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Agonist and antagonist specificities of decidual prostaglandin-releasing oxytocin receptors and myometrial uterotonic oxytocin receptors in pregnant rats

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This paper describes further pharmacological characterization of the decidual prostaglandin-releasing oxytocin receptors and the myometrial uterotonic oxytocin receptors in the uterus of the pregnant rat. The effects of oxytocin, arginine-vasopressin and their related agonists and antagonists on the release of $PGF_{2\alpha}$ were studied *in vitro* on isolated uteri from rats on day 19–20 of pregnancy that had been incubated in Krebs buffer, pH 7.4, at 37°C. The concentration of $PGF_{2\alpha}$ in the media was measured using specific radioimmunoassays. It was found that the decidual and myometrial oxytocin receptors exhibit different ligand specificities. Of the agonists tested, oxytocin and arginine-vasopressin stimulated $PGF_{2\alpha}$ release in a dose-dependent manner. Arginine-vasopressin has only 3% of the uterotonic potency of oxytocin, but was found to have 16% of its $PGF_{2\alpha}$-releasing activity. [4-Threonine, 7-glycine]oxytocin, a highly potent and selective uterotonic oxytocin analogue, had no detectable prostaglandin-releasing activity at a dosage 30 times higher than oxytocin. However, 1-deamino-[8-D-arginine]vasopressin, a highly potent and selective antidiuretic arginine-vasopressin analogue, which has only 10% of the uterotonic activity of arginine-vasopressin, was as potent as arginine-vasopressin in prostaglandin-releasing activity. Of the oxytocin antagonists tested, it was confirmed that [1-penicillamine, 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin and its close congener [1-penicillamine, 2-*p*-methyl-phenylalanine, 4-threonine]ornithine-vasotocin are partial oxytocin antagonists and that 9-desglycinamide-[1-(β-mercapto-β-β-cyclopentamethylene-propionic acid)2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin, [1-(β-mercapto-β-β-cyclopentamethylenepropionic acid)2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin and 1-deamino-penicillamine [2-*O*-methyl-tyrosine]ornithine-vasotocin are full oxytocin antagonists. The two partial oxytocin antagonists blocked the uterotonic action of oxytocin but had agonistic activity on decidual receptors, stimulating release of $PGF_{2\alpha}$. The full oxytocin antagonists blocked both the uterotonic and $PGF_{2\alpha}$-releasing actions of oxytocin. Thus, the myometrial and decidual oxytocin receptors have different ligand specificities for agonists and antagonists. We propose that the two uterine oxytocin receptor subtypes be designated as OT_{1a} for the myometrial uterotonic receptors and OT_{1b} for the endometrial or decidual prostaglandin-releasing receptors.

Introduction

The mechanism that triggers the onset of labour is not known. The onset of parturition is preceded by a marked increase in the concentrations of oxytocin receptors (Alexandrova and Soloff, 1980; Soloff, 1985) and the formation of gap junctions (Garfield *et al.*, 1980; Verhoeff and Garfield, 1986) in the myometrium. The development of these two biomolecular events may have an important role in the initiation of labour. Oxytocin has a dual action in the uterus: a uterotonic action on myometrial cells and a prostaglandin-releasing action on endometrial and decidual cells (Chan, 1980; Fuchs *et al.*, 1982). We have shown that oxytocin receptors and gap junctions in the pregnant rat are stimulated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), while inhibiting prostaglandin synthesis suppresses their formation and delays the onset of labour (Chan *et al.*, 1991; Chan and Chen, 1992). Others have demonstrated that uterine tissues express the oxytocin gene during pregnancy, and that the rate of expression increases markedly near term (Lefebvre *et al.*, 1992, 1993; Chibbar *et al.*, 1993). These findings suggest that a paracrine or autocrine oxytocin system is involved in initiating

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labour. We postulate that oxytocin may regulate its own receptor formation and gap junction development in the myometrium via its $PGF_{2\alpha}$-releasing action in the endometrium and decidua.

Preliminary findings showed that myometrial and decidual oxytocin receptors in the uterus of pregnant rats could be differentiated by oxytocin antagonists into two subtypes (Chan *et al.*, 1993). Presently available oxytocin antagonists may block only the uterotonic action or may block both the uterotonic and $PGF_{2\alpha}$-releasing actions of oxytocin. Delineating the two oxytocin receptor subtypes and their ligand specificities is important both for the fundamental study of the action of oxytocin and to the clinical management of preterm labour. Characterizing the two uterine oxytocin receptor subtypes could lead to the development of myometrial-selective and endometrial- or decidual-selective oxytocin antagonists. Oxytocin antagonists selective for different receptor subtypes would provide powerful probes to investigate the role of oxytocin in the initiation of labour, and potential tocolytic agents for the treatment of preterm labour.

In this paper, we report further studies on the characterizations of the myometrial uterotonic oxytocin receptors and the decidual prostaglandin-releasing oxytocin receptors in pregnant rats. We present data showing that the two oxytocin receptor subtypes can be differentiated not only by appropriate oxytocin antagonists but also by their affinity and specificities for agonists.

Materials and Methods

Materials

The following oxytocin and vasopressin agonists and antagonists were used (with the exceptions noted below, these were synthesized in the laboratory by M. Manning): 1-deamino-[8-D-arginine]vasopressin (dDAVP) (Zaoral *et al.*, 1967; Manning *et al.*, 1976); [4-threonine, 7-glycine]oxytocin ([Thr^4, Gly^7]OT) (Lowbridge *et al.*, 1977); 1-deamino-penicillamine [2-*O*-methyl-tyrosine]ornithine-vasotocin (dP[Tyr-$(Me)^2$]OVT) (Sawyer *et al.*, 1980); 1-(β-mercapto-β-β-cyclopentamethylenepropionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin (d$(CH_2)_5$[Tyr$(Me)^2$, Thr^4]OVT) and 9-desglycinamide-[1-(β-mercapto-β-β-cyclopentamethylene-propionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin (desGly-NH_2, d$(CH_2)_5$[Tyr$(Me)^2$, Thr^4]OVT) (Manning *et al.*, 1989). [1-Penicillamine, 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin (P[Tyr$(Me)^2$, Thr^4]OVT) and [1-penicillamine, 2-*p*-methyl-phenylalanine, 4-threonine]-ornithine-vasotocin (P[Phe$(Me)^2$, Thr^4]OVT) (Chan *et al.*, 1986, 1987) were synthesized in V. J. Hruby's laboratory (University of Arizona). Oxytocin and arginine-vasopressin were purchased from Peninsula Laboratories (Belmont, CA). Multi-labelled [^{3}H]$PGF_{2\alpha}$ was purchased from DuPont NEN (Boston, MA) and prostaglandin standards and anti-prostaglandin sera from PerSeptive Diagnostics (Cambridge, MA).

Animals

Pregnant Wistar rats, 60–70 days old, were purchased from Hilltop Laboratory Animals (Scottsdale, PA). Rats were mated in the morning and were examined in the afternoon for the presence of vaginal plugs (presence of plug indicated day 1 of pregnancy). Those found with plugs were identified, separated, and delivered to us on day 13 of pregnancy. The rats were housed individually in shoebox cages in our central animal care facility under a controlled photoperiod and air-conditioning. Water and food were freely available. The use of animals in this protocol was approved by the Institutional Animal Care and Use Committee.

Experimental protocol

The effects of oxytocin agonists and antagonists on uterine $PGF_{2\alpha}$ release *in vitro* were determined on isolated uteri from rats on day 19–20 of pregnancy. Rats were killed by cervical dislocation and the uterine horns quickly removed and placed in ice-cold Krebs–bicarbonate buffer at pH 7.4. The uterine horns were cut open and fetal tissues removed. Each uterine horn was divided longitudinally in half over the placental attachment sites, forming a matched pair. Thus, four matched tissues were obtained from each rat. Each uterine tissue was rinsed clean in buffer at 37°C, and was hung in an incubation chamber under a tension of 2 g. The tissues were incubated in 20 ml Krebs buffer that was aerated continuously with 95% O_2 and 5% CO_2 at 37°C.

After 20 min, the experimental incubation period was started. The incubation medium was withdrawn and replaced at 30 min intervals. After two control intervals (each of 30 min), an oxytocin agonist or an oxytocin antagonist was added to the incubation medium. The incubation continued for a further two or three 30 min periods. In each incubation experiment, one matched tissue was used as a control and was incubated only in the Krebs buffer. Three concentrations of each test peptide were studied. Only one test peptide and one concentration were tested in a given tissue preparation. Thus, from one rat, the four matched tissues were allocated to a control and three concentrations of a test peptide. In experiments in which the effects of oxytocin antagonists on the $PGF_{2\alpha}$-releasing activity of oxytocin were determined, the antagonist was introduced 5 min before oxytocin. At the conclusion of the incubation experiment, the wet masses of the uterine tissues were measured.

$PGF_{2\alpha}$ in the control and treated incubates was extracted and determined by radioimmunoassays as described below. The rate of $PGF_{2\alpha}$ release was expressed in ng g^{-1} tissue min^{-1}. The release rate during the second control (pretreatment) period for each tissue was taken as the basal release rate for that tissue preparation (generally between 0.20 and 0.35 ng g^{-1} min^{-1}) and was designated the value of 100%. $PGF_{2\alpha}$ release rates in all subsequent samples were expressed as a percentage of their respective basal rates. The $PGF_{2\alpha}$ release rate of the second post-treatment incubation sample was used as the measure of response. The $PGF_{2\alpha}$ release rate of the control tissue for the corresponding period was used as the baseline stability reference. Preliminary studies showed that this experimental protocol yielded reproducible linear dose–response curves to oxytocin (Chan *et al.*, 1993). Only the release of $PGF_{2\alpha}$ was measured, since our previous experiments have shown that other prostanoids are not significantly released by oxytocin (Chan, 1987; Chan *et al.*, 1993).

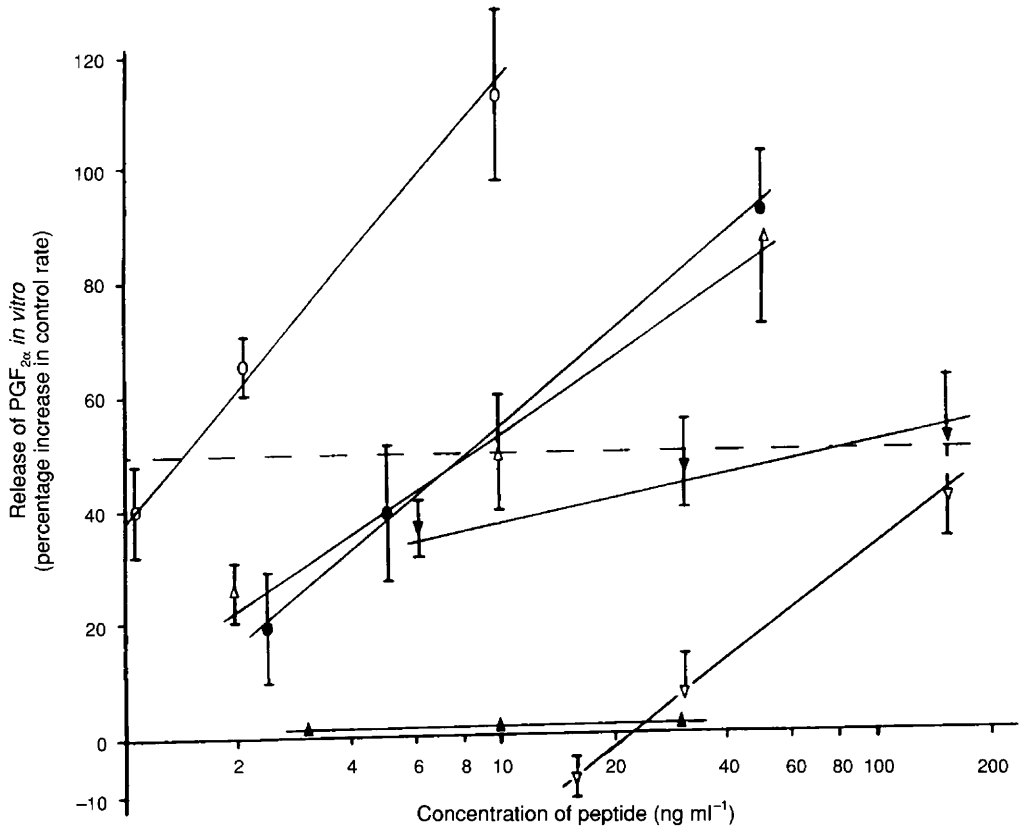

Fig. 1. Effects of oxytocin and vasopressin agonists (○: oxytocin; ●: arginine-vasopressin; △: 1-deamino-[8-D-arginine]vasopressin; ▲: [4-threonine, 7-glycine]oxytocin) and antagonists (▼: [1-penicillamine, 2-*p*-methyl-phenylalanine, 4-threonine]ornithine-vasotocin; ▽: [1-penicillamine, 2-*O*-methyl-tyrosine, 4-threonine] ornithine-vasotocin) on the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) *in vitro* from rat uteri on day 19–20 of pregnancy. Each point on the dose–response curves represents the mean value of at least three experiments ± SEM. The dashed line marks the relative potencies of the agonists; that is, the dose required to increase the rate of release to 50% more than the control basal rate.

Extraction and radioimmunoassays of $PGF_{2\alpha}$

$PGF_{2\alpha}$ from each incubation sample was extracted and eluted with ethyl acetate through a Sep-Pak C_{18} cartridge (Waters, Milford, MA). The extracts were dried at 40°C under N_2 and the residue stored at − 20°C until used for radioimmunoassay. Details of the extraction and radioimmunoassay procedures were described by Chan (1987). $PGF_{2\alpha}$ concentrations were determined in each sample and in duplicate. The anti-$PGF_{2\alpha}$ antibody used was highly specific, with no significant crossreactions (< 0.5%) with other prostaglandins present in the uterus. The sensitivity of the radioimmunoassay was 15 pg per assay tube. The intra-assay coefficient of variation was < 10% and the interassay coefficient of variation was < 15%.

In vitro *oxytocin and anti-oxytocin assays*

In vitro oxytocin assays were performed on isolated uteri from Wistar rats that had been pretreated the afternoon before with 50 μg diethylstilboestrol in oil per rat injected s.c. Mg^{2+}-free van Dyke–Hasting solution (Munsick, 1960) was used as the bathing medium. Agonistic potencies were determined against the USP standard using the four-point bioassay design (Holton, 1948). Antagonistic potencies of the oxytocin antagonists were measured against oxytocin and expressed as pA_2 values (Schild, 1947).

Statistical analyses

All data were expressed as sample means ± SEM and analysed by analysis of variance. Significant differences between sample means were analysed by paired Student's *t* test at the $P = 0.05$ level.

Results

Effects of oxytocin/vasopressin agonists on $PGF_{2\alpha}$ release in vitro *in the uteri of pregnant rats*

The $PGF_{2\alpha}$-releasing activity of the four agonists (oxytocin and its uterotonic-selective analogue [Thr^4, Gly^7]OT; and arginine-vasopressin and its antidiuretic-selective analogue dDAVP) were determined *in vitro* in the rat uterus on day 19–20 of pregnancy.

Oxytocin, arginine-vasopressin and dDAVP stimulated $PGF_{2\alpha}$ release in a dose-dependent manner (Fig. 1). The dose range for oxytocin, 1–10 ng ml^{-1}, represents 0.5–5.0 mU

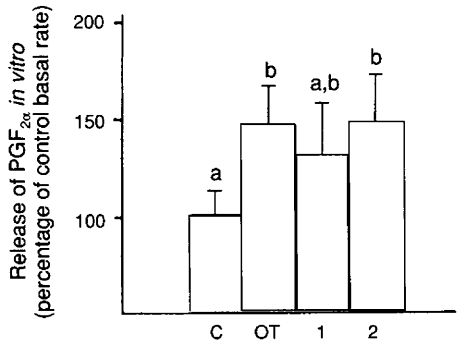

Fig. 2. Effects of [4-threonine, 7-glycine]oxytocin ([Thr^4, Gly^7]OT) on oxytocin-induced release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) *in vitro* from rat uteri on day 19–20 of pregnancy. The bar columns show the group means of four experiments in each group ± SEM. Group means with different letters are significantly different from each other ($P < 0.05$). C: control; OT: 4 ng oxytocin ml^{-1}; treatment 1: 4 ng oxytocin ml^{-1} plus 30 ng [Thr^4, Gly^7]OT ml^{-1}; treatment 2: 4 ng oxytocin ml^{-1} plus 300 ng [Thr^4, Gly^7]OT ml^{-1}.

oxytocic activity ml^{-1}, which is in the low–moderate dose range in rat oxytocic assays *in vitro*. Unexpectedly, the highly potent and selective uterotonic analogue [Thr^4, Gly^7]OT had no $PGF_{2\alpha}$-releasing activity at concentrations up to 30 ng ml^{-1}. In tests for antagonisim, [Thr^4, Gly^7]OT had no inhibitory effect on the $PGF_{2\alpha}$-releasing activity of oxytocin at doses of up to 300 ng ml^{-1} (Fig. 2).

There was no apparent correlation between the uterotonic and the $PGF_{2\alpha}$-releasing activities of the peptides (Table 1).

Effects of oxytocin antagonists on $PGF_{2\alpha}$ release in vitro

The five potent oxytocin antagonists (anti-oxytocic) were examined for their effects on $PGF_{2\alpha}$ release and their antagonism of the $PGF_{2\alpha}$-releasing activity of oxytocin *in vitro* in rat uteri on day 19–20 of pregnancy. The anti-oxytocin potencies of these five oxytocin antagonists range from pA_2 values of 7.30 to 7.95. The concentrations of the oxytocin antagonists used in the experiments (30–750 ng ml^{-1}) cover the half-maximal to maximal anti-uterotonic doses of these antagonists in the pA_2 bioassay. They were effective inhibitory doses of the uterotonic response to the dose of oxytocin (2–4 ng ml^{-1}) used to stimulate $PGF_{2\alpha}$ release in the pregnant rat uterus.

In confirmation of preliminary studies (Chan *et al.*, 1993), P[Phe(Me)^2, Thr^4]OVT was found to be a partial oxytocin antagonist and desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT a full oxytocin antagonist.

P[Tyr(Me)^2, Thr^4]OVT, a close congener of P[Phe(Me)^2, Thr^4]OVT, also acts as a partial oxytocin antagonist. It blocked the uterotonic response to oxytocin but stimulated $PGF_{2\alpha}$ release (Table 2). The $PGF_{2\alpha}$-releasing dose–response curves of these two partial oxytocin antagonists are shown (Fig. 1), together with the agonist peptides tested.

dP[Tyr(Me)^2]OVT and d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT are full oxytocin antagonists. They, like desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT, blocked both the uterotonic and the $PGF_{2\alpha}$-releasing actions of oxytocin. The inhibition of oxytocin-induced $PGF_{2\alpha}$ release *in vitro* by the three full oxytocin antagonists is dose dependent, and desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT was the most potent (Fig. 3).

Discussion

In one of our early studies, we found that treating pregnant rats during the last 3 days of gestation with P[Phe(Me)^2, Thr^4]OVT, a long-acting oxytocin antagonist, significantly prolonged the duration of parturition, but did not delay the onset of labour or suppress oxytocin receptor and gap junction formation (Chan *et al.*, 1991; Chan and Chen, 1992). Subsequently, we determined that P[Phe(Me)^2, Thr^4]OVT was a partial oxytocin antagonist, which blocked the uterotonic action of oxytocin but had agonistic action on decidual receptors stimulating $PGF_{2\alpha}$ release (Chan *et al.*, 1993). This could account for the failure of this antagonist to suppress oxytocin receptor formation and delay the onset of labour. The study reported here provided further evidence that the myometrial and decidual oxytocin receptors represent two subtypes.

The myometrial and decidual oxytocin receptors each had different ligand specificities for agonists and antagonists. To study the effects of agonists, we compared the uterotonic and $PGF_{2\alpha}$-releasing potencies of oxytocin and [Thr^4, Gly^7]OT, a highly potent and selective uterotonic oxytocin analogue, and of arginine-vasopressin and dDAVP, a highly potent and

Table 1. Comparison of uterotonic and prostaglandin-releasing activities of oxytocin agonists in rats

Peptides	Oxytocic potency		Prostaglandin-releasing potency	
	U mg^{-1}[a]	Relative potency	ED_{50}[b] (ng ml^{-1})	Relative potency
Oxytocin	520	1.00	1.4	1.00
[Thr^4, Gly^7]OT	857[c]	1.76	30 (no effect)	0.00
Arginine-vasopressin	14	0.03	9	0.16
dDAVP	1.5	0.003	10	0.14

[a]Rat oxytocic activity *in vitro* in USP units assayed in previous studies (Manning *et al.*, 1976; Lowbridge *et al.*, 1977).
[b]Concentrations that increased the release of prostaglandins by 1.5 times the basal rate in isolated rat uteri on day 19–20 of pregnancy incubated in Krebs buffer at 37°C.
[c]Assayed in 0.5 mmol Mg^{2+} l^{-1}. All assays of prostaglandin release were with Mg^{2+}.
dDAVP: 1-deamino-[8-D-arginine] vasopressin; [Thr^4, Gly^7]OT: [4-threonine, 7-glycine]oxytocin.

Table 2. Comparison of effects of oxytocin antagonists on prostaglandin release *in vitro* from rat uteri on day 19–20 of pregnancy

Oxytocin antagonists	Anti-uterotonic potency, pA_2[a]	Effects on prostaglandin release	
		Spontaneous[b]	Oxytocin-induced[c]
P[Phe(Me)^2, Thr^4]OVT	7.30	Stimulate	No effect
P[Tyr(Me)^2, Thr^4]OVT	7.30	Stimulate	No effect
desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT	7.95	No effect	Decrease
d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT	7.84	No effect	Decrease
dP[Tyr(Me)^2]OVT	7.70	No effect	Decrease

[a]pA_2 Values represent the negative logarithm to the base 10 of the average molar concentration of an antagonist that will reduce the biological response to $2x$ units of agonist to equal the response given by x units of agonist in the absence of antagonist. *In vitro* assays on nonpregnant rat uteri.
[b]Studied in the dose range 30–750 ng ml^{-1}.
[c]Studied in the dose range 150–750 ng ml^{-1}.
d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT: [1-(β-mercapto-β-β-cyclopentamethylene propionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin; desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT: 9-desglycinamide-[1-(β-mercapto-β-β-cyclopentamethylene propionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin; dP[Tyr(Me)^2]OVT: 1-deamino-penicillamine [2-*O*-methyl-tyrosine]-ornithine-vasotocin; P[Phe(Me)^2, Thr^4]OVT: [1-penicillamine, 2-*p*-methyl-phenylalanine, 4-threonine]ornithine-vasotocin; P[Tyr(Me)^2, Thr^4]OVT: [1-penicillamine, 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin.

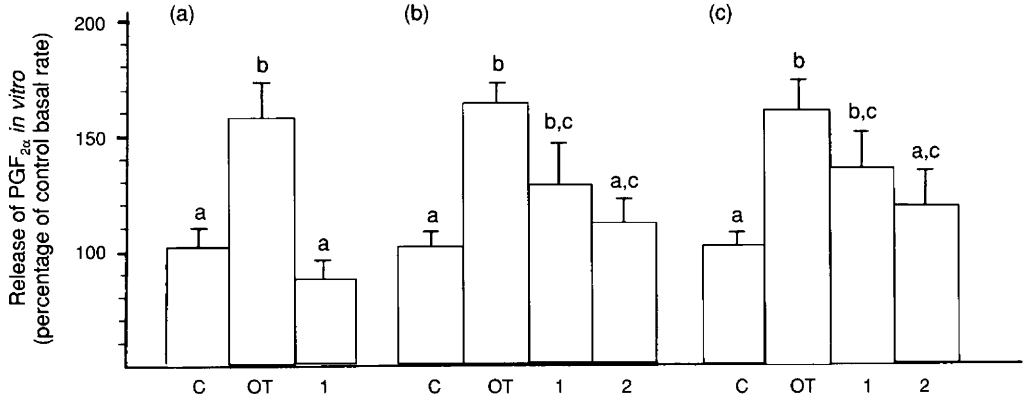

Fig. 3. Inhibition of oxytocin-induced release of prostaglandin $F_{2\alpha}$ *in vitro* in rat uteri on day 19–20 of pregnancy by the oxytocin antagonists (a) 9-desglycinamide-[1-(β-mercapto-β-β-cyclopentamethylenepropionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin, (b) [1-(β-mercapto-β-β-cyclopentamethylene-propionic acid) 2-*O*-methyl-tyrosine, 4-threonine]ornithine-vasotocin and (c) 1-deamino-penicillamine [2-*O*-methyl-tyrosine]ornithine-vasotocin. The bar columns show the group means of at least four experiments in each group ± SEM. Group means with different letters in each panel are significantly different from each other ($P < 0.05$). C: control; OT: 4 ng oxytocin ml^{-1}; treatment 1: 4 ng oxytocin ml^{-1} plus 150 ng antagonist ml^{-1}; treatment 2: 4 ng oxytocin ml^{-1} plus 750 ng antagonist ml^{-1}.

selective antidiuretic vasopressin (V_2 receptor) analogue *in vitro*. The rank orders of their relative uterotonic potencies and their $PGF_{2\alpha}$-releasing potencies were found to be markedly different. Arginine-vasopressin possesses only a fraction (3%) of the uterotonic activity of oxytocin but was found to have 16% of the $PGF_{2\alpha}$-releasing activity of oxytocin. [Thr^4, Gly^7]OT, the highly selective uterotonic oxytocin agonist with negligible vasopressor (V_{1a} receptor) or antidiuretic (V_2 receptor) activities and nearly two times more potency than oxytocin in uterotonic activity, when assayed in 0.5 mmol Mg^{2+} l^{-1} (Lowbridge *et al.*, 1977), had no detectable $PGF_{2\alpha}$-releasing activity at a dosage 30 times higher than that of oxytocin. dDAVP is a highly potent and selective V_2 agonist (Zaoral *et al.*, 1967; Manning *et al.*, 1976). It has an antidiuretic activity of 1200 U mg^{-1} versus 320 U mg^{-1} for arginine–vasopressin, but a uterotonic activity of only 10% of that of arginine–vasopressin and 0.3% of that of oxytocin (Manning *et al.*, 1976). Its $PGF_{2\alpha}$-releasing activity was found to be equal to that of arginine–vasopressin. The effects of the oxytocin antagonists P[Phe(Me)^2, Thr^4]OVT and its close congener P[Tyr(Me)^2, Thr^4]OVT (Chan *et al.*, 1986, 1987), and of desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT and its nontruncated structure d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT (Manning *et al.*, 1989), and dP[Tyr(Me)^2]OVT (Sawyer *et al.*, 1980), were also compared. We confirmed our preliminary findings (Chan *et al.*, 1993) that P[Phe(Me)^2, Thr^4]OVT is a partial oxytocin antagonist and desGly-NH_2, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT is a full oxytocin antagonist. P[Tyr(Me)^2, Thr^4]OVT, like its Phe(Me)^2

congener, was also found to be a partial oxytocin antagonist, both of these partial antagonists blocked the uterotonic action of oxytocin but stimulated $PGF_{2\alpha}$ release in the isolated pregnant rat uterus. However, their $PGF_{2\alpha}$-releasing potencies were 2–3 orders of magnitude weaker than oxytocin.

The peptides $d(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT and dP[Tyr-(Me)^2]OVT, like the truncated desGly-NH_2, $d(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT, were found to be full oxytocin antagonists, which blocked both the uterotonic and prostaglandin-releasing actions of oxytocin. However, all three oxytocin antagonists were much more potent in antagonizing the uterotonic action than the $PGF_{2\alpha}$-releasing action of oxytocin. The concentrations of the antagonists used in the anti-prostaglandin-release assays (30–750 ng ml^{-1}) were at least a thousand times higher than those used in the pA_2 anti-uterotonic assays. The dose range used in the anti-prostaglandin-release assays produced maximal inhibitions of the oxytocin-induced contractions in the isolated pregnant uterus. The high doses required to block the oxytocin-induced $PGF_{2\alpha}$ release indicate that these oxytocin antagonists are poor ligands for the decidual $PGF_{2\alpha}$-releasing oxytocin receptors.

The striking differential effects between the different oxytocin antagonists on uterotonic inhibition and inhibition of $PGF_{2\alpha}$ release and the lack of $PGF_{2\alpha}$-releasing activity of the highly potent and selective oxytocin agonist [Thr^4, Gly^7]OT strongly support our postulate that the myometrial uterotonic oxytocin receptors and the endometrial/decidual prostaglandin-releasing oxytocin receptors represent two distinct subtypes. We propose, as suggested by Chan *et al.* (1993), that the myometrial uterotonic oxytocin receptors be designated as the OT_{1a} subtype and the endometrial/decidual $PGF_{2\alpha}$-releasing oxytocin receptors as the OT_{1b} subtype. Although the number of agonists and antagonists investigated in this study was too small to allow a characterization of the ligand specificities of the two receptor subtypes, the findings do appear to suggest that deleting the amino-terminal amino group may be a requirement for inhibiting prostaglandin release. It also appears that a structure selective for V_2 receptors may enhance binding to decidual oxytocin receptors, as suggested by the relative $PGF_{2\alpha}$-releasing activity of arginine-vasopressin and dDAVP to their respective uterotonic activities. Further analysis of structure–activity relationships could lead to the discovery of decidual-selective and myometrial-selective oxytocin agonists and antagonists.

Premature birth is a major medical, societal and economic problem. Premature labour affects nearly 10% of pregnancies, and premature birth is the leading cause of neonatal mortality and morbidity in developed countries (Vital Statistics of US, 1982; Main, 1988). Safe and effective therapeutic intervention of preterm labour has yet to be developed. The mechanism that triggers the onset of labour is poorly understood. The marked increases in myometrial oxytocin receptor concentrations and gap junction densities at term (Soloff, 1985; Verhoeff and Garfield, 1986; Chan *et al.*, 1991; Chan and Chen, 1992) and the recent new findings that the pregnant uterus is a major site of oxytocin synthesis at term (Lefebvre *et al.*, 1992, 1993; Chibbar *et al.*, 1993) suggest that a paracrine or autocrine oxytocin system may play an important role in the initiation of labour. The identification of the two oxytocin receptor subtypes in the uterus with different ligand specificities has important implications both for our understanding of the mechanism of initiation of labour and for the clinical application of oxytocin antagonists in the treatment and prevention of preterm labour.

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