

胎儿; 培养的细胞; 药物协同作用

**目的:** 研究抗孕唑(DL111-IT)与米非司酮(Mif)合用抗早孕的作用点。 **方法:** 用组织学和细胞培养法观察对细胞的损伤; 放射配基分析及 PAP 免疫组化观察子宫胞浆孕酮受体(PR)的影响。 **结果:** 两药合用, 单用均造成蜕膜细胞坏死, 胚胎生长抑制, 以合用药组损伤力最强; 药物作用于人蜕

膜细胞 24 h 的  $LD_{50}$  ( $mg \cdot L^{-1}$ ) 为: DL111-IT 18.1 (15.1 - 21.4), Mif 25.0 (23.1 - 26.9), 合用 DL111-IT 5.0 + Mif 3.5 (1.8 - 6.5)。 **im** DL111-IT 24 h 及 48 h 的 PR 与对照组比, 含量下降 47%, 与 Mif 的结合率不变。 **结论:** DL111-IT 使 PR 数量下降, 不影响 Mif 与 PR 的结合率, 少量 Mif 即可有效拮抗孕酮; DL111-IT 增强 Mif 对蜕膜细胞的损伤作用, 影响胚胎发育而终止早孕。

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### SK&F 105494, a $V_2$ -vasopressin receptor antagonist, blocks oxytocin receptors in porcine myometrium

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**KEY WORDS** SK&F 105494; muscle contraction; L-366948; oxytocin receptors; lypressin; myometrium

**AIM:** To study the effects of SK&F 105494 (SK&F), a  $V_2$ -vasopressin receptor antagonist, on lypressin (Lyp)- and oxytocin (Oxy)-induced increases in myometrial contractility. **METHODS:** Uterine strips isolated from sows in the luteal phase of the estrous cycle were used to study the contractility. The receptor binding assays were performed to study the Oxy receptor blocking activity. **RESULTS:** Lyp and Oxy caused a concentration-dependent increase in myometrial contractility with  $EC_{50}$  of  $50 \pm 14 \text{ nmol} \cdot L^{-1}$  and  $170 \pm 32 \text{ pmol} \cdot L^{-1}$ , respectively. SK&F  $30 - 300 \text{ nmol} \cdot L^{-1}$  antagonized the contractile effects of Lyp and Oxy in a concentration-dependent manner. When the concentration-dependent inhibition of [<sup>3</sup>H]Oxy binding by SK&F was compared with Lyp and L-366948 [(cyclo-(L-Pro-D-2-naphthyl-Ala-L-Ile-D-pipecolic acid-L-pipecolic acid-D-His))], a potent antagonist specific for Oxy

receptors, the  $K_i$  values for SK&F, Lyp, and L-366948 were  $1.73 \pm 0.32$ ,  $14.36 \pm 1.73$ , and  $1.79 \pm 0.35 \text{ nmol} \cdot L^{-1}$ , respectively. **CONCLUSION:** SK&F has a potent Oxy receptor blocking activity in addition to its known  $V_2$ -receptor blocking activity.

The vasopressin (VP) analog SK&F 105494 [1-des cysteine, cyclo(2-O-ethyl)-D-tyrosine, 6-L-(2-amino-6,6-cyclopentamethylene suberic acid), 4-valine, 7-arginine, 8-D-arginine, 9-des glycine]-vasopressin, SK&F] is a potent  $V_2$ -antagonist for the antidiuretic action of VP in rats<sup>[1]</sup>, dogs<sup>[2]</sup>, and rhesus monkeys<sup>[3]</sup>, demonstrating no or minimal agonistic activity. SK&F also binds rat  $V_2$ -receptor with a  $K_B$  of  $0.21 \text{ mol} \cdot L^{-1}$ <sup>[4]</sup>.

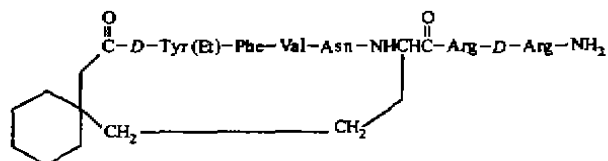
To illustrate the mechanisms by which lypressin (Lyp) increases myometrial contractility in porcine uterine strips, we used SK&F as a  $V_2$ -receptor antagonist. SK&F not only antagonized the contractile effect of Lyp, but also blocked the effect of oxytocin (Oxy) (unpublished observations). SK&F may have Oxy receptor blocking activity in addition to its  $V_2$ -receptor blocking activity, the present study was undertaken to determine how SK&F blocks the activities of both

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Received 1995-08-07

Revised 1996-03-21

Lyp and Oxy in the porcine myometrium. We employed L-366948, a potent antagonist specific for Oxy receptors<sup>15,6)</sup>, to help us solve the problem.



SK & F 105494

**MATERIALS AND METHODS**

**Drugs** Oxy, Lyp, and SK&F were dissolved in 5 g bovine serum albumin/L of phosphate buffer 0.05 mol · L<sup>-1</sup>. L-366948 and carbachol were dissolved in distilled water. [<sup>3</sup>H]Oxy was diluted in the radioligand binding assay buffer. Drugs were obtained as follows: carbachol, Oxy, and Lyp (Sigma, USA), [<sup>3</sup>H]Oxy (DuPont NEN, USA), SK&F (SmithKline & Beecham, USA), and L-366948 (Merck Sharp & Dohme, USA).

**Uterine strips** Uterine strips from sows during luteal phase were prepared<sup>(7,8)</sup>. The contraction experiments were performed using our previously published method<sup>(8)</sup> with modifications. In the first part of the experiments, uterine strips of 10 mm × 2 mm with minimal or no spontaneous contractions (≥90 % of strips) were equilibrated for ≥30 min, and then exposed to carbachol 1 μmol · L<sup>-1</sup> for 3 min to generate near maximal contractions. The strips that did not respond to carbachol were discarded. After 3 washes with 10 mL Tyrode's solution, the strips were exposed to carbachol 1 μmol · L<sup>-1</sup> for 10 min, followed by 3 more washes. They were allowed to equilibrate at the 2-g tension. The variability of the responses to carbachol between myometrial preparations was <20 %. Oxy and Lyp were added at 10-min intervals into the baths in cumulative concentration using 0.1 mL volumes. SK&F was added 10 min before the first dose of agonist was applied, because antagonism by SK&F of Lyp-induced contractions reached equilibrium within 10 min addition and there were no differences between 10 and 30 min of administration of SK&F. Different uterine strips from the same uterus were randomly assigned to all treatment groups.

**Oxy receptor binding assays** Oxy receptor binding experiments were performed. The total binding was determined with duplicate tubes containing 950 μL of membrane suspension (200 μg of membrane protein) and 50 μL of [<sup>3</sup>H]Oxy 40 nmol · L<sup>-1</sup> at 30 °C for 40 min. The nonspecific binding was determined with a parallel set of tubes that, in addition to the membrane protein and [<sup>3</sup>H]Oxy, contained a final concentration of unlabeled Oxy 1 μmol · L<sup>-1</sup>. Specific binding was 60 % - 85 % of total binding. For displacement of [<sup>3</sup>H]Oxy, duplicate tubes containing 950 μL

of membrane suspension and 50 μL of increasing concentrations of various unlabeled drugs (or assay buffer for the control group) were incubated for 5 min. [<sup>3</sup>H]Oxy 50 μL (2 nmol · L<sup>-1</sup> final concentration) was then added and the mixture was incubated at 30 °C for 40 min. Filtration and <sup>3</sup>H counting were done.

**Data analysis** All values are shown as  $\bar{x} \pm s$ . In contractility experiment, for each 10-min interval, the area under the contraction curve was calculated using a scanning program (SigmaScan, USA). These values were expressed as a percentage of response to carbachol 1 μmol · L<sup>-1</sup>.

Radioligand binding data were analyzed by ANOVA. The conservative *F* test was used for the treatment effects. The least significant difference test was used to evaluate the difference between means of end points for which the ANOVA indicated a significant (*P* ≤ 0.05) *F* ratio.

**RESULTS**

**Myometrial contractility** Lyp (30 nmol · L<sup>-1</sup>) and Oxy (0.1 nmol · L<sup>-1</sup>) caused similar phasic contraction patterns in the myometrium isolated from sows in the luteal phase of the estrous cycle, which lasted for ≥10 min (Fig 1). Both Lyp (0.01 - 1 μmol · L<sup>-1</sup>) and Oxy (0.03 - 3 nmol · L<sup>-1</sup>) increased myometrial contractility in a dose-dependent manner (Fig 2). The EC<sub>50</sub> values for Lyp and Oxy were 50.1 ± 14.5 nmol · L<sup>-1</sup> (*n* = 4)

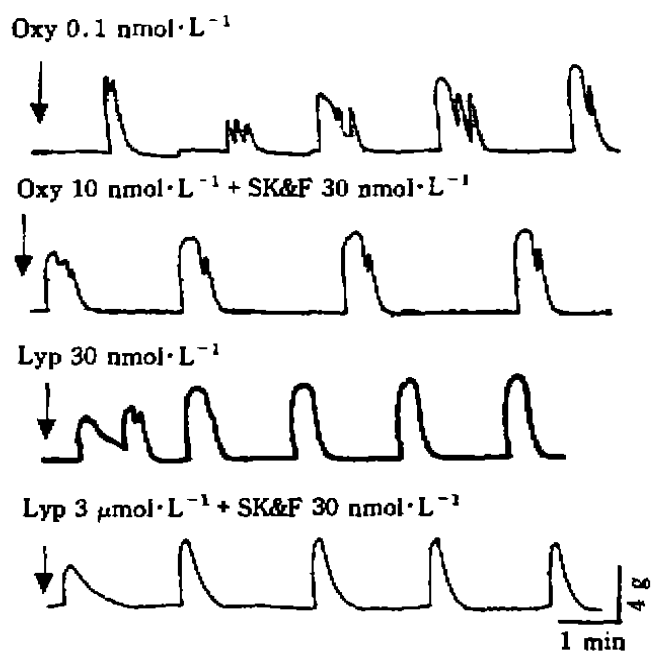


Fig 1. Uterine contraction (*n* = 6).

and  $0.17 \pm 0.03 \text{ nmol} \cdot \text{L}^{-1}$  ( $n = 5$ ), respectively. SK&F ( $0.03, 0.1, 0.3 \mu\text{mol} \cdot \text{L}^{-1}$ ) inhibited both Lyp- and Oxy-induced contractility dose-dependently and decreased the maximum response to Oxy and Lyp (Fig 1 & 2).

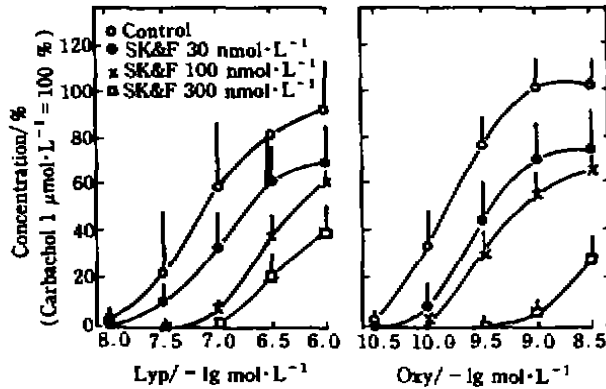


Fig 2. Effect of SK&F 105494 (SK&F) on the Lyp- and Oxy-induced increases in contractility of uterine strips.  $n = 6, \bar{x} \pm s$ . Control (○); SK&F  $30 \text{ nmol} \cdot \text{L}^{-1}$  (●); SK&F  $100 \text{ nmol} \cdot \text{L}^{-1}$  (×); SK&F  $300 \text{ nmol} \cdot \text{L}^{-1}$  (□).

#### [ $^3\text{H}$ ]Oxy binding in myometrial membranes

SK&F, Lyp, and L-366948, caused a concentration-dependent inhibition of [ $^3\text{H}$ ]Oxy binding to myometrial membranes (Fig 3). The rank order potency for displacing [ $^3\text{H}$ ]Oxy was SK&F = L-366948 > Lyp. The  $K_i$  values were  $1.73 \pm 0.32, 1.79 \pm 0.35,$  and  $14.36 \pm 1.73 \text{ nmol} \cdot \text{L}^{-1}$  for SK&F, L-366948, and Lyp, respectively. ( $n = 6$ ). The  $K_i$  values indicated that the apparent affinities for SK&F and L-366948 were 8.4 and 8.0 times greater than Lyp, respectively.

#### DISCUSSION

SK&F, a new  $V_2$ -receptor antagonist, markedly blocked the Oxy-induced increase in porcine myometrial contractility and inhibited [ $^3\text{H}$ ]Oxy binding to porcine myometrial membranes in a concentration-dependent manner. However, SK&F did not change the carbachol-induced myometrial contractions (unpublished results). SK&F inhibited [ $^3\text{H}$ ]Oxy binding as well as L-366948, a potent antagonist specific for Oxy receptors which is  $\geq 10,000$  times more selective for Oxy receptors than for VP receptors<sup>[5,6]</sup>. These

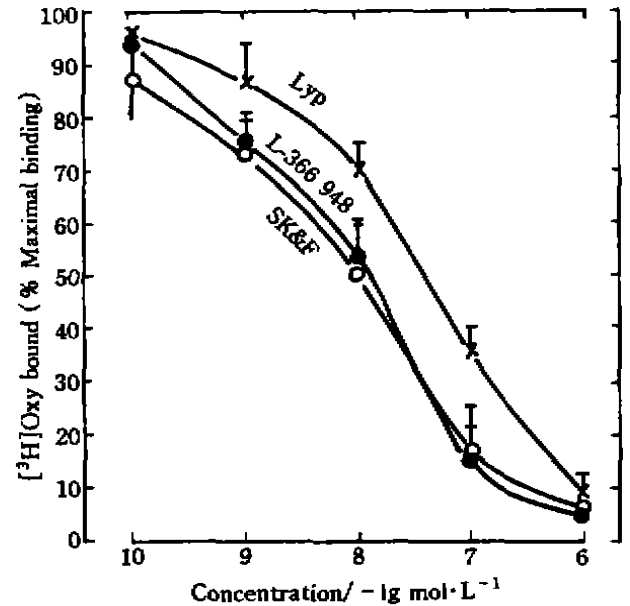


Fig 3. Inhibition of [ $^3\text{H}$ ]Oxy in porcine myometrial membranes by SK&F 105494 (○), L-366948 (●), and Lyp (×). Different contractions of these drugs were given 5 min before  $2 \text{ nmol} \cdot \text{L}^{-1}$  [ $^3\text{H}$ ]Oxy. The [ $^3\text{H}$ ]Oxy mixture was incubated for 40 min at  $30 \text{ }^\circ\text{C}$ .  $n = 6, \bar{x} \pm s$ .

results suggested that SK&F has potent blocking activity against Oxy receptors, in addition to its remarkable VP receptor blocking activity. Our findings further confirmed and extended those of the others in which SK&F binds to human Oxy receptors with a  $K_d$  of  $27 \text{ nmol} \cdot \text{L}^{-1}$ <sup>[4]</sup>.

Although Oxy exerts a potent contractile effect in the prepartum myometrium, it was found that this effect was not significantly different from that in the luteal myometrium of the sow<sup>[10]</sup>. Thus, the latter was used in the present study because of its better availability.

In the present study, SK&F blocked both Lyp- and Oxy-induced increases in myometrial contractility with a similar  $pA_2$  values. In addition, by comparing their  $EC_{50}$  values, Oxy was about 300 times more potent than Lyp in increasing porcine myometrial contractility. These results are consistent with those of our previous studies, which suggested that Lyp's myometrial contractile effect in the sow is mediated by Oxy receptors<sup>[8]</sup>. Although both Oxy and VP receptors may be found in the myometrium of other species<sup>[11]</sup>, in another

study of ours using a specific Oxy receptor antagonist L-366948 and a specific  $V_1$ -receptor antagonist  $d(CH_2)_5[D-Tyr(Me)^2]Arg^8$ -vasopressin, we found that the Oxy receptor antagonist was at least 30 times more potent than the  $V_1$ -receptor antagonist in blocking the Lyp-induced increase in porcine myometrial contractility in the luteal phase of the estrous cycle<sup>8)</sup>. Since the antagonists used in the present study have not been extensively characterized in the pig, the conclusion can be drawn only if it is assumed that the behavior of receptors and antagonists in the pig is similar to that in other species in which they have been more fully characterized. Nevertheless, our previous and present findings suggest that in the sow, Oxy receptors dominate over VP receptors in the regulation of myometrial contractions in the nonpregnant uterus.

SK&F was developed as an aquaretic agent, and acts by blocking  $V_2$ -receptors in the collecting duct of the kidney to decrease free water reabsorption<sup>13)</sup>. Indeed it is a fully effective aquaretic agent in rats, dogs, and rhesus monkeys, but is an anti-diuretic agent in humans<sup>4)</sup>. This study indicated that SK&F had potent blocking activity on Oxy receptors, and thus has great potential to be developed as a tocolytic agent to counteract the effect of Oxy. However, the aquaretic and antidiuretic effects of this compound may complicate its use for this purpose.

**ACKNOWLEDGMENTS** To Dr Paul HIEBLE and Roger FREIDINGER for donating SK&F and L-366948, respectively.

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365-368  
 **$V_2$  加压素受体拮抗剂 SK&F 105494 对猪子宫肌催产素受体的阻断作用**

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**关键词** SK&F 105494; 肌肉收缩; L-366948; 催产素受体; 赖氨酸加压素; 子宫肌层

**A 目的:** 确定  $V_2$  加压素受体拮抗剂 SK&F 105494 (SK&F)对催产素受体的阻断作用. **方法:** 采用猪离体子宫收缩试验和受体结合试验. **结果:** SK&F 对赖氨酸加压素(Lyp)和催产素(Oxy)引起的子宫收缩均有阻断作用, 并随 SK&F 浓度增加而加强.  $K_i$  值小于 Lyp, 近似于 Oxy 受体特异性阻断剂 L-366948, 分别为  $1.73 \pm 0.32$ ,  $14.36 \pm 1.73$  和  $1.79 \pm 0.35 \text{ nmol} \cdot \text{L}^{-1}$ . **结论:** SK&F 对 Oxy 受体有强烈的阻断作用

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