Br. J. Pharmacol. (1993), **110**, 1437–1440

Mechanism of rat uterine smooth muscle contraction induced by endothelin-1

[1]Hajime Tsunoda, *Takashi Miyauchi, Kayo Fujita, Takeshi Kubo & *Katsutoshi Goto

Department of Obstetrics and Gynecology, Institute of Clinical Medicine, and *Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

1 Endothelin (ET)-1 has been demonstrated to cause contraction of uterine smooth muscle. We investigated the role of ET receptor subtypes (ET_A and ET_B receptors) in ET-1-induced contraction of rat uterine smooth muscle by using the ET_A receptor antagonist BQ-123 and the ET_B receptor agonist BQ-3020.

2 ET-1 caused a contraction with superimposed oscillations of the rat isolated uterus suspended in Krebs-Ringer solution; both the amplitude of contraction as well as the oscillation frequency increased in a dose-dependent manner (10^{-11}–10^{-7} M).

3 BQ-123 (10^{-6} M) markedly shifted the dose-response curve of ET-1 for both contractile effects and oscillation frequency to the right.

4 BQ-3020 (10^{-11}–3×10^{-7} M) did not cause uterine contraction; neither did it affect the dose-response curve of ET-1 for either the contractile effect or the increase in oscillation frequency. Thus, stimulation of ET_B receptors is not involved in these responses.

5 The present findings suggest that ET-1-induced contractile responses and the increase in oscillation frequency in rat uterine smooth muscle is mediated through ET_A receptors, and that ET_B receptors play no role in these responses.

Keywords: Endothelin-1; ET_A receptor; ET_B receptor; BQ-123; BQ-3020; uterus; contraction

Introduction

Endothelin (ET)-1, a potent vasoconstrictor peptide, was first isolated from culture supernatant of endothelial cells (Yanagisawa *et al.*, 1988). Further studies revealed the existence of two related peptides, ET-2 and ET-3 (Yanagisawa & Masaki, 1989), and of two distinct ET receptor subtypes termed the ET_A receptor (ET-1 selective) and the ET_B receptor (equally sensitive to isopeptides of the endothelin family) (Arai *et al.*, 1990; Sakurai *et al.*, 1990; 1992). These peptides produce contractions of smooth muscle from various vascular and nonvascular tissues, such as the uterus, trachea, and stomach (Yanagisawa & Masaki, 1989; Sakurai *et al.*, 1992). It has been reported that ET-1-induced contraction of the porcine coronary artery is mediated mainly through ET_A receptors (Ihara *et al.*, 1992a), whereas ET-1-induced contraction of the guinea-pig trachea has been shown to be mediated mainly through ET_B receptors (Takai *et al.*, 1992).

Although it has been demonstrated that ET-1 causes contraction of the rat uterus (Bousso-Mittler *et al.*, 1989), the roles of the endothelin receptor subtypes (ET_A and ET_B receptors) in this response have not been established. In the present study, contraction of the rat uterus in response to ET-1 was analyzed by using the ET_A receptor antagonist BQ-123 (Ihara *et al.*, 1992a) and the ET_B receptor agonist BQ-3020 (Ihara *et al.*, 1992b).

Methods

Preparation and solutions

Virgin female Sprague-Dawley rats (200–250 g) were injected subcutaneously with 0.2 mg of β-oestradiol 24 h before the experiments. After anaesthetization with sodium pentobarbitone (50 mg kg^{-1}, i.p.), the uterine horns were removed and mounted in an organ bath containing 5 ml of Krebs-Ringer solution of the following composition (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.2, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.5. The Krebs-Ringer solution was maintained at 37°C and aerated with a mixture of 95% O_2–5% CO_2.

Mechanical activity

The isometric contraction of the uterus was measured with a force-displacement transducer (TB-612T, Nihon Kohden, Tokyo, Japan) connected to a polygraph system (amplifier, AP-601G, Nihon Kohden; thermal-pen recorder, WT-687G, Nihon Kohden). A resting tension of 1 g was applied to the tissue, and an equilibration period of 2 h was allowed during which the tissue was washed with fresh solution every 15 min. After equilibration, a maximum response to KCl (50 mM) was established repeatedly at 30 min intervals until a steady response was obtained (usually, three to four times). Thereafter, the dose-response relationship for ET-1 or BQ-3020 was determined by means of cumulative application. To examine the effects of BQ-123 on the dose-response curve of ET-1, BQ-123 (10^{-6} M) was applied 15 min before the cumulative application of ET-1. For normalization, the responses to ET-1 or BQ-3020 were expressed as % of the maximum response to KCl. Since ET-1 also induced oscillation-like contractions, we measured both the frequency of the oscillation and the increase in peak tension. To study the effect of BQ-3020 on the ET-1-induced uterine contraction, the dose-response curve of ET-1 in the presence of BQ-3020 was also determined.

Statistical analysis

Values are expressed as mean ± s.e.mean. Statistical analyses were performed by an unpaired Student's *t* test and a *P* value of less than 0.05 was considered significant.

Drugs

ET-1 was purchased from Peptide Institute Inc. (Osaka. Japan). The selective ET_A receptor antagonist BQ-123 (cyclo

[1] Author for correspondence.

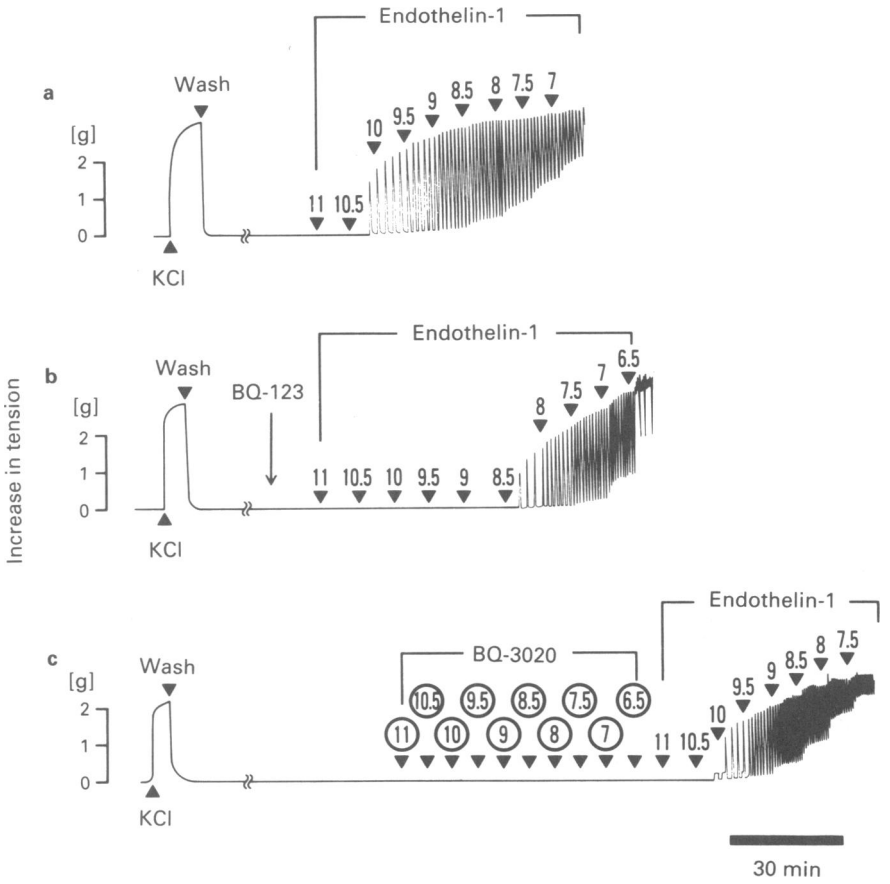

Figure 1 Representative traces of the responses to increasing concentrations of endothelin-1 of the rat uterus in the absence (a) or presence (b) of the ET_A receptor antagonist BQ-123 (10^{-6} M). As shown in (c), although the ET_B receptor agonist BQ-3020 caused no contraction of the uterus, ET-1 caused a contraction of the same tissue. Numbers, which are not encircled, in the figure indicate the − log molar concentration of endothelin-1. Encircled numbers in (c) indicate the − log molar concentration of BQ-3020. The transverse bar indicates time.

(-D-Trp-D-Asp-Pro-D-Val-Leu)), and the selective ET_B receptor agonist BQ-3020 (N-acetyl-LeuMetAspLysGluAlaValTyrPheAlaHisLeuAspIleIleTry) were synthesized at Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan).

Results

ET-1 produced dose-dependent contraction of the rat uterus (Figures 1a and 2a). This response to ET-1 included oscillation-like contractions superimposed on an increase in baseline tension. Since this oscillation-like response sometimes lacked the relaxation phase and became a tetanic contraction, we measured the contractile tension only at the peak of the systolic phase for the analysis in Figures 2a, 3 and 4. The dose-response relationship for the contractile effects of ET-1 on the rat uterus are shown in Figure 2a; the pD_2 value was 9.99 ± 0.26, $n = 7$. In the presence of BQ-123 (10^{-6} M), an ET_A receptor antagonist, the dose-response curve for the contractile effects of ET-1 was shifted significantly to the right (Figures 1b and 2a).

The calculated pK_B value of BQ-123 for antagonizing ET-1-induced contractile responses was 7.51 ± 0.04. ET-1 also caused an increase in the oscillation frequency in a dose-dependent manner (Figures 1a and 2b). Since ET-1-induced uterine contraction sometimes lacked the relaxation phase when ET-1 concentrations were high, we quantified oscillation frequency only up to a concentration of 10^{-8} M (Figure 2b). In the presence of BQ-123 (10^{-6} M), the dose-oscillation-frequency curve for ET-1 was shifted significantly to the right (Figure 2b). The calculated pK_B value of BQ-123 for blockade of the ET-1-induced increase in oscillation frequency was 7.39 ± 0.11.

BQ-3020 ($10^{-11} - 3 \times 10^{-7}$ M), a selective ET_B receptor agonist, produced no contraction of the uterine smooth muscle (Figures 1c and 3). Furthermore, BQ-3020 (3×10^{-7} M) did not modify the dose-response curve of ET-1 for either the contractile effect or the increase in oscillation frequency (Figure 4).

Discussion

We have shown that the ET_A receptor antagonist, BQ-123, effectively antagonized ET-1-induced contraction of the uterine smooth muscles, and that the ET_B receptor agonist BQ-3020 produced no contractile response on the tissues. Since it has been demonstrated that the potency of BQ-3020 with respect to stimulation of ET_B receptors is almost the same as that of ET-1 (Ihara *et al.*, 1992b), the dose of BQ-3020 used in the present study ($10^{-11} - 3 \times 10^{-7}$ M) was considered to be appropriate for the stimulation of ET_B receptors. We have also shown that BQ-123 effectively antagonizes the ET-1-induced increase in the oscillation frequency of the rat uterus. These data suggest that only ET_A receptors play a role in uterine contraction induced by ET-1 in rats, and that ET_B receptors are not involved in this response. The pK_B value (7.51) of BQ-123 for blockade of the ET-1-induced increase in the contraction was similar to that for antagonism of the ET-1-induced increase in the oscillation frequency (7.39). These pK_B values are similar to the pA_2 value (7.4) which has been determined for blockade of ET-1 by BQ-123 on the porcine isolated coronary artery (Ihara *et al.*, 1992a). Thus, it

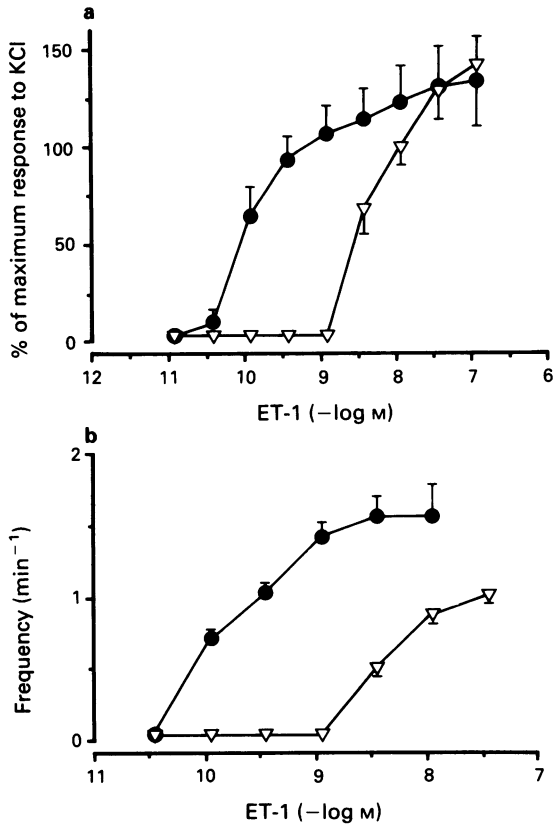

Figure 2 Dose-response curves for the contractile effects (a) and increase in oscillation frequency (b) induced by ET-1 on the rat uterus in the absence (●; $n = 7$) or presence of the ET_A receptor antagonist BQ-123 (10^{-6} M; ▽; $n = 7$). In (a), the contractile effects on the rat uterine smooth muscle are expressed as % of maximum contraction to 50 mM KCl. In (b), oscillation frequency was calculated per min for each dose until the contraction of some tissues became tetanic (up to 10^{-8} M). Points and vertical lines represent means ± s.e.mean, respectively.

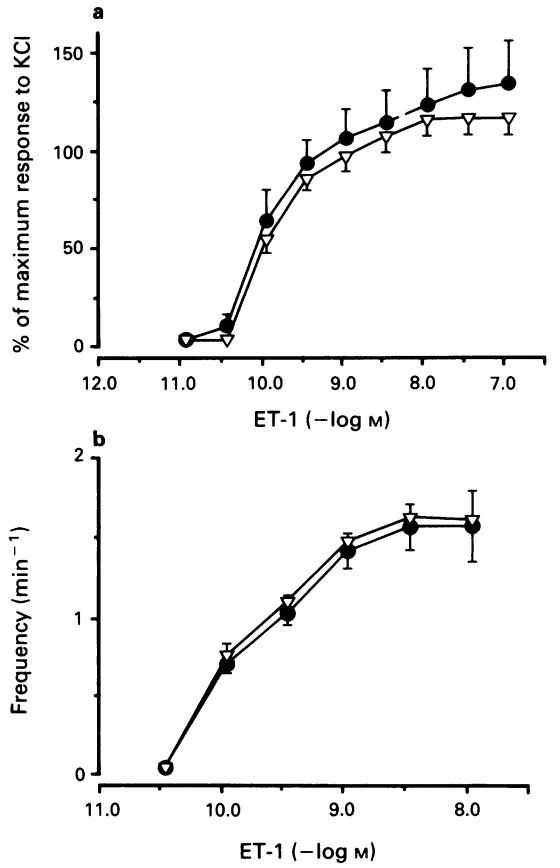

Figure 4 Dose-response curves for the contractile effects (a) and oscillation frequency (b) of ET-1 on the rat uterus in the absence (●; $n = 7$) or presence (▽; $n = 7$) of the ET_B receptor agonist BQ-3020 (3×10^{-7} M). In (b), oscillation frequency was calculated per min for each concentration until the contraction of some tissues became tetanic (up to 10^{-8} M). Points and bars represent means ± s.e.mean, respectively.

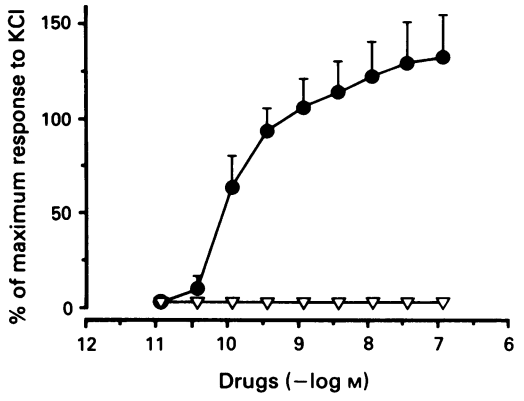

Figure 3 Effects of increasing doses of the ET_B receptor agonist BQ-3020 (▽; $n = 7$) or ET-1 (●; $n = 7$) on the rat uterus. Points show means and vertical lines s.e.mean.

is suggested that the ET-1-induced increase in both the contraction and the oscillation frequency is mediated through ET_A receptors.

The ET_A receptor shows far higher affinity for ET-1 than ET-3, whereas the ET_B receptor exhibits a high affinity for both ET-1 and ET-3 (Arai *et al.*, 1990; Sakurai *et al.*, 1990; 1992). Bousso-Mittler *et al.* (1989) and Sakata & Kuraki (1992) demonstrated that the potency of ET-1 for causing contraction of the rat uterus is markedly higher than that of ET-3. Based on these results, they speculated that the ET-1-induced contraction of the rat uterus is mediated mainly through ET_A receptors. The present data obtained using an ET_A receptor antagonist support this conclusion.

An important finding in the present study is that BQ-3020, a selective ET_B receptor agonist, had no effects *per se* on rat uterus. Further, neither the increases in the contractile tension nor the increases in oscillation frequency induced by ET-1 were affected by BQ-3020 in the rat uterus. Therefore, it can be concluded that activation of ET_B receptors neither stimulates the rat uterus nor affects the ET_A receptor-mediated uterine contraction. However, since the expression of ET_B receptor mRNA has been demonstrated in the rat uterus (Sakurai *et al.*, 1990), it is possible that ET_B receptors may play a role other than inducing contraction of the smooth muscle in this tissue.

It has been shown that ET-1 is produced by human endometrial tissues (Economos *et al.*, 1992). Furthermore, Usuki *et al.* (1990) reported that the maternal circulating ET-1 level increases at the end of pregnancy and during labour, and that a large amount of immunoreactive ET-1 exists in human amniotic fluid. These findings suggest that ET-1 may have a role in the modulation of the uterine function. Since ET-1 showed extremely potent contractile effects on estrogen-dominated uterine smooth muscles in rats through activation of ET_A receptors, possible alteration in the function and expression of ET receptor subtypes during the later stages of pregnancy may be relevant to the marked increase in drug sensitivity in this period. This possibility is now under investigation in our laboratory.

This work was supported by a grant of the Special Research Project on the Circulation Biosystem in the University of Tsukuba and by the University of Tsukuba Project Research. We thank Drs Masaru Nishikibe and Mitsuo Yano, at Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba, Japan for useful discussions and for the generous gifts of BQ-123 and BQ-3020. We also thank Mrs Yumiko Kawakura for her excellent technical assistance.

References

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H. & NAKANISHI, S. (1990). Cloning and expression of cDNA encoding an endothelin receptor. *Nature*, **348**, 730–732.

BOUSSO-MITTLER, D., KLOOG, Y., WOLLBERG, Z., BDOLAH, A., KOCHVA, E. & SOKOLOVSKY, M. (1989). Functional endothelin/sarafotoxin receptors in the rat uterus. *Biochem. Biophys. Res. Commun.*, **162**, 952–957.

ECONOMOS, K., MACDONALD, P.C. & CASEY, M.L. (1992). Endothelin-1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. *J. Clin. Endocrinol. Metab.*, **74**, 14–19.

IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKIBE, M. & YANO, M. (1992a). Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.*, **50**, 247–255.

IHARA, M., SAEKI, T., FUKURODA, T., KIMURA, S., OZAKI, S., PATEL, A.C. & YANO, M. (1992b). A novel radioligand [^{125}I]BQ-3020 selective for endothelin (ET_B) receptors. *Life Sci.*, **51**, 47–52.

SAKATA, K. & KARAKI, H. (1992). Effects of endothelin on cytosolic Ca^{2+} level and mechanical activity in rat uterine smooth muscle. *Eur. J. Pharmacol.*, **221**, 9–15.

SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., KIMURA, S., GOTO, K. & MASAKI, T. (1990). Cloning of a cDNA encoding a non-isopeptide-selective subtype of endothelin receptor. *Nature*, **348**, 732–735.

SAKURAI, T., YANAGISAWA, M. & MASAKI, T. (1992). Molecular characterization of endothelin receptors. *Trends Pharmacol. Sci.*, **13**, 103–108.

TAKAI, M., UMEMURA, I., YAMASAKI, K., WATANABE, T., FUJITANI, Y., ODA, K., URADE, Y,. INUI, T., YAMAMURA, T. & OKADA, T. (1992). A potent and specific agonist, Suc-[Glu^9, Ala^{11,15}]-endothelin-1(8–21), IRL 1620, for the ET_B receptor. *Biochem. Biophys. Res. Commun.*, **184**, 953–959.

USUKI, S., SAITOH, T., SAWAMURA, T., SUZUKI, N., SHIGEMITU, S., YANAGISAWA, M., GOTO, K., ONDA, H., FUJINO, M. & MASAKI, T. (1990). Increased maternal plasma concentration of endothelin-1 during labor pain or on delivery and the existence of a large amount of endothelin-1 in amniotic fluid. *Gynecol. Endocrinol.*, **4**, 85–97.

YANAGISAWA, M., KURIHARA, H,. KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 441–445.

YANAGISAWA, M. & MASAKI, T. (1989). Molecular biology and biochemistry of the endothelins. *Trends Pharmacol. Sci.*, **10**, 374–378.

(Received July 13, 1993
Revised August 9, 1993
Accepted August 18, 1993)