Gestational changes in endothelin-1-induced receptors and myometrial contractions in rat

S.Sakamoto1,3, T.Aso1, H.Masuda2, M.Goto2, S.Tamaoki2 and H.Azuma2

1Department of Obstetrics and Gynecology, Faculty of Medicine, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo 113-8519, and 2Department of Medicinal Chemistry, Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2–3–10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101-0062, Japan
3To whom correspondence should be addressed

The present experiments were performed to characterize the gestational changes in endothelin (ET)-1-induced myometrial contractions and ET receptors in rat. ET-1-induced contractions were composed of two types: increases in resting tone and rhythmic contractions. The increase in resting tone was decreased at 7 days of gestation, but increased at 20 days. The increase in amplitude and frequency of rhythmic contractions remained unchanged during days 7–14 of gestation. Continuous rhythmic contractions were not produced by ET-1 near the term. Both contractions were inhibited by the antagonists BQ 123 and Ro 46–2005 but not by RES 701–1 or BQ 788. In binding studies, total binding sites of [125I]-ET-1 were unchanged, however higher affinity binding sites appeared during pregnancy in addition to the lower affinity sites. The specific [125I]-ET-1 binding in non-pregnant and pregnant myometrium was completely inhibited by unlabelled ET-1 and Ro 46–2005. In contrast, the proportion which was inhibited by BQ 123 was decreased during pregnancy. In conclusion, characteristic gestational changes were the augmentation of ET-1-induced increased resting tone near term, and the appearance of high affinity ET-1 binding sites and an increase in BQ 123-resistant ET-1 binding sites during pregnancy. Further investigations are needed to understand the physiological role of these changes.

Key words: endothelin-1/ET_A receptor/ET_B receptor/gestation/myometrial contraction

Introduction

We have reported that endothelin-1 (ET-1) causes two types of myometrial contractions, increases in resting tone and rhythmic contractions, and that both contractions are mediated through the excitation of ET_A receptors, which are the predominate subtype in the oestrus rat myometrium (Sakamoto et al., 1997). Some physiological roles of ET-1-induced myometrial contraction were proposed for the initiation of parturition in rabbit (Peri et al., 1992), human (Wolff et al., 1993; Maggi et al., 1994; Osada et al., 1997) and rat (Yallampalli and Garfield, 1994; Izumi et al., 1995). They demonstrated that ET-1-induced myometrial contractions (total sum or maximum amplitude of the contractions) were increased during pregnancy. However, they did not refer to the two types of ET-1-induced myometrial contractions. Regarding the changes in ET receptor density during pregnancy, Maggi et al. (1994) reported that total ET binding sites were increased, due to the increase in ET_A receptors, while Osada et al. (1997) failed to demonstrate an increase in total ET-1 binding sites, which were accompanied by an increased proportion of ET_A receptors and by a decreased proportion of ET_B receptors in the human myometrium. In the rat myometrium, Yallampalli and Garfield (1994) reported that ET-1 binding sites were increased during delivery compared with those at the late pregnancy (18 days’ gestation). Therefore, the gestational changes in ET receptor density are still controversial. The present experiments were designed to investigate the changes in two types of ET-1-induced myometrial contractions and ET receptor density during the progression of pregnancy.

Materials and methods

Chemicals

The following chemicals were used: ET-1(human) was purchased from Protein Research Foundation, Osaka, Japan. Bacitracin, aprotinin, leupeptin, pepstatin A and bovine serum albumin (BSA; fraction V) were obtained from Sigma, St Louis, MO, USA. [125I]-ET-1(specific activity 2200 Ci/mmol) was purchased from Du Pont-New England Nuclear, Wilmington, DE, USA. Cyclo(Thr-Pro-D-Val-Leu-d- Trp)-[Gly-Asp-I-Pro-d-Val-l-Leu-d-Trp] (BQ 123), a selective antagonist for ET_A receptor subtype (Ihara et al., 1992), 4-tert-butyl-N-[6-(2-hydroxyethoxy)5-(3-methoxyphenox)-4-pyrimidinyl]-benzenesulphonamide (Ro 46–2005), a mixed-type antagonist for ET_A and ET_B receptors (Clozel et al., 1993), were synthesized by the Chemical Research Department, Teikoku Hormone Manufacturing, Tokyo, Japan and were generous gifts from the company. Cyclic (Gly^{1,Asp^{3}}) (Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp) (RES-701-1), a selective antagonist for ET_B receptor subtype (Tanaka et al., 1994) was a gift from Dr Matsuda, Tokyo Research Laboratories, Kyowa Hakko Kogyo, Tokyo, Japan. Cis-2,6-dimethylpiperidinocarbonyl-g-methylleucyl-d-Trp(1-CO_{2}CH_{3})-d-Nle-OH (BQ 788), a selective antagonist for ET_B receptor subtype (Ishikawa et al., 1994) was purchased from Novabiochem, L{"u}lfeltingen, Switzerland.

Animals and tissues

Female Sprague–Dawley rats, 10–15 weeks of age, were mated in the evening of the pro-oestrus cycle. If spermatozoa were observed...
in the vaginal smear next morning, that day was defined as day 0 of gestation. Rats in oestrus (non-pregnant), and on the seventh, 14th, and 20th days of gestation were anaesthetized with ether and underwent a hysterectomy. Immediately after the hysterectomy, the fetuses and placentas were removed from the uteri and the uteri were merged in oxygenated and ice-cold modified Kreb’s solution (NaCl 115 mmol/l, KCl 4.7 mmol/l, MgSO4 7H2O 1.2 mmol/l, CaCl2, 2H2O 2.5 mmol/l, KH2PO4 1.2 mmol/l, NaHCO3 25.0 mmol/l and glucose 10 mmol/l). For measurement of mechanical responses, uteri were cut into longitudinal strips, 2 mm wide and 5 mm long. For radioligand receptor binding assay, membrane fractions were prepared from homogenates of myometrium after removing the endometrium with a surgical knife. Because ET receptors also exist in the human (Bacon et al., 1995; Collett et al., 1996) and sheep (Riley et al., 1995) endometrium, histological examinations were performed to ensure the removal of the endometrium.

The study was conducted in compliance with the Animal Welfare Regulation of Tokyo Medical and Dental University and in accordance with UK legal requirements.

Measurement of mechanical responses
The mechanical responses were measured according to the methods described previously (Azuma et al., 1992). A longitudinal uterine strip was mounted vertically in an organ bath containing 5 ml of modified Kreb’s solution, continuously bubbled with 95% O2 and 5% CO2 at 37°C. One end of each strip was secured to the bottom of the organ bath, and the other was attached to a force-displacement transducer (TB-611T; Nihon Kohden Kogyo Co, Tokyo, Japan). Isometric changes in tension were recorded on a pen writing oscillograph (R-64; Rikadenki Kogyo Co, Tokyo, Japan). The length of the strips was adjusted several times until a stable tension of 400 mg was attained. Before beginning the experiments, strips were allowed to equilibrate for at least 60 min in the bathing solution and during this period the bathing solution was replaced every 20 min with fresh solution. After 60 min of equilibration, a single concentration of ET-1 was applied to one uterine strip and the responses were recorded for 20 min. In order to construct the concentration–response curves, the concentration of ET-1 in the organ bath was increased by one half log unit from 3×10⁻¹⁰ to 3×10⁻⁸ mol/l. For subtyping receptors which mediate myometrial contractions, each strip was incubated in the presence or absence of BQ 123, BQ 788, or Ro 46–2005. After a 20 min pretreatment with each antagonist, 3×10⁻⁸ mol/l ET-1 was added to each strip and the changes in developed tension were recorded for 20 min. Finally, 60 mmol/l KCl was added to obtain the reference contraction.

ET-1-induced myometrial contractions were assessed by changes in the resting tone (measured at 5, 10, 15 and 20 min after adding ET-1), amplitude of rhythmic contraction (maximal contraction minus basal resting tone, measured at 5, 10, 15 and 20 min after adding ET-1) and frequency of rhythmic contractions (the number of rhythmic contractions between 5–15 min after adding ET-1) (Figure 1). Increases in resting tone and amplitude of rhythmic contractions were given as percentages of 60 mmol/l KCl-induced contractions.

Preparation of crude membrane fractions
Crude membrane fractions were prepared according to the method of Azuma et al. (1994). Briefly, myometrial tissues were minced with scissors and homogenized in a Polytron at maximum speed for 20 s to a 25% homogenate in buffer A (20 mmol/l HEPES, 250 mmol/l sucrose, 5 mmol/l EGTA, 3 µg/ml leupeptin, 2 µg/ml aprotinin, 0.25 mg/ml bacitracin, 3 µg/ml pepstatin A, pH 7.4). The homogenate was centrifuged at 1200 g for 20 min at 4°C. The supernatant was removed and centrifuged at 80 000 g for 60 min at 4°C. The resultant pellet was resuspended in buffer A as a crude membrane fraction and stored at −80°C until use. Protein concentration was determined with the micro BCA kit (Pierce, Rockford, IL, USA).

Radioligand receptor binding assay
The radiolabelled ligand used for saturation analysis was [¹²⁵I]-ET-1. The medium for the assay was adjusted to 200 µl containing 20 µl crude membrane fraction (5 µg protein), 160 µl buffer B (30 mmol/l HEPES, 150 mmol/l NaCl, 5 mmol/l MgCl₂, 0.5 mg/ml bacitracin, 1 mg/ml BSA) and 20 µl [¹²⁵I]-ET-1 at eight different concentrations of 3.32–350.0 pmol/l. Each assay medium was shaken for 120 min at 25°C. After addition of 3 ml ice-cold buffer B, the mixture was filtered under reduced pressure through a Whatman GF/B glass-fibre filter (Whatman, Maidstone, UK). After two washes with 3 ml buffer, the filters were dried in an oven and transferred to counting tubes. Radioactivity was counted in a gamma counter (Auto-Gamma 800C, Packard, Merden, CT, USA). Specific binding was defined as total binding minus non-specific binding measured in the presence of 125 nmol/l unlabelled ET-1. To analyse the data, [Bound] / [Free] is plotted against [Bound], where [Bound] = specific binding and [Free] = concentration of free ET-1 (Scatchard plots) (Rosenthal 1967). If the binding site is single, plots will be straight and the slope and X-intercept will be –1/Kd and Bmax, where Kd and Bmax are the dissociation equilibrium constant and receptor density respectively. If two binding sites exist, plots will be a hyperbola, which is the sum of the two straight lines and each slope and X-intercept of straight line will represent –1/Kd and Bmax of two binding sites. Displacement of the specific binding of [¹²⁵I]-ET-1 (45 pmol/l) was performed with ET-1 (3×10⁻¹⁰ to 3×10⁻⁸ mol/l), BQ 123 (10⁻¹⁰ to 10⁻⁵ M), RES 701–1 (10⁻⁹ to 10⁻⁵ M) and Ro 46–2005 (10⁻⁸ to 3×10⁻⁸ mol/l).

Statistical analysis
All data are given as mean ± SE. The statistically significant differences between two means were determined by Student’s t-test. Multiple comparisons between two groups were made by one-way analysis of variance (ANOVA) with post-hoc test (Scheffé’s F test). Differences were considered significant when P < 0.05.
Results

Mechanical responses to ET-1

Figure 2 shows the representative mechanical responses of the myometrium to $3 \times 10^{-8}$ mol/l ET-1 and 60 mmol/l KCl from non-pregnant, 7, 14 and 20 days of gestation rats. ET-1 ($3 \times 10^{-10}$ to $3 \times 10^{-8}$ mol/l) caused contractions composed of two types: increases in resting tone and rhythmic contractions in a concentration-dependent manner. At 20 days of gestation, however, ET-1 greatly increased the resting tone with little change in the rhythmic contractions. The gestational changes in the $3 \times 10^{-8}$ mol/l ET-1-induced increase in resting tone expressed as percentage of 60 mmol/l KCl-induced reference contraction are shown in Figure 3. ET-1-induced increase in resting tone at 7 days of gestation was significantly smaller ($P < 0.001$) than that of non-pregnancy, but it was gradually augmented as the pregnancy progressed. At 20 days of gestation, the increased tone with ET-1 became larger ($P < 0.001$) than that of non-pregnancy. On the other hand, both the amplitude (expressed as percentage of 60 mmol/l KCl-induced reference contraction) and the frequency of rhythmic contractions induced by $3 \times 10^{-8}$ mol/l ET-1 remained unchanged at 7 and 14 days of gestation (Figure 4a,b). At 20 days’ gestation, continuous rhythmic contractions in response to ET-1 were not observed (Figure 3). The 60 mmol/l KCl-induced reference contraction was increased during pregnancy and was greatest at 14 days’ gestation (Figure 5).

Figure 6 shows the effects of ET receptor antagonists on the increase in resting tone of the myometrium at 20 days of gestation. Pretreatment with BQ 123 as a selective antagonist for ETA receptor and Ro 46–2005 ($3 \times 10^{-6}$ to $3 \times 10^{-5}$ mol/l) as a mixed type antagonist for ETA and ETB receptors greatly inhibited the increase in resting tone caused by $3 \times 10^{-8}$ mol/l ET-1 in a concentration-dependent manner. In contrast, BQ 788 as a selective antagonist for ETB receptors did not produce a significant inhibition even at the highest concentration of $3 \times 10^{-6}$ mol/l. The ET-1-induced increases in resting tone observed during non-pregnancy and at 7 and 14 days of gestation were also greatly inhibited by BQ 123 and Ro 46–2005 but not by BQ 788. The ET-1-induced increases in amplitude and frequency of rhythmic contractions observed during non-pregnancy and at 7 and 14 days’ gestation were also inhibited by the pretreatment with BQ 123 and Ro 46–2005.
Gestational changes in ET-1-induced myometrial contractions

**Figure 4.** Gestational changes in (a) amplitude and (b) frequency of the $3 \times 10^{-8}$ mol/l endothelin-1 (ET-1)-induced rhythmic contractions. The amplitude was expressed as percentage of 60 mmol/l KCl-induced reference contractions. ET-1-induced rhythmic contractions were unchanged during pregnancy in comparison with those during non-pregnancy. Continuous rhythmic contractions were not observed at 20 days of the gestation.

**Figure 5.** Gestational changes in amplitude of the 60 mmol/l KCl-induced myometrial contractions. Amplitude of the 60 mmol/l KCl-induced myometrial contractions were increased during pregnancy and largest at 14 days' gestation *$P < 0.001$ versus non-pregnancy; **$P < 0.05$ versus non-pregnancy; +$P < 0.01$ versus 7 days' gestation.

**Radioligand receptor binding assay**

The binding of $^{[125I]}$-ET-1 was saturable with high affinity. Scatchard plot analysis revealed that the binding site of $^{[125I]}$-ET-1 constituted a single population in the non-pregnant myometrium. Two populations of binding sites with low and high affinity were detectable in the pregnant myometrium. The dissociation equilibrium constant ($K_d$) and receptor density ($B_{max}$) values are shown in Table I. While the high affinity binding sites appeared, the low affinity binding sites were decreased during pregnancy. However, total binding sites during pregnancy were not different from that of non-pregnancy.

As shown in Figure 7, unlabelled ET-1 and Ro 46–2005 attained at $3 \times 10^{-8}$ mol/l of unlabelled ET-1 and $10^{-5}$ mol/l of Ro 46–2005 in all membrane preparations. In contrast, the $^{[125I]}$-ET-1 binding was not fully inhibited with BQ 123 even at the highest concentration of $10^{-6}$ mol/l. The binding sites not inhibited with BQ 123 were calculated to be $9.3 \pm 1.4\%$ ($n = 3$), $46.0 \pm 15.1\%$ ($n = 3$), $39.3 \pm 14.8\%$ ($n = 3$) and $24.7 \pm 7.8\%$ ($n = 3$) of the total specific binding in non-pregnant, 7, 14 and 20 days of gestation respectively. RES 701–1 at concentrations of $10^{-9}$ to $10^{-5}$ mol/l did not produce a significant inhibition of the specific $^{[125I]}$-ET-1 binding in all membrane preparations.

**Discussion**

ET-1-induced increase in resting tone was attenuated at 7 days of gestation and augmented at 20 days of gestation although
Table I. The dissociation constant ($K_d$) and receptor density ($B_{max}$) values of the myometrial membrane preparation of non-pregnant, and after 7, 14 and 21 days gestation. The binding site of $[\text{125I}]$-endothelin-1 (ET-1) constituted a single population during non-pregnancy, but two populations during pregnancy, because the higher affinity binding sites were detectable. While the high affinity binding sites were increased, the low affinity sites were decreased, and the total binding sites did not vary significantly during pregnancy.

<table>
<thead>
<tr>
<th>Gestational days</th>
<th>$K_d$ low (pmol/l)</th>
<th>$K_d$ high (pmol/l)</th>
<th>$B_{max}$ low (fmol/mg protein)</th>
<th>$B_{max}$ high (fmol/mg protein)</th>
<th>$B_{max}$ total (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>53.6 ± 6.4</td>
<td>1435.6 ± 226.0</td>
<td>1435.6 ± 226.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 (n = 4)</td>
<td>66.4 ± 8.7</td>
<td>27.4 ± 6.9</td>
<td>983.3 ± 229.1</td>
<td>417.6 ± 64.3</td>
<td>1400.9 ± 283.6</td>
</tr>
<tr>
<td>14 (n = 3)</td>
<td>60.0 ± 12.6</td>
<td>27.8 ± 1.5</td>
<td>925.3 ± 364.4</td>
<td>399.2 ± 143.1</td>
<td>1324.5 ± 507.4</td>
</tr>
<tr>
<td>20 (n = 4)</td>
<td>65.1 ± 7.7</td>
<td>26.6 ± 5.7</td>
<td>628.7 ± 224.1$^*$</td>
<td>308.7 ± 113.8</td>
<td>937.4 ± 328.7</td>
</tr>
</tbody>
</table>

$^*P < 0.05$ compared with non-pregnant.

Figure 7. Displacement of the $[\text{125I}]$-endothelin-1 (ET-1) binding to myometrial membrane preparations by unlabelled ET-1 ($\infty$), BQ 123 (O), Ro 46–2005 (□) and RES 701–1 (×). (a) non-pregnancy (oestrus cycle); (b) 7 days’ gestation; (c) 14 days’ gestation; (d) 20 days’ gestation. Complete inhibition was attained at $3 \times 10^{-8}$ mol/l of unlabelled ET-1 and $10^{-5}$ mol/l of Ro 46–2005 in all membrane preparations. In contrast, $[\text{125I}]$-ET-1 binding was not fully inhibited with BQ 123 even at the highest concentration of $10^{-5}$ mol/l. The binding sites not inhibited with BQ 123 were calculated to be 9.3 ± 1.4% (n = 3), 46.0 ± 15.1% (n = 3), 39.3 ± 14.8% (n = 3) and 24.7 ± 7.8% (n = 3) of the total specific binding in non-pregnant, and at 7, 14 and 20 days of gestation respectively. RES 701–1 at concentrations of $10^{-8}$ to $3 \times 10^{-5}$ mol/l did not produce a significant inhibition of the specific $[\text{125I}]$-ET-1 binding in all membrane preparations.

the KCl-induced myometrial contraction was augmented throughout pregnant periods, indicating that the contractile force of the myometrium itself was increased during pregnancy and that the time-dependent changes in the increase in resting tone with ET-1 were a characteristic of pregnancy. The attenuated increase in resting tone with ET-1 at 7 days of gestation may play a role in maintaining the pregnancy, whereas the augmented tonic contraction with ET-1 at late pregnancy may be involved in parturition. Kajihara et al. (1996) reported that ET-1 is produced in the myometrium and that the production of ET-1 in myometrium increases toward parturition and is highest in the early post-partum period. Therefore, they considered that the increased ET-1 might be involved in afterbirth pain to control bleeding from the placental bed. We are in agreement with Kajihara et al. (1996), since an augmented increase in resting tone with ET-1 could be observed after delivery (unpublished observations).

We reported that ET-1-induced myometrial contractions were evoked by the increase in the cytoplasmic free Ca$^{2+}$ concentration; the rhythmic contractions were mediated by the increase in Ca$^{2+}$ influx via voltage-dependent Ca$^{2+}$ channels, while the increase in resting tone was mediated by Ca$^{2+}$ influx via another mechanism (Sakamoto et al., 1997). It has been reported that oestrogens inhibit the transmembrane Ca$^{2+}$ cur-
rents in pregnant rat myometrium (Yamamoto, 1995), while they enhance Ca\(^{2+}\) channels in ovine gonadotrophs (Heyward and Clarke, 1995). Oestrogens also stimulate K\(^{+}\) channels and increase intracellular free Ca\(^{2+}\) concentration in the endothelium of rabbit aorta (Rusko et al., 1995). It is well established that not only oestrogens, but also progesterone, are increased during pregnancy. Therefore, it is possible to assume that the hormonal changes modulate Ca\(^{2+}\) mobilization with ET-1 in a manner to attenuate and augment the increase in resting tone with ET-1 at the early and late stages of gestation respectively.

Both the increases in resting tone and rhythmic contractions with ET-1 were abolished in the presence of BQ 123, a selective antagonist for ETA receptor subtype, but unaffected by BQ 788, a selective antagonist for ETB receptor subtype. In addition, the potency and inhibition profiles of the non-pregnant and pregnant myometrium were not different. It is, therefore, suggested that two types of ET-1-induced contractions are mediated mainly through the excitation of ETA receptors.

Our binding study revealed that total \([^{125}\text{I}]\)-ET-1 binding sites were unchanged during pregnancy, although high affinity ET-1 binding sites appeared after pregnancy was achieved. In the displacement experiments, the mode of action of ET-1, Ro 46–2005 and RES 701–1 (or BQ 788) on the \([^{125}\text{I}]\)-ET-1 binding was not different between non-pregnancy and pregnancy. However, the inhibitory activity of BQ 123 was clearly decreased during pregnancy. Recently, heterogeneity of ETA receptors has been reported because of the different sensitivity to BQ 123 as a selective antagonist for ETA receptors in rabbit saphenous vein (Sudjarwo et al., 1994; Nishiyama et al., 1995), human saphenous vein (White et al., 1994), human coronary artery (Bax et al., 1994), goat cerebral arteries (Salom et al., 1993), human omental vessels (Riezebos et al., 1994), and rat and mouse vas deferens (Eglezos et al., 1993; Maas et al., 1995). In an earlier report (Sakamoto et al., 1997), we suggested the presence of BQ 123-resistant ET-1 binding sites (putative non-ETA/non-ETB receptors) in the non-pregnant rat myometrium. The present experiments demonstrated that the undefined ETA-1 binding sites were clearly increased during pregnancy. Furthermore, high affinity binding sites were detected only in the pregnant myometrium. It seems possible to assume that the appearance of the high affinity binding sites corresponds to the BQ 123-resistant binding sites. However, further investigations were needed to demonstrate the correspondence and the physiological role of these binding sites. The BQ 123-resistant binding sites may not be involved in causing myometrial contractions, since the contractions were abolished by BQ 123 or Ro 46–2005 both in the non-pregnant and pregnant myometrium. In human myometrium, Maggi et al. (1994) and Wolff et al. (1996) reported that total ET binding sites were increased because ET\(_B\) receptors were increased during pregnancy, whereas Osaka et al. (1997) reported that total ET-1 binding sites were unchanged with an increased proportion of ETA receptors and a decreased proportion of ETB receptors. We demonstrated that total \([^{125}\text{I}]\)-ET-1 binding sites are unchanged during pregnancy in rat myometrium. In this regard, our data were comparable with those of Osada et al. (1997). However, BQ 123-sensitive ET\(_A\) receptors were increased in human, but decreased in rat myometrium. Yallampalli and Garfield (1994) reported that ET-1 binding sites were increased during delivery as compared to those at the late pregnancy (18 days of the gestation) in rat. Although we did not perform the \([^{125}\text{I}]\)-ET-1 binding study in myometrium from rats in labour, total ET binding sites are possibly increased only around the onset of labour. Variations in the timing of a Caesarean section may reflect the different results of Maggi et al. (1994), Wolff et al. (1996) and Osada et al. (1997) in human myometrium.

In conclusion, the main change in ET-1-induced myometrial contractions was augmentation of ET-1-induced-increases in resting tone near term and this change may be involved in parturition. The characteristic gestational changes in ET receptors in myometrium were the appearance of high affinity ET-1-binding sites and the increase in BQ 123-resistant binding sites. Further investigations are needed to understand the physiological role of these ET-1-binding sites, including the detailed characterization of high K\(_d\) binding sites that appear during pregnancy.

Acknowledgements
This study was supported in part by Grants-in-Aid for Scientific Research (08457436) from the Ministry of Education, Science and Culture of Japan.

References


Received on July 23, 1998; accepted on November 9, 1998