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Endothelin ET_A receptors mediate human uterine smooth muscle contraction

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Abstract

Receptors mediating endothelin-induced contraction of myometrium were investigated in the human uterus. Endothelin-1 and endothelin-3 (10 pM to 0.3 μM) caused concentration-dependent contraction of myometrial strips. Endothelin-1 was approximately ten times more potent than endothelin-3, with pD_2 values of 8.24 and 7.20, respectively. By contrast, two endothelin ET_B receptor selective agonists, BQ 3020 (*N*-acetyl-[$Ala^{11,15}$]endothelin-1-(6–21)) and sarafotoxin 6c (up to 0.3 μM), did not induce contraction of human myometrium. The endothelin ET_A receptor selective antagonist, FR139317 (1-hexahydroazepino-CO-Leu-D-Trp(CH_3)-D-(2-pyridyl)alanine) (0.1, 0.3 and 1 μM), competitively antagonized the endothelin-1-elicited contraction, with a pA_2 value of 7.10, whereas another endothelin ET_A receptor-selective blocking drug, BQ 123 [cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu)], behaved as a non-competitive antagonist. Pretreatment of myometrial strips with an endothelin ET_B receptor selective antagonist, IRL 1038 ([Cys^{11}-Cys^{15}]endothelin-1-(11–21)), had no effect on contractions induced by endothelin-1. All these data indicate that only endothelin ET_A receptors mediate endothelin-1-induced contractions of human myometrium.

Keywords: Endothelin; Endothelin ET_A receptor; Endothelin ET_A receptor selective antagonist; Myometrium, human

1. Introduction

Numerous studies have provided evidence for the involvement of endothelins in contraction of various smooth muscles including blood vessels and uterus (see review: Masaki, 1993). The physiological effects of endothelins are mediated by at least two types of G-protein-coupled receptors, ET_A and ET_B, which have been cloned and sequenced. The endothelin ET_A receptor has a greater affinity for endothelin-1 than for endothelin-3 (Arai et al., 1990), whereas endothelin the ET_B receptor does not discriminate between the two peptides (Sakurai et al., 1990). Until recently, only the endothelin ET_A subtype was believed to be involved in endothelin-1-induced vasoconstriction (Schoeffter et al., 1993). However, with the development of compounds showing selectivity for endothelin ET_A and ET_B receptors, some studies have suggested that endothelin ET_B receptors are also capable of mediating contraction in some parts of the vascular bed (Sumner et al., 1992; La Douceur et al., 1993). Although it has been demonstrated that endothelin-1 causes contraction of the human uterus (Word et al., 1990; Wolff et al., 1993), the roles of endothelin receptor subtypes (ET_A and ET_B) in this response remain to be established. The uterus contains a predominance of endothelin ET_A receptors and several lines of evidence have recently suggested that endothelin-1 activates primarily endothelin ET_A receptors to produce uterine contractility in rat and premenopausal women (Rae et al., 1993; Tsunoda et al., 1993; Do Khac et al., 1994; Bacon et al., 1995). In addition, we previously reported the presence of both endothelin ET_A and ET_B receptors coupled to phospholipase C in the human myometrium (Breuiller-Fouché et al., 1994). To assess the disparate roles of the multiple endothelin receptor subtypes in uterine contractility, it is important to study the precise pharmacological properties of the two individual endothelin receptor subtypes. The aim of the present study was to characterize pharmacologically the endothelin receptor subtype(s) that mediate the

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contraction of the human myometrium by using endothelin ET_A receptor-selective antagonists (e.g. BQ 123, [cyclo-(D-Trp-D-Asp-Pro-D-Val-Leu)], Ihara et al., 1992; and FR 139317, (1-hexahydroazepino-CO-Leu-D-Trp(CH_3)-D-(2-pyridyl)alanine), Aramori et al., 1993) and an endothelin ET_B receptor-selective antagonist (e.g. IRL 1038, ([Cys^{11} – Cys^{15}]endothelin-1-(11–21)), Urade et al., 1992).

2. Materials and methods

2.1. Experimental protocol

Myometrial tissue was obtained from 23 cycling women (42–54 years old) undergoing hysterectomy for benign gynecological indications such as leiomyomas. The operations were performed with the patients under thiopental sodium-succinylcholine anesthesia.

Tissue samples were excised with a scalpel from the uterine corpus from normal muscle (myometrial outer layer); they were dissected free of serosa and immediately placed in physiological salt solution with the following composition (mM): glucose 2.8; KCl 6.2; NaCl 144; $CaCl_2$ 2.5; $MgCl_2$ 0.5; NaH_2PO_4 1; $NaHCO_3$ 30.

Uterine strips ($5–7 \times 2 \times 1$ mm) were processed in groups of eight. They were suspended in 10-ml organ baths containing physiological solution at 32°C bubbled with 95% O_2 and 5% CO_2, resulting in a final pH of 7.4. Contractile activity was measured isometrically using Bioscience UF1 tension transducers and recorded on an IBM-PC and Gould recorder. The preparations were allowed to equilibrate for 2–3 h under a resting tension of 0.5 g until spontaneous contractions became regular in frequency and intensity. During this period, the bath solution was renewed every 20 min. As previously published (Leroy et al., 1991), spontaneous contractile activity varied among individuals and/or samples in terms of frequency, duration and amplitude. Muscle strips which did not develop spontaneous contractions at this stage were considered not to be viable and were discarded.

Eight myometrial strips were taken from the same patient and were tested in parallel. Since the effect of endothelin is slowly reversible, and since maintained responses to this peptide were observed in human myometrium in a number of cases, even after extensive washing, only one agonist was tested on each myometrial strip, with comparisons being made across tissues. After equilibration, agonist peptides (endothelin-1, endothelin-3 and sarafotoxin 6c) were cumulatively added (0.5 log-concentration increments) at 10-min intervals. When endothelin receptor antagonists were used, myometrial preparations were exposed for 30 min to one of these antagonists or its vehicle (paired control preparations) and then to cumulative additions of endothelin-1. The effects of antagonists were investigated in myometrial strips adjacent to those used as controls. All concentrations are given as final bath concentrations. Protease inhibitors (1 μg ml^{-1} leupeptin, 20 μg ml^{-1} aprotinin, 20 μg ml^{-1} pepstatin, 15 μg ml^{-1} bacitracin) did not affect the potency of endothelin-1 for inducing uterine contractility (data not shown).

The contractile effects in myometrial strip preparations were recorded by computerized calculation of the integral under the tension-time curve for 10 min after the agonist was added. Agonist concentration-response curves were fitted to the equation:

$$E_A = (E_{max} \times C^n / [C^n + EC_{50}^n]) + E_0$$

in which E_A is the pharmacological effect, E_{max} is the asymptote, C is the concentration of the agonist, EC_{50} is the concentration resulting in a response at $E_{max}/2$, n is the slope (pseudo-Hill coefficient) and E_0 is the starting basal activity.

The pA_2 value was calculated according to Arunlakshana and Schild (1959) by incubating the tissue with three increasing concentrations (one concentration per strip, 0.1, 0.3 and 1 μM) of the antagonist. pA_2 values are only reported for measurements satisfying apparent competitive inhibition criteria, i.e., no variation in agonist E_{max} in the presence of the antagonist, and slope of the Schild plot not significantly different from 1 (Kenakin, 1987). In other cases, antagonist potency was measured by calculation of apparent pK_B values: $pK_B = \log(CR - 1) - \log[B]$, where $[B]$ is the concentration of antagonist used and CR the concentration ratio calculated by dividing the EC_{50} value obtained in the presence of the antagonist by that obtained in its absence.

2.2. Chemicals

Endothelin-1, endothelin-3, sarafotoxin 6c, FR 139317, BQ 123, IRL 1038 and BQ 3020 were purchased from Neosystem Laboratoire (Strasbourg, France). Other drugs and chemicals used were of the highest quality available from Sigma Chemical Co (St Louis, MO, USA).

2.3. Statistics

Results are presented as means ± S.E. of n myometrial strips isolated from different patients. Contractions were expressed as g/10 min or percentage of the maximal tension (E_{max}) elicited by the agonist. Individual EC_{50} values were calculated from the maximum contractile response to each agonist by computer analysis using non-linear regression (Inplot Computer Program, GraphPad Software, San Diego, CA). pD_2 ($-\log EC_{50}$) were computed from these values and expressed as means ± S.E. Linear regression was used

to fit the data for pA_2 determination. Significance of difference was assessed by one-way analysis of variance followed by unpaired Student's t-test. P values < 0.05 were considered to be significant.

3. Results

3.1. Agonist activities

Spontaneous contractile activity was observed after a period of stabilization (at least 2 h) in approximately 95% of the myometrial strips studied.

Fig. 1 shows a representative tracing of cumulative application of endothelin-1 on the spontaneous contractility of the outer myometrial layer. Concentrations of endothelin-1 as low as 1 nM increased the frequency of contractions. The administration of 0.1 μM endothelin-1 produced an additional effect, namely an increase in smooth muscle tone, culminating in marked tetanic contraction. The endothelin-1 response was poorly reversible, in that two changes of bath solution after the addition of 0.1 μM endothelin-1 were needed to cancel the effect of the peptide.

Fig. 2 compares the effects of cumulative addition of endothelin-1, endothelin-3 and sarafotoxin 6c on the contractile activity of human myometrial strips. Endothelin-1 (10 pM to 0.3 μM) produced a concentration-dependent contraction of myometrial strips, with a mean pD_2 value of 8.24 ± 0.12 (Table 1). Maximal stimulatory effect (2.27 g/10 min) was obtained with a concentration of 30 nM. Endothelin-3 was slightly less effective and less potent than endothelin-1 in stimulating myometrial contraction, with a mean pD_2 value of 7.20 ± 0.07. Sarafotoxin 6c showed no agonist activity even at a concentration of 0.3 μM. No change in contractile activity was observed following cumulative addition of the endothelin ET_B receptor-selective agonist BQ 3020 up to 0.3 μM (data not shown).

Responses to endothelin-1 were unaffected by indomethacin (10 μM) and N^G-methyl-L-arginine (10 μM) (data not shown).

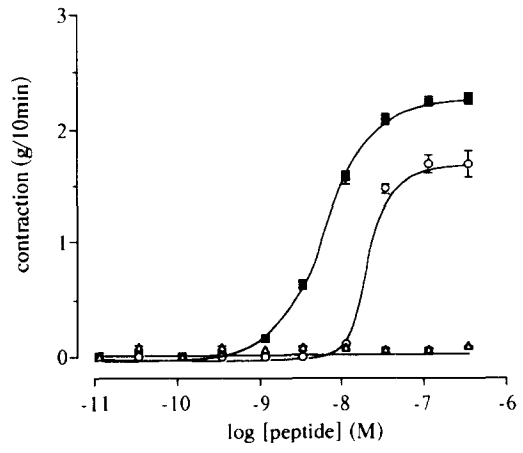

Fig. 2. Contractile effects of endothelins and sarafotoxin on human myometrium. Increasing concentrations of endothelin-1 (closed squares), endothelin-3 (open circles) or sarafotoxin 6c (open triangles) were added to myometrial strips every 10 min. Contraction is expressed as grams of tension/10 min. Results are means ± S.E. for 24 (endothelin-1) or 10 (endothelin-3, sarafotoxin 6c) myometrial strips isolated from 5 to 12 different patients.

Table 1
Maximal contractile response (E_{max}) and pD_2 values of endothelins and sarafotoxin 6c in human myometrium. Effects of endothelin ET_A and ET_B receptor antagonists

Treatment	E_{max} (g/10 min)	pD_2	n
Endothelin-1	2.27 ± 0.04	8.24 ± 0.12	24
Endothelin-3	1.70 ± 0.12	7.20 ± 0.07 [a]	10
Sarafotoxin 6c	0.15 ± 0.01 [a]	–	10
FR139317			
0.1 μM	3.30 ± 0.07	7.72 ± 0.04 [a]	14
0.3 μM	2.63 ± 0.17	7.45 ± 0.06 [a]	13
1 μM	1.94 ± 0.04	7.03 ± 0.07 [a]	8
BQ 123			
0.1 μM	2.31 ± 0.09	7.80 ± 0.11 [a]	9
0.3 μM	2.25 ± 0.04	7.69 ± 0.10 [a]	16
1 μM	1.77 ± 0.20	7.63 ± 0.06 [a]	10
IRL 1038			
0.3 μM	2.86 ± 0.17	8.51 ± 0.08	4
1 μM	3.06 ± 0.43	8.21 ± 0.17	5

Increasing concentrations of endothelin-1, endothelin-3 or sarafotoxin 6c were added every 10 min to myometrial strips. When present, BQ 123, FR 139317 or IRL 1038 was added 30 min before cumulative addition of increasing concentrations of endothelin-1. Data are means ± S.E. from n myometrial strips isolated from different patients. [a] Significantly different from values obtained with endothelin-1 ($P < 0.05$).

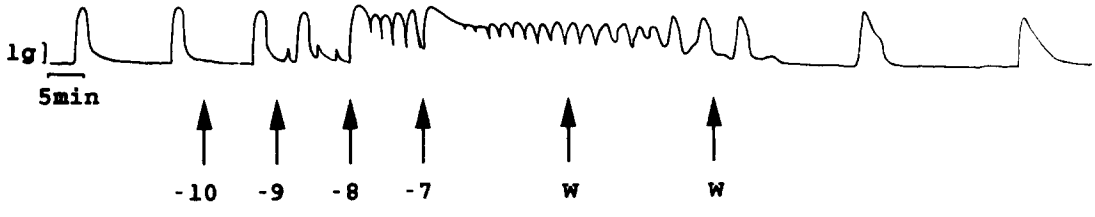

Fig. 1. Representative tracing of the effect of cumulative addition of endothelin-1 on the contractile activity of the human myometrium in vitro. Increasing concentrations of endothelin-1 were applied every 10 min. Arrows represent timing of peptide application. Concentrations of endothelin-1 are expressed as logarithms. The bath solution was changed every 20 min (W) after the final addition of endothelin-1. Similar records were obtained in eleven other experiments.

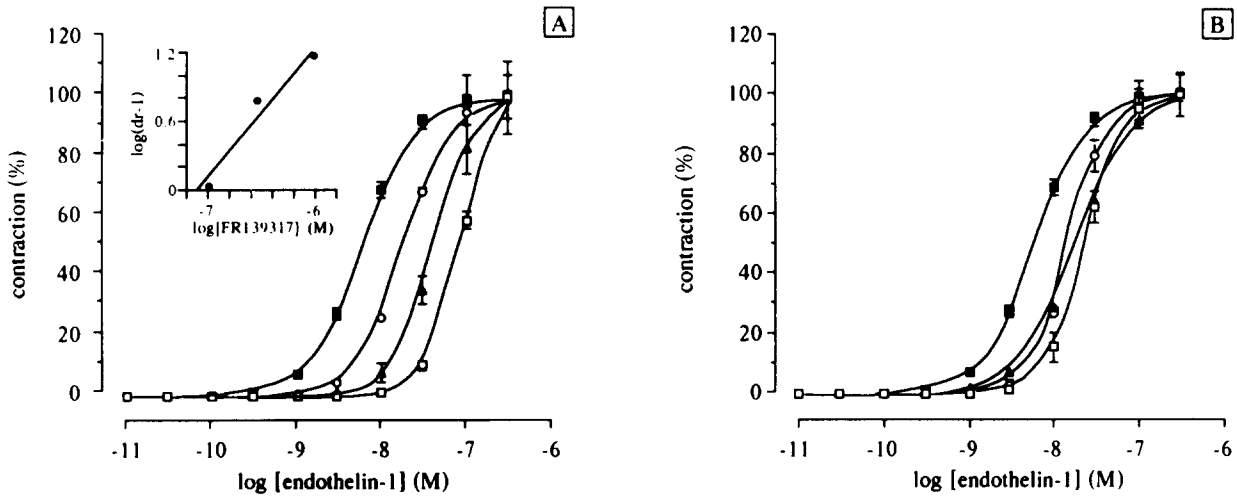

Fig. 3. Influence of endothelin ET_A receptor antagonists on endothelin-1-induced contraction. 30 min before addition of endothelin-1, myometrial strips were incubated with different concentrations (0.1 μM, open circles; 0.3 μM, closed triangles; 1 μM, open squares) of FR 139317 (panel A) or BQ 123 (panel B). Closed squares represent the dose-response curve for endothelin-1 obtained in the absence of the antagonist. Results are expressed as means ± S.E. for 8 to 16 myometrial strips isolated from 4 to 8 different patients. Inset represents Schild plot regression for blockade of endothelin-1-induced contractions by FR 139317. The contraction of each segment tested was calculated as a percentage of the maximal contraction obtained for each concentration of antagonist.

3.2. Antagonist potencies

The presence of endothelin ET_A and ET_B receptor antagonists (up to 1 μM) was without any effect on spontaneous contractions of myometrial strips (data not shown).

Pretreatment of human myometrial strips for 30 min with FR 139317 (0.1, 0.3 or 1 μM) resulted in a dose-related rightward shift of the endothelin-1 response curve (Fig. 3A). No significant changes in the maximal responses were observed according to ANOVA calculations (Table 1). Schild plot analysis yielded a pA_2 value of 7.10 ± 0.64 with a slope of 1.13 (not significantly different from unity).

BQ 123 (0.1 μM) was found to be able to shift the response curve induced by endothelin-1 to the right, but higher doses of the antagonist (0.3 and 1 μM) did not produce any additional rightward shift. BQ 123 did not significantly attenuate the maximal response elicited by endothelin-1 (Fig. 3B, Table 1). Apparent pK_B values were 7.24 ± 0.07, 6.92 ± 0.03 and 6.48 ± 0.15 using 0.1, 0.3 and 1 μM, respectively.

The endothelin ET_B receptor antagonist IRL 1038 (0.3 and 1 μM) did not alter spontaneous contractions and did not significantly modify endothelin-1-induced contractions of human myometrium (Table 1).

4. Discussion

The major finding in this study is that, of the two endothelin receptor subtypes previously evidenced in the human myometrium (Breuiller-Fouché et al., 1994), only endothelin ET_A receptors can mediate contractile response. This is supported by the fact that endothelin-1 was more potent than endothelin-3 in contracting the human myometrium, with pD_2 values of 8.24 and 7.20, respectively. This rank order of potency is consistent with the current pharmacological definition of the endothelin ET_A receptor (Arai et al., 1990). In agreement with these observations was the lack of any effect of the two selective agonists of endothelin ET_B receptors, sarafotoxin 6c and BQ3020 (up to 0.3 μM), confirming that uterine contraction, at least in vitro, is exclusively an endothelin ET_A receptor-mediated phenomenon. Similarly, endothelin-1-induced-contraction of rat uterus has been shown to be mediated mainly, if not exclusively, by endothelin ET_A receptor activation (Rae et al., 1993; Tsunoda et al., 1993; Do Khac et al., 1994). The apparent lack of endothelin ET_B receptor-mediated responses may reflect the small number of endothelin ET_B receptors in human myometrial tissue, in which endothelin ET_B receptors represent less than 25% of the total endothelin receptors. The absence of functional endothelin ET_B receptors has been reported in cultured human myometrial cells (Maggi et al., 1994; Héluy et al., 1995). The results of these studies indicate that endothelin ET_A receptors, but not endothelin ET_B receptors, mediate endothelin-1-induced IP accumulation and the subsequent increase in intracellular calcium. At the present time, the physiological function of endothelin ET_B receptors in myometrium from different species is still unknown. Additional evidence for a functional link between endothelin ET_A receptor stimulation and uterine contraction was provided by the finding that endothelin-1-induced contractions were

inhibited by BQ 123 and FR 139317, two selective endothelin ET_A receptor antagonists. Indeed, pretreatment of myometrial strips with FR 139317 resulted in a concentration-dependent rightward shift in the endothelin-1-induced dose-response curve, without any effect on the maximal contraction. FR 139317 acted as a purely competitive antagonist, yielding a Schild plot regression line with a slope which did not differ from unity, and an estimated pA_2 value of 7.10, comparable to those obtained in vascular smooth muscle (Adner et al. 1994). In contrast, our results suggest noncompetitive antagonism of endothelin-1-induced contraction by BQ 123, although the latter had a potency in the same range as that found in the guinea-pig iliac artery (Schoeffter et al., 1993) and in human neuroblastoma cells (Hiley et al., 1992). In other studies and tissues, including rat myometrium (Rae et al., 1993), BQ 123 was characterized as a competitive antagonist. A simple hypothesis which might account for these results is that of recently reported pharmacological differences between rat and human endothelin ET_A receptors (Elshourbagy et al., 1993).

Several explanations can be advanced for these apparently conflicting data. An obvious one is that diffusion of BQ 123 is limited in human myometrium. One possible consequence is that increasing concentrations of BQ 123 are unable to displace the endothelin-1 curve. Recent evidence for this hypothesis has been provided by Bacon et al., (1995) in human myometrium. Similarly to what we found, these authors reported that FR 139317 was able to block endothelin-1-induced uterine contractions but surprisingly a concentration as high as 10 μM of BQ 123 was ineffective in this system. Nor can we exclude the observations of Vigne et al. (1993) and Sakamoto et al. (1994), who reported that BQ 123 acts as a competitive or noncompetitive inhibitor of endothelin-1 action, depending on the type of experimental conditions used. These authors demonstrated an apparent non-competitive antagonism by BQ 123 for endothelin ET_A receptors when their experiments were performed in a non-equilibrium state. This is probably the case in human myometrium, where true equilibrium conditions have not been attained for [^{125}I]endothelin-1 binding. Indeed we previously showed that binding of [^{125}I]endothelin-1 was poorly reversed by unlabeled endothelin-1 (1 μM) for up to 5 h (Breuiller-Fouché et al., 1994). Finally, since we recently hypothesized the existence of two distinct subtypes of endothelin ET_A receptors in human myometrial cells in culture (Héluy et al., 1995), endothelin ET_A receptor heterogeneity was also expected in the present study. Nevertheless, the apparent differences in the pharmacological responses of BQ 123 and FR 139317 observed in in vitro uterine contractile assays were not sufficient to postulate the existence of several endothelin ET_A receptors in human myometrial tissue. Hopefully, the development of new endothelin ET_A receptor antagonists will help to elucidate the separate roles of endothelin ET_A receptor subtypes in the uterus. Atypical responses to sarafotoxin 6b, which differs from endothelin-1 by only seven amino acids (Kloog et al., 1988), have recently been observed in vascular smooth muscle with endothelin ET_A receptor-selective compounds (see review: Bax and Saxena, 1994) and suggest further heterogeneity of endothelin ET_A receptors. Accordingly, the use of a wide spectrum of both agonists and antagonists will be needed to clarify the putative class of endothelin ET_A receptors in human myometrium.

It is possible that endothelin-1 locally produced by cells in the vicinity of myometrium, such as endometrial cells (Economos et al., 1992; O'Reilly et al., 1992; Cameron et al., 1993), regulates human myometrial contractility in a paracrine fashion. An autocrine role for endothelins could also be envisaged since gene expression of prepro-endothelins was detected in human myometrium (Mac Mahon et al., 1993).

In conclusion, the present study demonstrates that only the endothelin ET_A receptors are coupled to human uterine contractile responses. There is no evidence at the present time that activation of endothelin ET_B receptor elicits contractile behavior.

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