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Correlation between α_1/β-adrenoceptor ratio and spontaneous uterine motor activity in the post-partum rat

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The spontaneous uterine motor activity of the post-partum rat was investigated in parallel with the in-vitro determination of the density of the α_1 and β- adrenergic receptors of the myometrium. The in-vivo experiments were performed by an improved method, using a Millar catheter fitted with a latex microballoon. The spontaneous contractility of the post-partum rat uterus was found to be highest 24 h after delivery, indicating that this time is the most suitable for pharmacological examinations of tocolytic agents. A very close correlation was found between the results of the in-vivo experiments and the α_1/β-adrenergic receptor ratio assessed by an in-vitro receptor assay, thus indicating that the state of the adrenergic receptor system fundamentally determines the contractility of the uterus. This conclusion is supported by the fact that the pharmacological sensitivity of the rat uterus to prazosin and fenoterol changed as a function of the post-partum time in accordance with the α_1/β-adrenoceptor ratio. These results and the relevant data available reveal a crucially important role of an α_1-adrenoceptor-mediated process, implicating α_1-blockers as theoretically potent agents for inhibition of premature uterine contractions.

Key words: adrenergic receptors/post-partum rat/tocolysis/uterine contractility

Introduction

In humans, premature delivery is an unsolved clinical problem, affecting ~7% of pregnancies in the developed countries (McCombs, 1995). The authorized drugs for treatment of this state include β_2-selective adrenergic agonists and magnesium sulphate. The side-effect profile and efficacy of these drugs are not ideal; moreover, there is quite a large group of patients for whom these agents (mostly β_2-sympathomimetics) are strictly contraindicated. On the other hand, β-adrenergic receptors in the pregnant myometrium and other organs are down-regulated after exposure to an agonist, leading to a decrease or complete loss of effectiveness of tocolytic treatment with β_2-adrenergic agonists (von Mandach, 1994; Engelhardt *et al*., 1997). These facts indicate that tocolytic therapy is one of the major challenges in obstetric practice. Novel and more specific agents should be introduced with more favourable side-effects. Research on such compounds necessitates an appropriate and reliable animal model. Most investigations involve the use of in-vitro preparations, which is a widely accepted method. However, this does not take into consideration effects which are not exerted directly on the uterus, but which can modify the uterine activity (e.g. blood pressure, metabolism and electrolyte state). On the other hand, in most experiments the tocolytic agent is tested against agonist-induced contractions, which means that interactions are, in fact, investigated. Among the in-vivo animal models for testing uterine motility, one of the most generally used methods is the registration of intrauterine pressure in the post-partum period. After delivery, the uterus of the rat has a spontaneous activity which can be modified by the tested agent. As the post-partum uterine motility is basically determined by the involutional state, and the involution of the rat is quite fast, it is crucial to perform the measurement at the proper time after delivery. The aim of the present study was to elaborate a reproducible and standardized method by determining the post-partum time at which the spontaneous uterine activity is the greatest and hence the optimal time for testing tocolytic agents. Since adrenergic receptors are known to be deeply involved in the control of uterine activity (Riemer and Roberts, 1986), we assume that there could well be a relationship between the uterine motility as a function of time and the state of the adrenergic receptor system. A substantial amount of data on the density of adrenergic receptors in the uterus during gestation is available (Cohen-Tannoudji *et al*., 1991; Legrand *et al*., 1993). However, characterization of the involution in the rat at the level of these receptors in connection with the uterine motor activity is not sufficient. An additional aim of the present study was to search for some connection between the adrenergic receptor density, the pharmacological sensitivity to agents acting on these receptors (prazosin and fenoterol) and the spontaneous motility of the post-partum rat uterus.

Materials and methods

Experimental design

The method applied for the measurement of intrauterine pressure was based on the classical microballoon experiments described by Csapo (1963). Female Sprague–Dawley rats were anaesthetized with urethane (1 g/kg, i.p.) after spontaneous delivery. The abdominal cavity was opened and a Millar catheter fitted with a liquid-filled latex microballoon was inserted into the uterus through a small section

above the cervical part. After a 45 min equilibration period, the intrauterine pressure was recorded (Hewlett-Packard 7702B) and the spontaneous uterine activity was assessed by measuring the integrated tension relating to a 5 min period. When the pharmacological reactivity to prazosin or fenoterol was investigated, the above-mentioned process was preceded by cannulation of the jugular vein. The effects of the tested drugs were assessed by expressing the integrated tension in the first 5 min after i.v. administration of each compound as a percentage of the average for three 5 min periods before administration. Effective dose (ED_{50}) values were calculated by means of the computer program Grafit 3.01. All of the presented data are the averages of the results of six independent experiments. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research at Albert Szent-Györgyi Medical University, Szeged, Hungary.

Fenoterol and prazosin were purchased from Boehringer Ingelheim, Germany, and Sigma-Aldrich Ltd, Hungary, respectively. Both chemicals were dissolved in physiological saline. Control animals were treated with physiological saline, which caused no change in myometrial contractility.

Radioligand receptor assay

The myometrial tissue samples obtained from post-partum rats at different times after delivery were homogenized in 10 volumes of ice-cold buffer (10 mM Tris–HCl, 0.25 M sucrose, 1 mM EDTA, pH 7.4) and the homogenates were centrifuged at 500 *g*. The supernatants were filtered and centrifuged at 40 000 *g*. The resulting pellets were washed twice in buffer and resuspended in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 1 mM ascorbic acid, pH 7.4). Protein was determined by the method of Bradford (1976). Radioligand binding experiments were performed in triplicate using [^{3}H]-prazosin (an α_1-adrenergic antagonist) and [^{3}H]-dihydroalprenolol (a β-adrenergic antagonist) as radioligands. Radioligands were purchased from Amersham International plc (UK). Under standard assay conditions, the incubate in a final volume of 300 µl consisted of membranes, radioligand, and incubation buffer alone or with unlabelled ligands as indicated. Following incubation at 30°C for 30 min, the membranes were collected on a Whatman GF/C filter, using a Skatron Cell Harvester. Filters were collected in liquid scintillation vials and the radioactivity was measured with an LKB–Wallac liquid scintillation counter. Specific binding was defined as the difference between the amounts of radioligand bound in the absence (total binding) and presence (non-specific binding) of 10 µM prazosin (α_1) or propranolol (β). Saturation experiments were individually analysed with the program LIGAND to determine the receptor number (B_{max}) (Munson and Rodbard, 1980).

Statistical analysis

Statistical analyses were performed with the unpaired Student's *t*-test to assay significant differences in uterine activity (assessed as area under the curve) and α_1- and β-adrenergic receptor densities at specific times after delivery.

Results

Measurements of uterine motor activity were carried out 1, 8, 12, 24 and 48 h after delivery, and a characteristic curve was found (Figure 1a). The spontaneous contractions were strongest after 24 h, though the values after 12 and 48 h were also significantly increased as compared to the 1-hour value. In addition to quantitative differences, the contractions yielded a characteristic pattern (Figure 2). Shortly after delivery (1–8 h), the contractions were sufficiently strong but irregular, and this period was therefore not suitable for pharmacological investigations. The contractions of the uterus were found to be the most reliable, and hence optimal for testing tocolytics, 24 h after delivery. After 48 h, the contractions were quite regular but insufficiently strong. With regard to the density of adrenergic receptors, that of the β-receptors was found to be decreased throughout the first 48 h after labour, while that of the α_1-receptors described a biphasic curve, culminating at 24 h (Figure 1b). A very close correlation ($r = 0.93$) was found between the α_1/β-receptor density ratio and the spontaneous contractility of the uterus at different times (Figure 1c). The

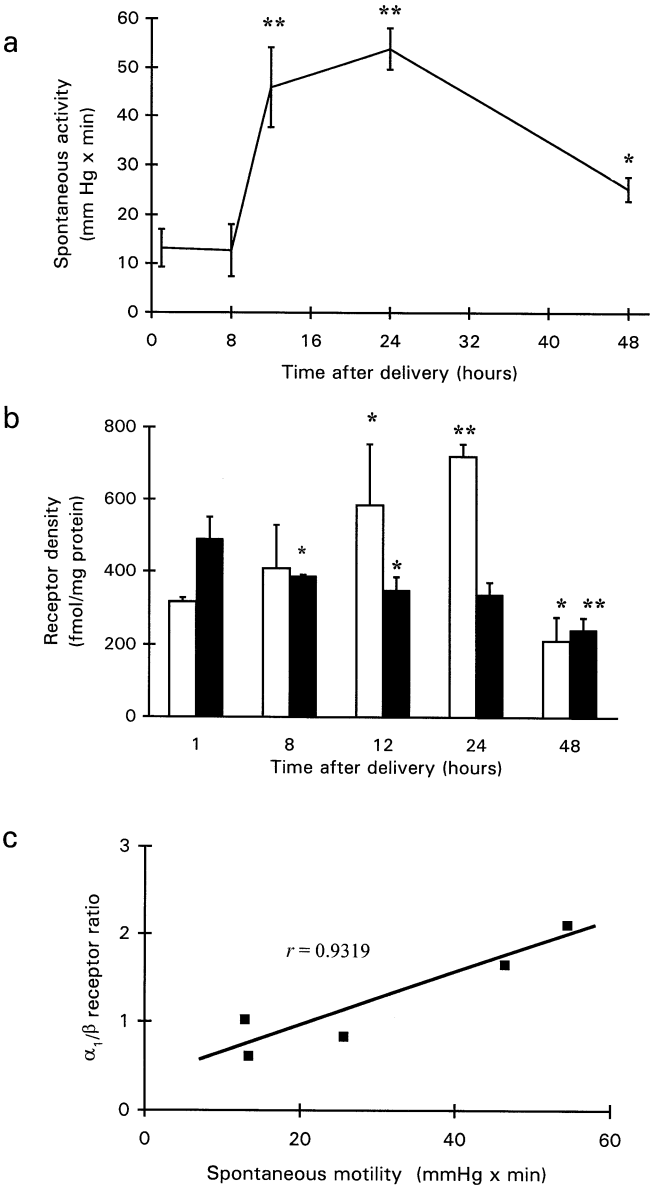

Figure 1. (**a**) Spontaneous motor activity of the post-partum uterus during the first 48 h. (**b**) Density of α_1 (open columns) and β-receptors (solid columns) as a function of post-partum time elapsed. The data are the averages of the results from six experiments ± SEM. $*P < 0.05$, $**P < 0.01$ versus the 1 h value. (**c**) Correlation between α_1/β-adrenergic receptor ratio and spontaneous uterine activity relating to the same post-partum time.

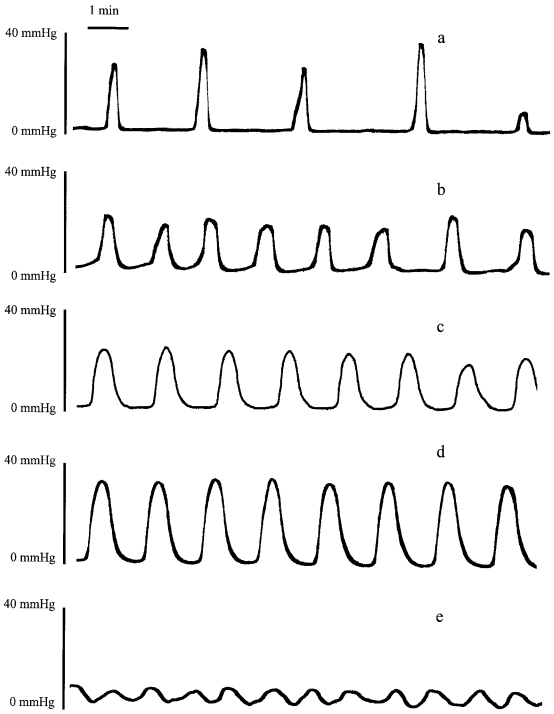

Figure 2. Characteristic patterns of intrauterine pressure (**a**) 1, (**b**) 8, (**c**) 12, (**d**) 24 and (**e**) 48 h after delivery.

binding affinities of the adrenergic receptors did not change during the investigated period. The K_D values for the α_1 and β-receptors were found to be 1.98–2.16 and 1.86–1.99 nM respectively. The tocolytic effects of prazosin and fenoterol were investigated 12 and 24 h after spontaneous delivery (Figure 3). The pharmacological sensivities to the tested compounds were assessed by calculating their tocolytic ED_{50} values (the doses at which 50% of the maximal inhibition of the uterine motor activity is exerted) 12 and 24 h after spontaneous delivery. ED_{50} for prazosin was 0.1103 ± 0.0125 mg/kg and 0.0732 ± 0.0045 mg/kg at 12 and 24 h respectively, after labour. The calculated tocolyic ED_{50} of fenoterol was significantly higher at 24 h (1.0 ± 0.1 μg/kg) in comparison with 12 h (0.52 ± 0.02 μg/kg) after delivery.

Discussion

Mostly in-vitro methods are used for the investigation of uterine contractility and the action of drugs upon it. When tocolytic agents are examined by these methods, usually agonist-induced or electric field-triggered contractions are antagonized by the tested compound. These experimental conditions are fairly different from the clinical situation in which tocolytic therapy must be initiated. On the other hand, some of the traditionally used tocolytics (salbutamol and hexoprenaline) do not inhibit the spontaneous activity of myometrial strips obtained from the pregnant human uterus (Poli *et al*., 1990). These facts urge the development of a reproducible and reliable animal model. It is obvious that the greater the spontaneous contractility, the more suitable the preparation for testing active tocolytic agents. This is why it is important to know the period of the involution at which the

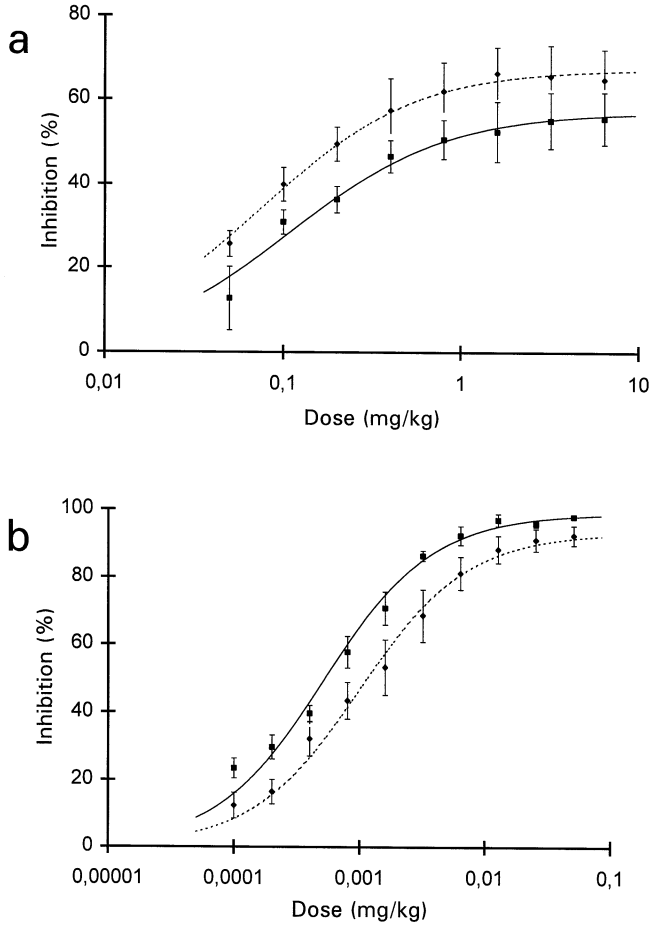

Figure 3. Inhibitory effects of (**a**) prazosin and (**b**) fenoterol on post-partum uterus of the rat 12 (■, solid line) and 24 h (◆, dotted line) after spontaneous delivery. The data are the averages of the results of six independent experiments ± SEM.

motor activity is the highest. When the activity of the uterus of the rat was characterized with regard to the post-partum time elapsed, it was found that the uterine contractility was the greatest at 24 h after delivery, thus indicating that this is the time most suitable for pharmacological investigations. In the present study, the spontaneous uterine contractility *in vivo* was assessed in parallel with the in-vitro assay of α_1 and β-adrenoceptors. A very close correlation ($r = 0.93$) was found between the α_1/β ratio and the motor activity of the uterus. A comparison of the pharmacological reactivities to α_1-blockade and β-stimulation at 12 and 24 h after delivery demonstrated that the ED_{50} value for prazosin decreased slightly during this 12 h period, indicating an increased sensitivity to prazosin. As concerns the ED_{50} of fenoterol, this was markedly increased within this time interval (0.52 compared with 1.0 μg/kg), which lends support to our conclusion that the α_1/β-adrenoceptor ratio determines not only the spontaneous motor activity of the rat uterus, but also the potency of agents with a tocolytic effect. The subject of our experiments (post-partum rat) may differ from the clinical situation in advanced human pregnancy. However, we conclude that the basic mechanism of the control of myometrial contractility is similar, thus making the conclusions useful in clinical research. The correlation outlined above underlines the fact that the adrenergic system funda-

mentally determines the uterine contractility, and that the onset of delivery may be postponed by means of adrenergic modulation (Smiley and Finister, 1996). This pharmacological intervention may involve drugs acting on α_1-receptors besides β-mimetics. These data lead to the speculation that an α_1-blockade may have a reliable and beneficial effect on the activity of the uterus similarly to that of β-agonists. It is generally accepted that the adrenergic innervation of the uterus is dramatically reduced during pregnancy, probably promoted by fetal factors (Owman *et al*., 1975; Sporrong *et al*., 1981), and a similar phenomenon has been documented in humans (Thorbert *et al*., 1979). However, α_1-blockers extensively inhibit the parturition process of the rat (Legrand and Maltier, 1986), indicating that α-adrenergic receptors function in advanced pregnancy. These α-receptors have recently been shown to be situated extrasynaptically (Gáspár *et al*., 1998). A group of α_1-blockers (e.g. prazosin and terazosin) have significant advantages over β-sympathomimetics, including a more favourable side-effect profile and the absence of tachyphylaxis. Treatment with these drugs may mean an alternative to magnesium sulphate as therapy for the group of patients in whom β-agonists are contraindicated (e.g. those with diabetes, tachycardia and cardiac arrhythmias). There is a quite large body of data advocating the usage of α_1-antagonists as tocolytics (Legrand and Maltier, 1986; Lechner *et al*., 1990); however, controlled clinical trials on a sufficient number of subjects for a reliable evaluation of these agents for this purpose have not yet been performed. Since the subtype selectivity of agents acting on α-adrenergic receptors is currently undergoing rapid development, it is probable that a selective and uterus-specific α-blocker will be the key to the unsolved obstacle of tocolysis.

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References

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72,** 248–254.

Cohen-Tannoudji, J., Vivat, V., Heilmann, J. *et al*. (1991) Regulation by progesteron of the high-affinity state of myometrial beta-adrenergic receptor and adenylate cyclase activity in the pregnant rat. *J. Mol. Endocrinol*., **6,** 137–145.

Csapo, A. I. (1963) Model experiments and clinical trials in the control of pregnancy and parturition. *Am. J. Obstet. Gynecol.*, **85,** 359–379.

Engelhardt, S., Zieger, W., Kassubek, J. *et al*. (1997) Tocolytic therapy with fenoterol induces selective down-regulation of beta-adrenergic receptors in human myometrium. *J. Clin. Endocrinol. Metab*., **82,** 1235–1242.

Gáspár, R., Márki, A., Zupkó, I. *et al*. (1998) Evidence of non-synaptic regulation of post-partum uterine contractility in the rat. *Life Sci.*, **62,** 1119–1124.

Lechner, W., Keene, F., Daxenblicher, G. *et al*. (1990) Uterine relaxation by the α_1-blocking agent doxazosin. *Int. J. Feto–Maternal Med*., **3,** 6–8.

Legrand, C. and Maltier, J.P. (1986) Evidence for a noradrenergic transmission in the control of parturition in the rat. *J. Reprod. Fertil*., **76,** 415–424.

Legrand, C., Mhaouty, S., Rigolot, C. *et al*. (1993) Heterogenity of alpha-2 adrenoceptors in pregnant rat uterus: identification of subtypes and autoradiographic distribution. *J. Pharmacol. Exp. Ther.*, **266,** 439–449.

McCombs, J. (1995) Update on tocolytic therapy. *Ann. Pharmacother.*, **29,** 515–522.

Munson, P.J. and Rodbard, D. (1980) Ligand: a versatile computerized approach for characterization of ligand-binding system. *Anal. Biochem*., **107,** 220–239.

Owman, C., Alm, P., Rosengren, E. *et al*. (1975) Variations in the level of uterine norepinephrine during pregnancy in the guinea pig. *Am. J. Obstet. Gynecol.*, **122,** 961–964.

Poli, E., Merialdi, A. and Coruzzi, G. (1990) Characterization of the spontaneous motor activity of the isolated human pregnant myometrium. *Pharmacol. Res*., **22,** 115–124.

Riemer, R.K. and Roberts, J.M. (1986) Endocrine modulation of myometrial response. In Huszar, G. (ed.), *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor.* CRC Press Inc, Boca Raton, USA, pp. 53–71.

Smiley, R.M. and Finister M. (1996) Do receptors get pregnant too? Adrenergic receptor alterations in human pregnancy. *J. Matern. Fetal. Med*., **5,** 106–114.

Sporrong, B., Alm, P., Owman, C. *et al*. (1981) Pregnancy is associated with extensive adrenergic nerve degeneration in the uterus. An electromicroscopic study in the guinea-pig. *Neuroscience*, **6,** 1119–1126.

Thorbert, G., Alm, P., Bjorklund, A.B. *et al*. (1979) Adrenergic innervation of the human uterus. Disappearance of the transmitter and transmitter-forming enzymes during pregnancy. *Am. J. Obstet. Gynecol*., **135,** 223–226.

von Mandach, U. (1994) Adrenergic beta-2 receptors and cyclic AMP in lymphocytes and their relationship to uterine contractility. *Z. Geburtshilfe Perinatol.*, **198,** 201–203.

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