted from C-cells of the thyroid gland, is to modulate plasma Ca^{2+} concentrations via its actions on bone and kidney[1]. The hypocalcemic effect of calcitonin is mainly caused by inhibition of osteoclastic bone resorption which is mediated by cyclic AMP-dependent protein kinase (PKA)[1]. Calcitonin can be used for treatment of metabolic bone disorders including osteoporosis and Paget's disease[2]. Elcatonin, developed in Japan, is a synthetic analog of eel calcitonin ([Aus[1,7]] eel calcitonin) and is more stable than natural eel calcitonin[3]. Elcatonin has biological activity comparable to that of natural eel calcitonin; elcatonin suppresses bone resorption[4] and is frequently used for treatment of hypercalcemia, Paget's disease, and osteoporosis[4-6]. Elcatonin is also effective against experimentally induced osteoporosis in dog and rat[7,8].

It has been reported that elcatonin and calcitonin reduce systemic blood pressure in normotensive and hypertensive rats[9,10]. In addition, these peptides cause flushing of the face and hands[6,11,12] and increase renal blood flow in man[13]. These effects seem to be largely due to local vasodilation in response to these peptides, but mechanisms underlying the vasodilating effect of elcatonin and its related peptides are not fully understood. It has been suggested that elcatonin-induced vasodilation is closely connected with increases in plasma 5-hydroxytryptamine (5-HT) and vasoactive intestinal peptide concentrations[12,14]. Since calcitonin reduces the production of prostaglandins (PGs) and thromboxane A_2 from arachidonic acid in guinea-pig lung preparation[15], the vasodilation in response to elcatonin may also be ex-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2620

plained by the reduced production of vasocontractile prostanoids. Elcatonin does not affect vasoconstrictor responses to norepinephrine (NE), angiotensin II, and vasopressin in rats, suggesting that the peptide is neither an β-adrenergic, angiotensin nor vasopressin receptor antagonists[10,16]. Although it has been shown that calcitonin causes an increase in Ca^{2+} uptake in sarcoplasmic reticulum vesicles of human cardiac muscle via an activation of adenylate cyclase[17], an enzyme responsible for the increased synthesis of cyclic AMP, it remains unknown whether or not stimulated production of cyclic AMP and the increase in the Ca^{2+} uptake in sarcoplasmic reticulum in blood vessels accounts for the elcatonin-induced vasorelaxation.

On the other hand, it has been found that calcitonin contracts skeletal blood vessels in dogs and mans[16,18]. Since the vasocontractile effect is not inhibited by phentolamine, it seems unlikely that calcitonin is an α-adrenoceptor agonist in these blood vessels[16]. In addition, calcitonin and elcatonin inhibit skeletal blood flow increase observed in patients with Paget's disease[2] and with Sudeck's atrophy of the foot[5], respectively. Thus, elcatonin and its related peptides may be the vasodilator or vasoconstrictor, depending on species, vascular regions, or preparations used. However, it is very likely that these peptides induce opposite biological responses in target cells, since calcitonin receptors are capable of coupling with effector systems that lead to activation of both PKA and protein kinase C (PKC) via GTP-binding proteins (Gs and Gi, respectively)[19,20].

Although several studies have shown the development of osteoporotic bone disorders as well as hypertension in spontaneously hypertensive rats (SHR)[21,22], little attention has been given to the effects of elcatonin on the development of hypertension in SHR[10]. Since abnormal responses of blood vessels from SHR to vasoactive drugs have frequently been reported[23,24], we have studied effects of elcatonin on systemic blood pressure and on responses of isolated femoral artery to certain vasoactive drugs in SHR.

METHODS AND MATERIALS

Vascular preparations and tension measurement SHR (♂, 230 – 280 g) were obtained from Charles River Breeding Laboratories (Shizuoka, Japan) at the ages of 9 – 11 weeks. The rats were treated sc with elcatonin, 3 times a week for 2 weeks at doses of 0.5 U/kg and 5 U/kg, and the control rats were treated with saline at 0.1 mL/kg. They were housed in stainless steel cages in groups of three, maintained under a 12 h day-night cycle, and given free access to food and water. Femoral arteries were isolated 2 d after the last administration of elcatonin or saline and were cut into helical strips. The preparation was suspended under a resting tension of 500 mg in 20 mL of warmed (37 ℃) and oxygenated (5 % CO_2 in O_2) Krebs-Henseleit bicarbonate (KHB) buffer. During an equilibration period of 60 min, the preparations were stretched until a stable resting tension was obtained. The preparation was contracted twice with KCl (40 mmol/L) for 10 min and then twice with NE (1 μmol/L) for 3 min at 40-min intervals between each determination. In some experiments, the endothelium was removed by mechanical rubbing of the intimal surface with a cotton swab. The successful removal of functional endothelium from the preparation was confirmed by the response to acetylcholine (ACh, 1 μmol/L) during contractions elicited by NE (1 μmol/L). Isometric tension changes were recorded through force-displacement transducers (TB-621T, Nihon Kohden Kogyo Co, Japan) coupled to a pen recorder.

Contractile responses Concentration-response curves for KCl, 5-HT, and $PGF_{2\alpha}$, in endothelium-denuded artery, were obtained by cumulative addition of the drugs to the organ chamber, while those for NE in the presence of propranolol (2 μmol/L) and desipramine (0.2 μmol/L) were obtained in endothelium-intact artery.

Relaxant responses To observe relaxant response to drugs, the femoral artery was contracted to 90 % – 80 % and 50 % – 40 % of maximum agonist contractions with NE 0.5 μmol/L (in the presence of propranolol) and 5-HT 0.1 μmol/L, respectively. After the contraction in response to NE had reached a steady state, concentration-response curves for ACh- and ATP-induced relaxation in endothelium-intact artery were determined in the presence of indomethacin (10 μmol/L). In some experiments, the preparation was incubated with nitro *L*-arginine (*L*-NA, 100 μmol/L), tetraethylammonium (TEA, 3 mmol/L), and charybdotoxin (0.1 μmol/L) for 30 – 60 min before and during the determination of the concentration-response curve for ACh. In the presence of *L*-NA, the artery was contracted by NE 0.3 μmol/L, since *L*-NA did not affect the basal vascular tone but augumented the contractile response to NE. Thus, the contraction elicited by NE 0.3 μmol/L in the presence of *L*-NA was similar to that elicited by NE 0.5 μmol/L in the absence of *L*-NA. Concentration-

response curves for isoproterenol (ISO)-induced relaxation was determined in endothelium-denuded, 5-HT-contracted arteries which had been treated for 60 min with phenoxybenzamine (2 μmol/L). Relaxation response to sodium nitroprusside (SNP) was determined in endothelium-denuded, 5-HT-contracted artery. At the end of the experiments, papaverine (0.1 mmol/L) was added to obtain the maximum relaxation. Since papaverine relaxed the preparations back to almost baseline tension, relaxation responses to vasorelaxants were expressed as percentages of the papaverine-induced relaxation (deduction of the tension obtained with papaverine from the contraction elicited by NE or 5-HT[25]).

Determination of pA_2 values for muscarinic cholinoceptor antagonists Four sequential concentration-response curves for ACh-induced relaxation were made in the endothelium-intact femoral artery precontracted with 5-HT (0.1 μmol/L) with an interval of 120 min between each determination. The artery was treated for 30 min with increasing concentrations of muscarinic cholinoceptor antagonists before the determination of the second, third, and fourth concentration-response curves. Concentration-ratio (CR) values were obtained by dividing the ACh EC_{50} value obtained in the presence of the antagonist by the ACh EC_{50} value obtained in the absence of the antagonist. Schild plots, ie, plots of log (CR-1) versus log molar concentration of the antagonist (log[B]), were obtained. Linear least-squares regression analysis was used to obtain the line of the best fit, using the combined data points from a number of animals; the x-intercept was taken as the pA_2 value of the antagonist[26]. EC_{50} values are the molar concentrations producing 50 % of the maximum ACh response.

Measurement of blood pressure Blood pressure was measured by indirect systolic blood pressure plethymography using a tail-cuff apparatus in rats without thermal stress at 30 ℃ (BP-98A, Softron, Japan)[23].

Reagents Acetylcholine chloride (ACh), adenosine 5′-triphosphate (ATP), charybdotoxin, 5-hydroxytryptamine creatinine sulfate (5-HT), (±)-isoproterenol hydrochloride (ISO), (−)-norepinephrine bitartrate (NE), N^G-nitro *L*-arginine (*L*-NA), pirenzepine dihydrochloride, and *DL*-propranolol hydrochloride were obtained from Sigma Chemical Company (USA) and dissolved in distilled water. 4-Diphenylacetoxy-*N*-methylpiperidine methobromide (4-DAMP) was a gift from Dr R B Barlow (University of Bristol, UK). 11-[[2-[(Diethyl-amino)methyl]-1-piperidinyl]acetyl-5,11-dihydroxy-6H-pyrido-[2,3-b][1,4]benzodiazepin-6-on (AF-DX 116) was a gift from Dr K Thomae Gmbh (Boehringer Ingelheim, Germany) and was dissolved in hydrochloric acid (0.1 mol/L). Cromakalim (Sigma) and indomethacin (Sigma) were dissolved in ethanol. Papaverine hydrochloride, sodium nitroprusside (SNP), and tetraethylammonium chloride (TEA) were obtained from Wako, Japan. Elcatonin ([$Asu^{1,7}$]-eel calcitonin) and prostaglandin (PG) $F_{2\alpha}$ were injectable preparations for animal and human use and obtained from Asahi Chemical Co (Japan) and Ono Pharmaceutical Co (Japan), respectively. KHB buffer contains NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and dextrose 10 mmol/L (pH 7.4).

Statistics Results are reported as $\bar{x} \pm s$ of the number (n) of observations. The one way analysis of variance (ANOVA) followed by Fisher's PLSD was used for statistical analysis. Difference between the pA_2 values for cholinoceptor antagonists in the elcatonin-treated SHR and control SHR was analyzed by the use of unpaired Student's t tests. Statistical significance was assumed when the P value was less than 0.05.

RESULTS

Blood pressure Mean systolic blood pressure was determined 2 d after the last administration of elcatonin at doses of 0.5 and 5 units/kg. Elcatonin did not change blood pressure in SHR; control (172 ± 22) mmHg, elcatonin 0.5 U/kg (181 ± 29) mmHg, elcatonin 5 U/kg (190 ± 36) mmHg (n = 12 animals in each group).

Contractile responses KCl (7 − 60 mmol/L), NE (1 nmol/L − 10 μmol/L), and 5-HT (10 nmol/L − 10 μmol/L) elicited concentration-dependent contractions of the femoral arteries (Fig 1). The absolute values for the maximum responses to KCl, NE, and 5-HT (expressed as 100 %) in the control artery were 363 mg ± 48 mg, 365 mg ± 64 mg, and 574 mg ± 101 mg (n = 6 − 8 arteries), respectively. Elcatonin (0.5 and 5 U/kg) did not change the response of the artery to these drugs (Fig 1). $PGF_{2\alpha}$ (0.1 − 60 μmol/L) produced concentration-dependent contraction in the endothelium-denuded femoral artery and elcatonin (0.5 and 5 units/kg) did not alter the response to $PGF_{2\alpha}$ (data not shown).

Relaxant responses ACh (1 nmol/L − 1 μmol/L) and ATP (0.1 − 100 μmol/L) relaxed the femoral artery in a concentration-dependent manner (Fig 2, upper panels). The response was abolished by removal of the

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

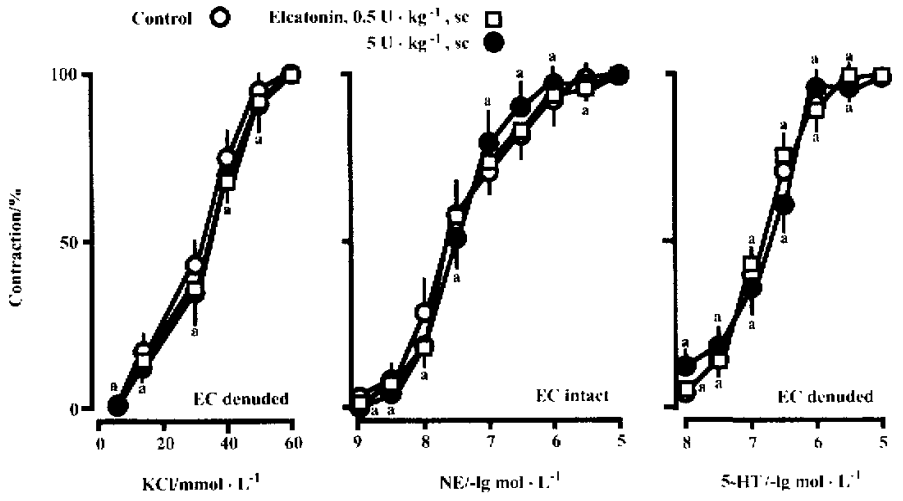

Fig 1. Cumulative concentration-response curves for KCl (left), NE (middle), and 5-HT (right) in femoral arteries. The rats were treated with elcatonin at doses of 0.5 (□) and 5 U/kg (●) and with saline (○). The concentrations of KCl are expressed as concentrations increased by addition of appropriate amounts of KCl into the KHB buffer (containing KCl, 5.9 mmol/L). Ordinate: maximum contraction responses to KCl (60 mmol/L), NE (10 μmol/L), and 5-HT (10 μmol/L) are expressed as 100 %. $n = 6-8$ arteries. $x \pm s$. $^aP > 0.05$ *vs* control.

endothelium (data not shown). When the rats were treated with elcatonin at 0.5 and 5 U/kg, the femoral artery from these rats responded more markedly by relaxation to ACh and ATP than did the control artery. There was no significant difference in the responses to ACh and ATP between the groups treated with elcatonin at 0.5 and 5 U/kg. ISO (10 nmol/L – 10 μmol/L) and SNP (0.1 – 300 nmol/L) relaxed the endothelium-denuded artery in a concentration-dependent manner (Fig 2, bottom panels). Elcatonin (0.5 U/kg) did not cause significant changes in the response of the artery to ISO, but the peptide at 5 U/kg decreased the response to ISO. The relaxant responses to SNP were not significantly altered by elcatonin at 0.5 and 5 U/kg.

In the presence of indomethacin (Fig 3A, B, and C), the relaxant response to ACh was inhibited by *L*-NA 100 μmol/L and completely abolished by a combination of *L*-NA 100 μmol/L and TEA 3 mmol/L (Fig 3A). On the other hand, the relaxant response of elcatonin (0.5 U/kg)-treated femoral artery to ACh was partly inhibited by *L*-NA, and the residual relaxation was further inhibited but not completely abolished by TEA (Fig 3B). Increased doses of elcatonin to 5 U/kg did not cause further changes in the response to ACh and in the inhibitory effects of *L*-NA and TEA (Fig 3C). In the presence of indomethacin and *L*-NA (Fig 3D, E, and F), the relaxant response to ACh was abolished by charybdotoxin (Fig 3D). When the rats were treated with elcatonin (0.5 U/kg), the relaxation in response to ACh was partly but not completely inhibited by charybdotoxin (Fig 3E). Again, the increase in the dose of elcatonin (5 U/kg) did not cause further changes in the inhibitory effect of charybdotoxin (Fig 3F).

Cromakalim elicited a concentration-dependent relaxation in the endothelium-denuded femoral artery (Fig 4). The response to cromakalim was not altered by elcatonin (0.5 and 5 U/kg).

pA_2 values for cholinoceptor antagonists

The relaxant response to ACh was competitively inhibited by pirenzepine, AF-DX 116, and 4-DAMP in the femoral artery. The pA_2 values for these drugs in the elcatonin (5 U/kg)-treated rat were not significantly different from those in the control rat (Tab 1). The slopes of the

Tab 1. pA_2 values and slopes of the Schild plots for muscarinic cholinoceptor antagonists in the femoral arteries from SHR treated with elcatonin at 5 U/kg (ACh as an agonist). $n = 4$ observations from 4 rats. $x \pm s$.

Antagonist	Control		Elcatonin	
	pA_2 value	Slope	pA_2 value	Slope
Pirenzepine	6.99 ± 0.09	1.03 ± 0.19	6.77 ± 0.22	0.93 ± 0.19
AF-DX 116	6.30 ± 0.19	1.03 ± 0.16	6.51 ± 0.19	1.03 ± 0.16
4-DAMP	9.41 ± 0.08	0.90 ± 0.25	9.26 ± 0.18	0.90 ± 0.25

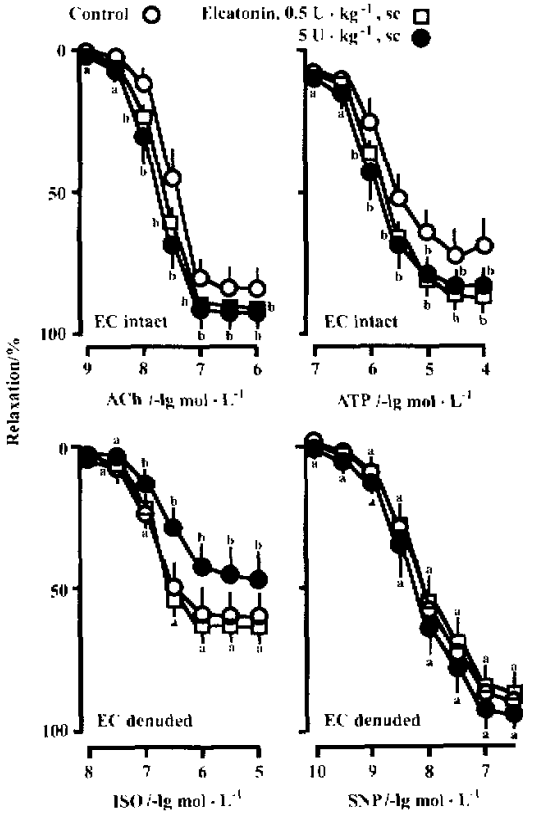

Fig 2. The cumulative concentration-response curves for ACh, ATP, ISO, and SNP in femoral arteries from rats treated with elcatonin at doses of 0.5 (□) and 5 (●) U/kg. Control, ○. Ordinate; papaverine-induced relaxations are expressed as 100 %, and the absolute values for the response to papaverine were 294 mg ± 85 mg (n = 18 arteries, top panels) and 238 mg ± 91 mg (n = 12 – 16 arteries, bottom panels). $x \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Schild plots did not differ significantly from unity.

DISCUSSION

Antihypertensive effects of calcitonin and elcatonin have been demonstrated in deoxycorticosterone-hypertensive rats and SHR, respectively[9,10]. In addition, these peptides could decrease or increase local blood flow in man[6,11-13,18] and animals[16,27]. On the contrary, it was shown that calcitonin changed neither blood pressure in SHR[28] and in normotensive rats[29] nor the bone blood flow increase observed in paraplegic rats[30]. Thus, the vascular effects of these peptides seemed to be dependent upon species, vascular regions, and experimental protocols as well as the source of peptides. Since there was the heterogeneity of vascular smooth muscle cells in their responsiveness to vasoactive substances, we attempted to determine effects of elcatonin on contractile and relaxant responses of femoral arterial preparations from SHR to vasoactive drugs.

SHR had sc application of elcatonin at the doses of 0.5 and 5 U/kg, 3 times a week for 2 weeks, since these doses had been reported to decrease blood pressure in the hypertensive rats[9,10]. We found that elcatonin did not decrease systemic blood pressure in SHR. The discrepancy between the present and earlier observations remains unclear.

Elcatonin or calcitonin did not affect vasocontractile responses to NE, angiotensin II, and vasopressin in rats and dogs[10,16]. We expanded this finding by demonstrating that elcatonin did not alter the contractile responses of the rat femoral artery to KCl, NE, 5-HT, and $PGF_{2\alpha}$.

We have previously reported that β-adrenoceptor-mediated, endothelium-independent relaxation of femoral artery of SHR is diminished as compared with that of normotensive rats[24,31]. Elcatonin at 5 U/kg but not 0.5 U/kg further attenuated ISO-induced relaxation in the endothelium-denuded artery of SHR. Similarly, calcitonin inhibited parathyroid hormone-induced vasodilation which was mediated by cyclic AMP in dogs[27]. The mechanism underlying the diminished ISO-induced relaxation in the elcatonin-treated SHR remains a matter for speculation. It was probable that repeated administrations of elcatonin led to parathyroid stimulation, with secondary increases in cyclic AMP production. Alternatively, like calcitonin[17], elcatonin activated adenylate cyclase through its receptor. In fact, elcatonin increased urinary excretion of cyclic AMP in man[5]. The increase in cyclic AMP concentrations, if any in the femoral artery, then might cause down-regulation of β-adrenoceptors through an activation of PKA.

ACh and ATP elicited endothelium-dependent relaxations of the rat femoral artery[26]. We suggested that the relaxation was due to stimulated releases of prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) from endothelial cells[32,33]. In the present experiments, however, the KHB buffer contained indomethacin to avoid a possible production of prostacyclin. Therefore, we could rule out the possibility that, the ACh- and ATP-induced relaxation was accounted for evoking the release of prostacyclin. We found that, in the control artery, the ACh-induced

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

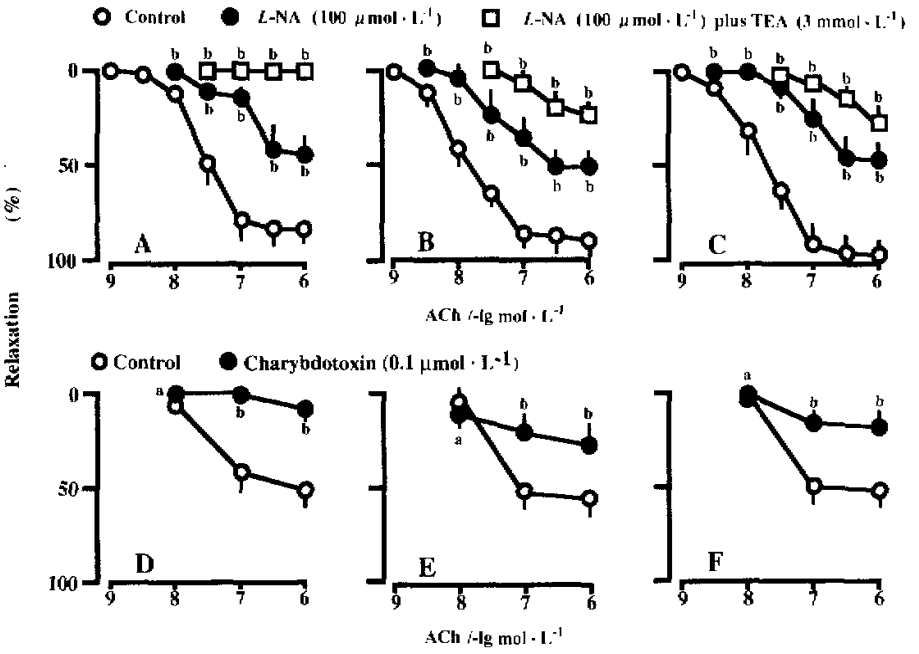

Fig 3. Effects of *L*-NA, TEA, and charybdotoxin on the ACh-induced relaxation in femoral arteries from control (panels A and D), elcatonin (0.5 U/kg; panels B and E), and elcatonin (5 U/kg; panels C and F)-treated rats. The experiments were carried out in the presence of indomethacin (panels A, B, and C) and in the presence of indomethacin and *L*-NA (panels D, E and F). In top panels, control (○), *L*-NA-treated (●), and *L*-NA and TEA-treated (□). In bottom panels, control (○) and charybdotoxin-treated (●). Ordinate, papaverine-induced relaxation are expressed as 100 %. $n = 5-8$ arteries. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

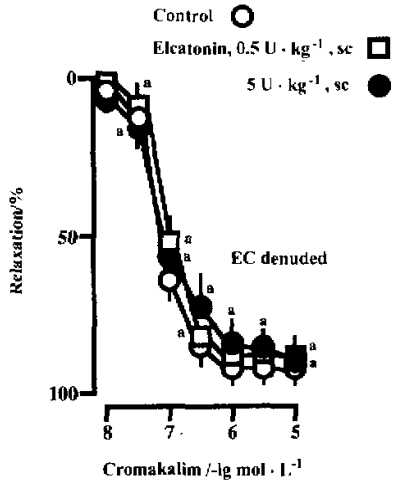

Fig 4. Effects of elcatonin on cromakalim-induced relaxation of femoral arteries. The rats were treated with saline (○) and elcatonin at doses of 0.5 (□) and 5 (●) U/kg. Ordinate, the absolute value (100 %) were 203 mg ± 44 mg. $n = 12$ arteries. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

relaxation was partly reduced by *L*-NA (an inhibitor of NO synthase) and the residual relaxation due to ACh in the presence of *L*-NA was completely abolished by TEA or charybdotoxin. Since the relaxation mediated by EDHF was reduced by Ca^{2+}-activated K^+ channel inhibitors including TEA and charybdotoxin in certain blood vessels[33,34], the present results suggest that the ACh-induced relaxation is mediated by NO and EDHF in the femoral artery of SHR. On the contrary, the contribution of EDHF to the ACh-induced relaxation was reported to be absent in mesenteric resistance artery of SHR[35]. This discrepancy may be in part explained by differences in rat strains and vascular regions between our and their studies. ACh-induced relaxation was impaired in blood vessels of SHR as compared with that in normotensive rats[36]. Elcatonin at 0.5 U/kg increased the relaxant response of the SHR femoral artery to ACh and ATP. The response was not further changed when the dose of elcatonin was increased to 5 U/kg, suggesting that the lower dose increases the response to a maximum extent. In the presence of *L*-NA, the response to ACh

in the elcatonin-treated rats (Fig 3B and C) was similar to that in the control rats (Fig 3A), and elcatonin did not alter relaxant response to SNP, a NO donor. These results suggest that the increased response to ACh in the artery treated with elcatonin is due to increased NO production or release from the endothelium rather than changes in the responsiveness of vascular smooth muscles to NO. Unlike that in the control artery, the ACh-induced, *L*-NA-resistant relaxation in the elcatonin-treated rat artery was partly attenuated but not abolished by TEA or charybdotoxin, suggesting that the relaxation to ACh in the elcatonin-treated rats involves an endothelium-derived relaxing factor distinct from NO and EDHF.

The response of the endothelium-denuded artery to cromakalim (an ATP-sensitive K^+ channel opener) was not altered by elcatonin, suggesting that the peptide does not change the relaxation mediated by ATP-sensitive K^+ channels.

We then determined whether or not the ACh-induced relaxation in the elcatonin-treated rat artery was mediated through M_3 subtypes of muscarinic cholinoceptors. The Schild plots for 3 muscarinic cholinoceptor antagonists tested were linear, with slopes of unity. pA_2 values obtained were similar to values in artery where ACh-induced relaxation was mainly mediated through M_3 subtypes of muscarinic receptors[26]. Since pA_2 values and slopes of the Schild plots were not significantly changed by elcatonin, the muscarinic cholinoceptor subtypes involved in the ACh-induced relaxation were M_3 in the elcatonin-treated as well as the control femoral arteries.

Since calcitonin and elcatonin in blood were reduced to half by 10 min – 30 min and disappeared within 4 h after iv injection[12,37,38], the effects of the peptides after repeated injections may be accounted for long-lasting alterations in neural activities or the development of a new vascular architecture. At present, it remains unclear whether or not elcatonin functions as a hypocalcemic hormone in SHR, since we did not estimate plasma Ca^{2+} concentrations and therefore we could not rule out the possibility that the effects of elcatonin observed herein were secondary to altered Ca^{2+} metabolism. The doses of elcatonin used in the present experiments were quite comparable with recommended doses of the peptide in patients with osteoporosis and other metabolic bone diseases (0.2 – 1 U/kg). Consequently, the results obtained will provide information about the vasorelaxant profile of elcatonin in these patients.

In summary, elcatonin at the doses which did not alter systemic blood pressure in SHR, attenuated the β-adrenoceptor-mediated, endothelium-independent relaxation, and increased the ACh- and ATP-induced, endothelium-dependent relaxation in femoral arteries. Elcatonin may alter local blood flow by changing vascular responsiveness to these substances.

ACKNOWLEDGEMENT Elcatonin ([Asu^{1,7}] eel calcitonin) was generously donated by Asahi Chemical Industry Co, Ltd (Tokyo, Japan).

REFERENCES

1 Marcus R. Agents affecting calcification and bone turnover. In: Hardman, JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. The pharmacological basis of therapeutics; ninth edition. New York; McGraw-Hill; 1995. p 1519 – 53.

2 Wootton R, Tellez M, Green JR, Reeve J. Skeletal blood flow in Paget's disease of bone. Metab Bone Dis Relat Res 1981; 3: 263 – 70.

3 Morikawa T, Munekata E, Sakakibara S, Noda T, Otani M. Synthesis of eel-calcitonin and [Asu^{1,7}]-eel-calcitonin; contribution of the disulfide bond to the hormonal activity. Experientia 1976; 32: 1104 – 6.

4 Yamamoto K, Maeyama I, Kishimoto H, Hagino H, Teshima R, Nishimura A. Effect of [Asu^{1,7}]-eel calcitonin (elcatonin) on bone mass and serum calcium in patients with primary osteoporosis. Jpn J Bone Metab 1986; 1: 89 – 99.

5 Nuti R, Vattimo A, Martini G, Turchetti V, Righi GA. Carbocalcitonin treatment in Sudeck's atrophy. Clin Orthop 1987; (215): 217 – 22.

6 Orimo H, Morii H, Inoue T, Yamamoto K, Minaguchi H, Ishii Y, *et al*. Effect of elcatonin on involutional osteoporosis. J Bone Miner Metab 1996; 14: 73 – 8.

7 Hori M, Takahashi H, Konno T, Inoue J, Haba T, Sakurada T, *et al*. Effect of elcatonin on experimental models for osteoporosis due to ovariectomy and low-calcium diet in dogs. Folia Pharmacol Jpn 1984; 84: 91 – 8.

8 Nakatsuka K, Nishizawa Y, Hagiwara S, Koyama H, Miki T, Ochi H, *et al*. Effect of calcitonin on total body bone mineral contents of experimental osteoporotic rats determined by dual photon absorptiometry. Calcif Tissue Int 1990; 47: 378 – 82.

9 Aldored JP, Luna PD, Zeedy RA, Bastian JW. Inhibition by salmon calcitonin (SCT) of desoxycorticosterone acetate (DOCA) induced hypertension in the rat. Proc Soc Exp Biol Med 1976; 152: 557 – 9.

10 Takayanagi N, Tozuka T, Toda N. Antihypertensive action of elcatonin in rats. Folia Pharmacol Jpn 1983; 82: 383 – 93.

11 Luengo M, Picado C, Del Rio L, Guanabens N, Montserrat JM, Setoain J. Treatment of steroid-induced osteopenia with calcitonin in corticosteroid-dependent asthma. A one-year

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

follow-up study. Ann Rev Respir Dis 1990; 142: 104 – 7.
12 Takeyama M, Ikawa K, Nagano T, Mori K. Elcatonin raises levels of vasoactive intestinal peptide in human plasma. J Pharm Pharmacol 1996; 48: 657 – 9.
13 Edwards IR, Smith AJ. Porcine calcitonin as a renal vasodilator in man. Clin Endocrinol 1972; 1: 837 – 50.
14 *Cantatore FP, Carrozzo M, Loperfido MC, D'Amore M,* Pipitone V. Effetti delle calcitonine sul metabolismo piastrinico. Ann Ital Med Int 1988; 3: 175 – 9.
15 Ceserani R, Colombo M, Olgiati VR, Pecile A. Calcitonin and prostaglandin system. Life Sci 1979; 25: 1851 – 6.
16 Driessens A, Vanhoutte PM. Effect of calcitonin, hydrocortisone, and parathyroid hormone on canine bone blood vessels. Am J Physiol 1981; 241: H91 – 4.
17 Hakim A. Effect of human calcitonin on the sarcoplasmic reticulum of human heart. Nat Wissenschaften 1973; 60: 53 – 60.
18 Porter RW, Hibbert C. Calcitonin treatment for neurogenic claudication. Spine 1983; 8: 585 – 92.
19 Chakraborty M, Chatterjee D, Kellokumpu S, Rasmussen H, Baron R. Cell cycle-dependent coupling of the calcitonin receptor to different G proteins. Science 1991; 251: 1078 – 82.
20 Suzuki H, Nakamura I, Takahashi N, Ikuhara T, Matsuzaki K, Isogai Y, *et al*. Calcitonin-induced changes in the cytoskeleton are mediated by a signal pathway associated with protein kinase A in osteoclasts. Endocrinology 1996; 137: 4685 – 90.
21 Izawa Y, Sagara K, Kadota T, Makita T. Bone disorders in spontaneously hypertensive rat. Calcif Tissue Int 1985; 37: 605 – 7.
22 Lucas PA, Brown RC, Drueke T, Lacour B, Metz JA, McCarron DA. Abnormal vitamin D metabolism, intestinal calcium transport, and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. J Clin Invest 1986; 78: 221 – 7.
23 Fujimoto S. α_1-Adrenoceptor subtypes mediating contraction of the femoral artery in spontaneously hypertensive rats. Can J Physiol Pharmacol 1994; 72: 862 – 9.
24 Fujimoto S, Fujimoto KS, Moriyama A. Diminished β-adrenoceptor-mediated relaxation of femoral arteries from young spontaneously hypertensive rats. J Auton Nerv Syst 2001; 87: 178 – 86.
25 Fujimoto S, Dohi Y, Aoki K, Matsuda T. Altered vascular beta adrenoceptor-mediated relaxation in deoxycorticosterone-salt hypertensive rats. J Pharmacol Exp Ther 1988; 244: 716 – 23.
26 Fujimoto S, Matsuda T. M_3 cholinoceptors and P_{2y} purinoceptors mediating relaxation of arteries in spontaneously hypertensive rats at prehypertensive stages. Eur J Pharmacol 1991; 202: 9 – 15.
27 Charbon GA, Pieper EEM. Effect of calcitonin on parathyroid hormone-induced vasodilatation. Endocrinology 1972; 91: 828 – 32.
28 Wegener LL, McCarron DA. Acute intravenous calcitonin: Failure to modify systemic blood pressure. Peptides 1988; 9: 1191 – 3.
29 Clement G, Rapisarda E, Fiore CE, Prato A, Amico-Roxas M, Millia C, *et al*. Effects of salmon calcitonin on plasma renin activity and systolic blood pressure in the rat. Neurosci Lett 1986; 66: 351 – 5.
30 Schoutens A, Verhas M, Dourov N, Bergmann P, Caulin F, Verschaeren A, *et al*. Bone loss and bone blood flow in Paraplegic rats treated with calcitonin, diphosphonate, and indomethacin. Calcif Tissue Int 1988; 42: 136 – 43.
31 Fujimoto S, Dohi Y, Aoki K, Matsuda T. Beta-1 and beta-2 adrenoceptor-mediated relaxation responses in peripheral arteries from spontaneously hypertensive rats. J Hypertens 1988; 6: 543 – 50.
32 Fujimoto S, Itoh T. Role of nitric oxide and nitric oxide-independent relaxing factor in contraction and relaxation of rabbit blood vessels. Eur J Pharmacol 1997; 330: 177 – 84.
33 Fujimoto S, Ikegami Y, Isaka M, Kato T, Nishimura K, Itoh T. K^+ channel blockers and cytochrome P450 inhibitors on acetylcholine-induced, endothelium-dependent relaxation in *rabbit mesenteric artery*. *Eur J Pharmacol* 1999; 384: 7 – 15.
34 Petersson J, Zygmunt PM, Hogestatt ED. Characterization of the potassium channels involved in EDHF-mediated relaxation in cerebral arteries. Br J Pharmacol 1997; 120: 1344 – 50.
35 Li J, Bian K, Bukoski RD. A non-cyclo-oxygenase, non-nitric oxide relaxing factor is present in resistance arteries of normotensive but not spontaneously hypertensive rats. Am J Med Sci 1994; 307: 7 – 14.
36 Sunano S, Watanabe H, Tanaka S, Sekiguchi F, Shimamura K. Endothelium-derived relaxing, contacting and hyperpolarizing factors of mesenteric arteries of hypertensive and normotensive rats. Br J Pharmacol 1999; 126: 709 – 16.
37 Yamauchi H, Shiraki M, Otani M, Matsuo M, Orimo H. Stability of [$Asu^{1,7}$]-eel calcitonin and eel calcitonin *in vitro* and *in vivo*. Endocrinol Jpn 1977; 24: 281 – 5.
38 Huwyler R, Born W, Ohnhaus EE, Fischer JA. Plasma kinetics and urinary excretion of exogenous human and salmon calcitonin in man. Am J Physiol 1979; 236: E15 – 9.

依降钙素在 SHR 股动脉介导的收缩和舒张反应

藤本征五[1], 藤本啓子[2]
(名古屋市立大学医学院药理教研室; [2]自然科学研究所, 名古屋 467-8601, 日本国)

关键词 乙酰胆碱; β肾上腺素受体; 胆碱能受体; 依降钙素; 血管内皮; 股动脉; 一氧化氮; 去甲肾上腺素; 近交 SHR 大鼠

(责任编辑 吕 静)