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European Journal of Pharmacology 281 (1995) 63–68

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Effects of the non-peptide inhibitor OPC-21268 on oxytocin and vasopressin stimulation of rat and human myometrium

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Received 9 February 1995; revised 5 April 1995; accepted 6 April 1995

Abstract

OPC-21268 (1-[-1-[4-(3-acetylaminopropoxy)benzoyl]-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone), a non-peptide vasopresssin V_1 receptor antagonist, inhibited oxytocin- and vasopressin-induced contractions of myometrial strips from rats and from full-term pregnant women. Administered intravenously in rats the drug also inhibited uterine contractions caused by infusion of oxytocin. When incubated with purified plasma membranes from rat or human myometrial tissue, OPC-21268 inhibited the specific receptor binding of tritiated oxytocin and vasopressin in a dose-dependent and reversible way.

Keywords: Oxytocin; Vasopressin; Myometrial receptor; OPC-21268; Vasopressin receptor antagonist

1. Introduction

In a number of studies the effect on oxytocin- and vasopressin-induced myometrial contractions of antagonists structurally derived from neurohypophysial peptides has been investigated (Atke et al., 1988; Melin and Trojnar, 1988; Wilson et al., 1990; Manning and Sawyer, 1993). Some of the antagonists (1-deamino-2-tyrosine-(*O*-ethyl)-oxytocin and 1-deamino-2-D-tyrosine-(*O*-ethyl)-4-threonine-8-ornithine-oxytocin) have been tested clinically in pregnant women with premature labour with the aim of arresting uterine contractions (Åkerlund et al., 1987).

Recently the non-peptide antagonist 1-[-1-[4-(3-acetylaminopropoxy)benzoyl]-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone (OPC-21268) of vasopressin V_1 receptors of smooth muscle was described (Yamamura et al., 1991; Chiba and Tsukada, 1992). This compound, because of its non-peptide structure, is orally active and has been shown to lower blood pressure in a patient with congestive heart failure (Miura et al., 1993) and in spontaneously hypertensive rats (Yamada et al., 1994). It also attenuates vasopressin induced bradycardia in rabbits (Masaki et al., 1993) and pressor responses in rats (Yamamura et al., 1991). It has further been demonstrated that OPC-21268 exerts its inhibitory action through binding to the vasopressin V_1 receptors in rat liver and kidney (Yamamura et al., 1991; Burrell et al., 1993). Finally, OPC-21268 was reported to be safe and non-toxic in healthy humans (Ohnishi et al., 1993).

Both in rats and humans the myometrium is sensitive to vasopressin and oxytocin. In rats the potency of oxytocin to contract the myometrium exceeds that of vasopressin, yet the binding affinities of the two peptides to myometrial receptors are roughly identical (Atke et al., 1989). In the human uterus, however, the non-pregnant myometrium is more sensitive to vasopressin than to oxytocin (Joelsson et al., 1966; Bengtsson, 1970). During pregnancy the ratio changes, and at term the sensitivity to oxytocin dominates (Fuchs et al., 1984; Maggi et al., 1990). Further, it has been demonstrated that oxytocin is able to displace receptor-bound [^{3}H]vasopressin and that vasopressin displaces receptor-bound [^{3}H]oxytocin (Atke et al., 1995; Fuchs et al., 1984), leaving the question open whether the two pep-

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SSDI 0014-2999(95)00225-1

tides exert their contractile effect on the myometrium through interactions with separate receptors or one class of receptors with affinity for both peptides. During the last few years the generally accepted opinion is that the myometrium possesses a selective oxytocin receptor as well as a receptor very similar to the classical vasopressin V_1 receptor, but still there are contradicting reports on this topic (Fuchs et al., 1985; Guillon et al., 1987; Atke et al., 1989; Ivanisevic et al., 1989; Chan et al., 1990; Maggi et al., 1994).

OPC-21268 is a specific vasopressin V_1 receptor antagonist and it was therefore found of interest to study its effect on the contractile action on the myometrium of vasopressin and oxytocin and to investigate its affinity for the myometrial receptors. In the present study we report specific binding of OPC-21268 to oxytocin and vasopressin receptors in rat myometrial plasma membranes as well as in full-term pregnant human myometrial plasma membranes. Furthermore, the in vitro effects of OPC-21268 on isolated myometrial strips from rats and full-term pregnant women, supplemented with in vivo experiments of the effects on oxytocin stimulation in anaesthetized rats, are described.

2. Materials and methods

2.1. Materials

OPC-21268 (1-[-1-[4-(3-acetylaminopropoxy) benzoyl]-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone) was a gift from Otsuka Pharmaceuticals, Rockville, MD, USA. Oxytocin and [Arg^8]vasopressin were kindly donated by Ferring Pharmaceuticals, Malmö, Sweden. Tritiated peptides were obtained from Amersham, Little Chalfond, UK. The specific activities were 39.0 Ci/mmol for oxytocin and 74.5 Ci/mmol for vasopressin. All chemicals used were analytical grade.

2.2. Human myometrial tissue

Human myometrial tissue specimens were obtained peroperatively from the upper part of the lower segment of the uterus of full-term pregnant women (gestational age > 39 weeks) undergoing elective Caesarean section. Informed consent from the women was obtained and the project was approved by the Ethics Committee. Patients undergoing hormonally or mechanically induced labour prior to operation were excluded. The tissue samples (1–5 g each) were placed in oxygenated Krebs-Ringer solution at 4° C and immediately (< 1 h) transported to the laboratory for dissection of strips for monitoring in vitro contractions or for preparation of purified myometrial plasma membranes.

2.3. In vitro measurement of uterotonic potency

In vitro assays on rat myometrial strips were performed as described by Melin et al. (1986) and Atke et al. (1988). Female Sprague-Dawley rats (200–250 g, the Panum Animal House, University of Copenhagen) given 75 μg oestradiol benzoate i.m. 2 days earlier were decapitated, the uterine horns were removed, freed of parametrial tissue and gently cut open longitudinally. A middle segment (20 mm) was mounted in an isometric myograph connected to a force transducer and placed in a 7 ml organ bath containing Munsick's solution (Munsick, 1969) at 30° C and constantly aerated with 5% CO_2 in O_2. Resting tension was adjusted to 1.0 g. Cumulative dose response relationships were determined as described by Van Rossum (1963) for vasopressin and oxytocin in the presence or absence of OPC-21268, and pA_2 values were calculated as described by Schild (1949) and Eggena et al. (1968), using the equation:

$$pA_2 = \div \log \frac{[OPC - 21268]}{\frac{[A_{50OPC-21268}]}{[A_{50}]} \div 1}$$

where A_{50} is the molar concentration of the agonist eliciting half-maximal response and $A_{50\ OPC\text{-}21268}$ the molar concentration of agonist giving half-maximal response in the presence of OPC-21268. Maximal responses were estimated by asymptotic approaches, or from supramaximal oxytocin stimulations.

Recordings from human myometrial strips (10 × 2 mm) were performed using the same procedure except that a Krebs-Ringer solution was used.

2.4. In vivo measurements of uterotonic potency

In vivo measurements were performed principally by the method described by Pliska (1969). Female Wistar rats (aged 2.5–3 months, the Konaravice Farm, Prague) were given 75 μg oestradiol dipropionate s.c. 48 h before the experiment. The animals were anaesthetized with 15% ethanol (6–8% of body weight) administered by stomach catheter. After laparotomy a ligature was passed round one uterine horn and a plastic cylinder filled with 1 ml of saline was introduced into the uterine cavity. The end of the ligature was fastened to the arm of a magnetoelectric compensation transducer (Zenisek and Barth, 1974) for measuring uterine contractions isometrically. Oxytocin and OPC-21268 were administered as infusions or injections through the femoral vein.

2.5. Preparation of myometrial plasma membranes

Rat myometrial plasma membranes were prepared as previously described by Atke and Vilhardt (1987).

Female Sprague-Dawley rats (200–250 g) given 75 μg oestradiol benzoate 48 h earlier were decapitated, the uterine horns were recovered and freed of parametrial tissue and endometrium was gently scraped off. After blending and homogenization in a glass-to-teflon homogenizer in a medium of 250 mmol/l sucrose, 40 mmol/l L-histidine, pH 7.3, stepwise differential centrifugation was performed to isolate a fraction of roughly purified plasma membranes. The preparation was further fractionated by centrifugation at 112000 × G_{av} on a discontinuous 10%:28% sucrose gradient for 120 min. The plasma membrane fraction was collected from the interface (Janis et al., 1977) and resuspended in distilled water and kept at −20° C for later receptor studies. All steps in the preparation were performed at 5° C and protein contents were determined by the method of Lowry et al. (1951).

Human myometrial membranes were prepared essentially by the same method, but omitting the gradient centrifugation step which appeared to reduce the binding capacity of the human plasma membranes.

2.6. Receptor binding assays

Receptor binding studies with human or rat myometrial plasma membranes were performed as previously reported (Atke and Vilhardt, 1987) in a medium of 50 mmol/l Tris-maleate, 10 mmol/l $MnCl_2$, 0.1% bovine serum albumin, pH 7.6. Volumes of 250 μl in incubation tubes were placed in a shaking bath at 22° C and contained various concentrations of tritiated vasopressin or oxytocin with and without unlabelled peptides and OPC-21268. The assays were started by addition of plasma membranes (25–100 μg per tube) and, after an incubation period of 60 min, further binding was stopped by addition of 5 ml ice-cold assay buffer followed by immediate filtration (Millipore SCWP 8.0 μm on top of PHWP 0.3 μm). The filters were washed with 2 × 5 ml ice cold buffer and counted in 10 ml of Bray's solution (Bray, 1960) in a liquid scintillation counter. Specific binding of the labelled peptides was calculated by subtraction of the activity counted in the presence of a 1000-fold excess of unlabelled hormone.

3. Results

3.1. Binding of OPC-21268 to rat myometrial receptors

Specific binding of OPC-21268 to oxytocin and vasopressin receptors of rat myometrial plasma membranes was demonstrated from drug displacement experiments in which increasing concentrations of OPC-21268 (1–100 μmol/l) were added to a fixed concentration (8.4 nmol/l) of either [^{3}H]oxytocin or [^{3}H]vasopressin (Fig. 1, left panel). Both curves displayed a concentration-dependent displacement of oxytocin and vasopressin from the receptors, indicating binding of OPC-21268 to both receptors. Computerized non-linear estimates (Munson and Rodbard, 1980) indicated that the con-

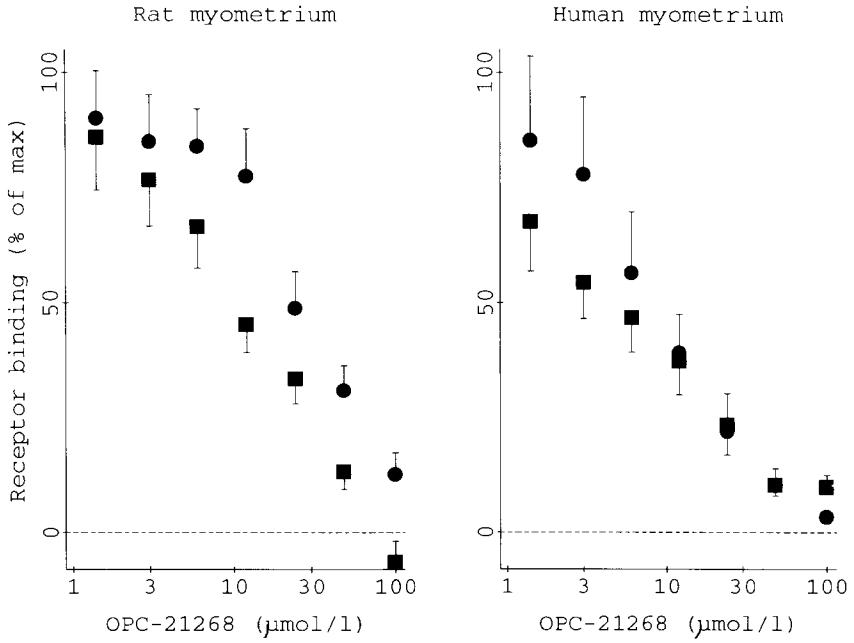

Fig. 1. Left panel: receptor binding of [^{3}H]oxytocin (8.4 nmol/l, •) or [^{3}H]vasopressin (8.4 nmol/l, ■) to rat myometrial plasma membranes as a function of the concentration of OPC-21268 added. Data are given as percentages of the binding in the absence of OPC-21268, means of seven individual experiments, error bars indicate S.E.M. Right panel: same as left panel for full-term pregnant human myometrial plasma membranes, means of six individual experiments.

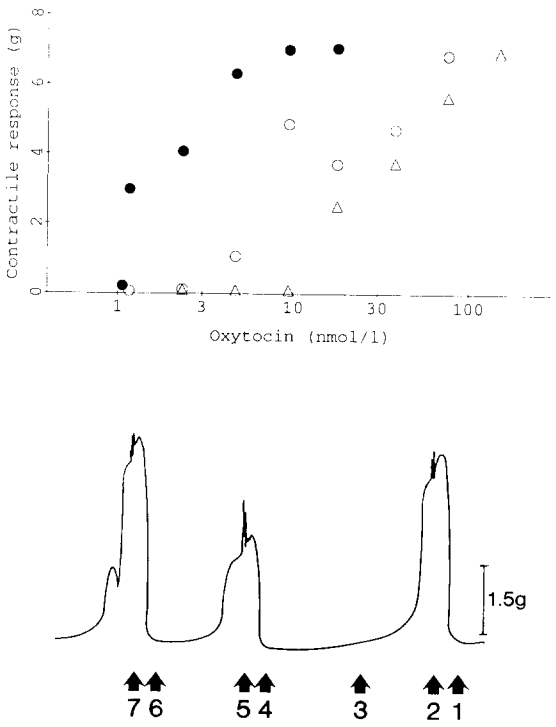

Fig. 2. Upper panel: contractile in vitro response of a rat myometrial strip, given as the resulting tension in g as a function of oxytocin concentration in the absence (●) or presence of 6.3 μmol/l (○) or 15.9 μmol/l (△) of OPC-21268. Lower panel: representative in vitro recording of a human full-term pregnant myometrial strip, displaying the effect of OPC-21268 on oxytocin stimulated contractions. 1, 4 and 6: addition of 0.625 nmol oxytocin; 2, 5 and 7: washing; 3: addition of 0.5 μmol OPC-21268. The curve reads from right to left, the vertical bar indicates 1.5 g of tension.

centrations of OPC-21268 giving half-maximal displacement were 9.1 ± 1.2 μmol/l for displacing vasopressin and 21.8 ± 2.6 μmol/l for displacing oxytocin (means ± S.E.M., $n = 7$).

3.2. Binding of OPC-21268 to human myometrial receptors

Using the same experimental conditions as for rat tissue (3.1) it was demonstrated that OPC-21268 binds specifically to human myometrial receptors for oxytocin and vasopressin (Fig. 1, right panel). Computerized non-linear fits (Munson and Rodbard, 1980) indicated that half-maximal displacement of oxytocin (8.4 nmol/l) to human receptors was achieved in the presence of 7.4 ± 0.5 μmol/l of OPC-21268 as compared with 5.8 ± 0.8 μmol/l for half-maximal displacement of 8.4 nmol/l vasopressin (means ± S.E.M., $n = 6$).

3.3. In vitro effect of OPC-21268 on rat myometrial strips

Fig. 2, upper panel, clearly demonstrates the inhibitory action of OPC-21268 on oxytocin stimulation of a representative rat myometrial strip in vitro. OPC-21268 inhibited the vasopressin-induced stimulation as well, but in itself displayed no contractile action (not shown). The inhibitory action was quantified by calculation of the apparent pA_2 values as suggested by Eggena et al. (1968), giving a value of 5.9 ± 0.3 for inhibition of vasopressin, and a value of 5.5 ± 0.2 for inhibition of oxytocin (3.2, 6.3 and 15.9 μmol/l of OPC-21268 tested, means ± S.E.M., $n = 6$). The effect of OPC-21268 was dose-dependent and fully reversible on washing of the preparation.

3.4. In vitro effect of OPC-21268 on human myometrial strips

Preliminary experiments with myometrial strips from full-term pregnant women demonstrated that OPC-21268 partially or completely suppressed, in a re-

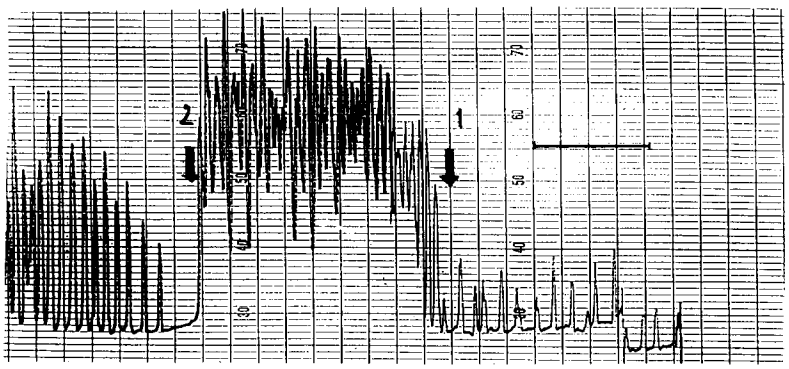

Fig. 3. Intrauterine pressure recording from an anaesthetized rat. 1: start of intravenous infusion of oxytocin (25.4 ng/h) followed by 2: intravenous injection of OPC-21268 (1 mg in 200 μl NaCl, 50% ethanol). The curve reads from right to left and the horizontal bar corresponds to 10 min.

versible way, oxytocin-stimulated contractions, as displayed in the representative trace in Fig. 2, lower panel. OPC-21268 in itself had no excitatory effect on the myometrium.

3.5. Effect of OPC-21268 on rat myometrial contractions in vivo

When OPC-21268 (1.0 mg) was injected in the femoral vein of anaesthetized rats, oxytocin-induced uterine contractions were completely blocked and the myometrium reverted to a state of relaxation (Fig. 3). Although contractions reappeared after 5–10 min the frequency was reduced and the base line remained low. In another series of experiments oxytocin injections (2.2–8.8 nmol) were given before and at intervals after bolus injections of 0.2–4.0 μmol OPC-21268. After exposure to OPC-21268 the myometrium exhibited a reduced sensitivity to oxytocin for a period of at least 1 h (data not shown), indicating a long-lasting but reversible inhibitory action.

4. Discussion

The results of the present study clearly demonstrate that OPC-21268 can inhibit myometrial receptor binding of neurohypophysial peptides in a competitive and reversible way. The prolonged inhibition of the oxytocin response seen in rats after a bolus injection of OPC-21268 can probably be related to the protracted effect of the drug because of its plasma half-life of several hours (Ohnishi et al., 1993). The results of the displacement binding assays show that OPC-21268 binds to the same receptors as oxytocin and vasopressin, although in parallel with other non-peptide antagonists (Gether et al., 1993) binding may occur at epitopes of the receptor molecule separate from the peptide binding sites. The molar concentrations needed to obtain half-maximal displacement of the labelled ligands were considerably higher than those previously reported in our laboratory for peptide-based antagonists to oxytocin and vasopressin (Atke et al., 1988), but parallelled the K_D values of OPC-21268 for other tissues.

In a previous study characterizing oxytocin and vasopressin binding to rat myometrial receptors we found that although the uterotonic potency of oxytocin (450 IU/mg) far exceeded that of vasopressin (17 IU/mg), the receptor binding affinities and binding capacities for the two peptides were not significantly different (Atke et al., 1989) and it was not possible to discern more than one class of receptor. Other groups have reported distinct oxytocin and vasopressin V_1 receptors, and generally the pregnant human myometrium is believed to have these two subtypes of receptors. The present study does not allow any definite conclusions as to the number and character of receptor types, but the affinity of OPC-21268 clearly emphasizes a close relationship between the myometrial receptors for oxytocin and vasopressin and the classical vasopressin V_1 receptors. However, the experiments demonstrating displacement of vasopressin by OPC-21268 in human myometrial plasma membranes indicate that a small fraction of the vasopressin bound is not displaced by OPC-21268 (Fig. 1, right panel). This might indicate the existence of a minor pool of selective vasopressin receptors different from the classical vasopressin V_1 receptors found in liver and smooth muscle cells. However, further experiments are needed before any conclusions are drawn about this question.

Whether OPC-21268 could be of therapeutical use, e.g. in preterm labour, remains to be seen. The advantage of the drug is that it can be administered perorally. However, other non-peptide antagonists and some peptide analogues are also active orally (e.g. the vasopressin analogue desmopressin; Vilhardt and Bie, 1984). Structural analogues of oxytocin with antagonistic properties appear to be more selective than the present non-peptide antagonist since they have no affinity for extrauterine vasopressin receptors. However, recently Williams et al. (1994) reported the synthesis of a series of *o*-totylpiperazine camphosulfonamides with considerably higher affinities for the oxytocin receptor than for the vasopressin receptor, at least in rats.

Acknowledgements

This study was supported by the Danish Research Council for Health Sciences, the Danish Biotechnology Programme and Brødrene Hartmann's Fond.

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