Progesterone Inhibits the Contractile Motility of the Guinea Pig Gallbladder

Takehira YAMAMURA, Toku TAKAHASHI, Masato KUSUNOKI, Yoshio ISHIKAWA, Masaru KANTOH and Joji UTSUNOMIYA

ABSTRACT: We studied the effects of progesterone on the contractile motility of the guinea pig gallbladder *in vitro*. Carbachol (10^{-6} M) induced contractions were reduced by the pretreatment with progesterone (10^{-8}–10^{-6} M) in a dose-dependent manner. Concentration-response curves for carbachol, histamine and CCK-OP showed inhibition by progesterone (5×10^{-7} M). These results suggest that progesterone has a direct inhibitory effect on gallbladder smooth muscle. Contractile responses to potassium (10–60 mM) or calcium (0.4–3.2 mM), which were thought to activate the contractile machinery by increasing the influx of extracellular calcium, were not affected by the pretreatment of progesterone. The direct inhibitory effects of progesterone on gallbladder smooth muscle might be explained by the inhibition of calcium release from the intracellular storage sites.

KEY WORDS: carbachol, histamine, cholecystokinin-octapeptide, calcium

INTRODUCTION

Progesterone has been shown to have an inhibitory effect on the contractility of gastrointestinal muscle.[1–6] It was also suggested that common complaints such as heart burn, nausea and constipation experienced by a large number of women during the course of pregnancy is partly due to increased serum progesterone concentrations.[2,4–6] An *in vitro* study indicated that progesterone inhibits the contractions evoked by gastrin and acetylcholine with a 20 min exposure time of the opossum lower esophageal sphincter.[2]

Although it has been known for over half a century that pregnancy increases the risk of cholesterol gallstone,[7–11] the mechanism responsible for the gallstone formation remains unknown. Some studies however, indicate that progesterone also reduces the motor activity of gallbladder smooth muscle and that retention of bile in the gallbladder contributes to gallstone formation.[12–14] Ryan and Pellecchia[13] found that chronic pretreatment with progesterone reduced contractions caused by acetylcholine and cholecystokinin octapeptide in the guinea pig gallbladder. It is unclear however, whether or not this inhibitory effect directly affects smooth muscle cells. We therefore studied the effects of progesterone on the contractile responses of isolated gallbladder muscle strips.

MATERIALS AND METHODS

As previously reported,[19,23] adult male guinea pigs, 300–500 g, were stunned and bled and their gallbladders were immediately removed. After removal of the mucosa by blunt

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dissection, the gallbladder was cut longitudinally into two portions, about 10 mm long and 3 mm wide, which were equally composed of fundus and body. Both sections were used for these experiments. The strip was suspended in a 20 ml organ bath in Krebs solution of the following composition (mM): NaCl 118; KCl 4.8; $CaCl_2$ 2.5; $NaHCO_3$ 25; KH_2PO_2 1.2; $MgSO_4$ 1.2 and glucose 11. The bath was continuously bubbled with 95 per cent O_2 and 5 per cent CO_2 and maintained at 37°C and pH 7.4. Contractile activity was recorded isometrically with a force displacement transducer (TB-612T, Nihon Kohden, Japan) and displayed on a recticorder (RJG-4128, Nihon Kohden). The strips were loaded with a tension of 1 g and allowed to equilibrate for 60–90 min before the experiment. Preliminary experiments indicated that a resting tension of 1 g was optimal for the measurement of reproducible contractions in response to various stimulations.[19,23]

Dose response curves were constructed on each muscle strip for carbamylcholine chloride (carbachol, Wako, Japan), histamine dihydrochloride (histamine, Wako), cholecystokinin octapeptide (CCK-OP) (Protein Research Foundation, Japan) and potassium chloride (Wako), alone, and in combination with progesterone (Merck, U.S.A.). Progesterone was added to the organ bath 20 mins before the application of various agonists. All paired observations were made on the same muscle strip. The final concentrations of drugs were expressed as molar concentrations.

To investigate the effects of progesterone on the stimulatory effect of calcium ions, 0.2–3.2 mM $CaCl_2$ was added to the organ baths in calcium-free Krebs solution containing 1 mM EDTA after the contractions evoked by 30 mM KCl were completely abolished. Results were compared with the data obtained from similar experiments after the pretreatment of progesterone for 20 mins on the same muscle strip.

Data were analysed by the Student's t-test.

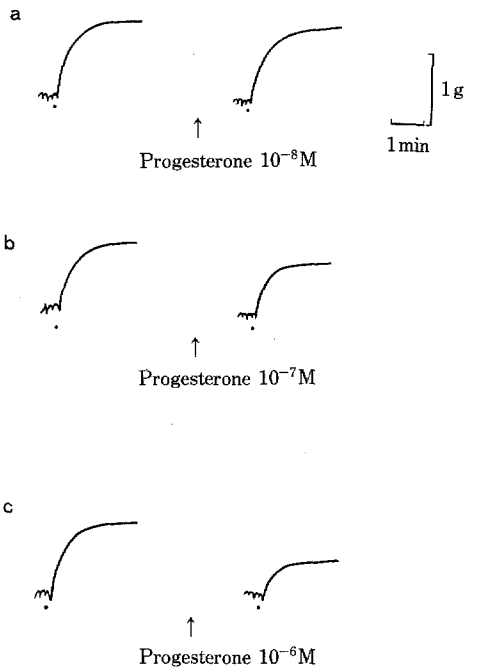

Fig. 1. Effects of progesterone (10^{-8} M, 10^{-7} M, 10^{-6} M) on 10^{-6} M carbachol induced contractions. Dots indicate the application of 10^{-6} M carbachol. Progesterone was added to the organ bath 20 mins before the application of 10^{-6} M carbachol.

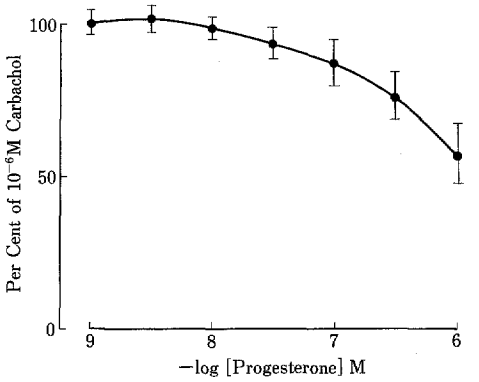

Fig. 2. Effects of progesterone (10^{-9} M–10^{-6} M carbachol induced contractions, expressed as a percentage of the response without progesterone. Results were obtained on 5 determinations.

Unless otherwise stated, results are expressed as mean ± SEM.

Results

Muscle strips in the organ bath showed phasic spontaneous contractions and the addition of 10^{-6} M carbachol produced tonic contractions. High concentrations of progesterone, 10^{-8} M–10^{-6} M, inhibited 10^{-6} M carbachol induced contractions in a dose dependent manner without affecting the resting tone (Fig. 1, 2). This inhibitory effect of progesterone was abolished by washing out the progesterone.

As shown in Fig. 2, drugs at various concentrations caused contractions of different tensions in the muscle preparations. Because bombesin, pentagastrin, prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) had less potent stimulatory effects than other agonists (Fig. 3), we examined the effects of progesterone on the contractile responses to carbachol, histamine and CCK-OP.

Pretreatment with 5×10^{-7} M progesterone for 20 mins reduced the contractile responses evoked by carbachol (Fig. 4a), histamine (Fig. 4b) and CCK-OP (Fig. 4c). The contractile responses to 10^{-4} M carbachol, in the concentration that produced the maximal response, were decreased 23.4 ± 8.4 per cent with the pretreatment of 5×10^{-7} M progesterone. Although the magnitude of the contractile responses to carbachol were altered with progesterone, no significant differences were seen between the dose response curves with respect to the ED_{50}, dose producing one-half the maximal response. The ED_{50} for the control curve was $1.5 \pm 0.4 \times 10^{-6}$ M and $1.8 \pm 0.5 \times 10^{-6}$ M for carbachol plus progesterone. At each point along the dose-response curve, the progesterone pretreated contractile responses to carbachol were reduced when compared with the control data. Progesterone, 5×10^{-7} M, decreased the contractile responses to a maximal dose of 10^{-4} M histamine and 3×10^{-7} M CCK-OP by 21.5 ± 7.2 per cent and 25.4 ± 6.6 per cent, respectively.

Elevating the extracellular potassium level

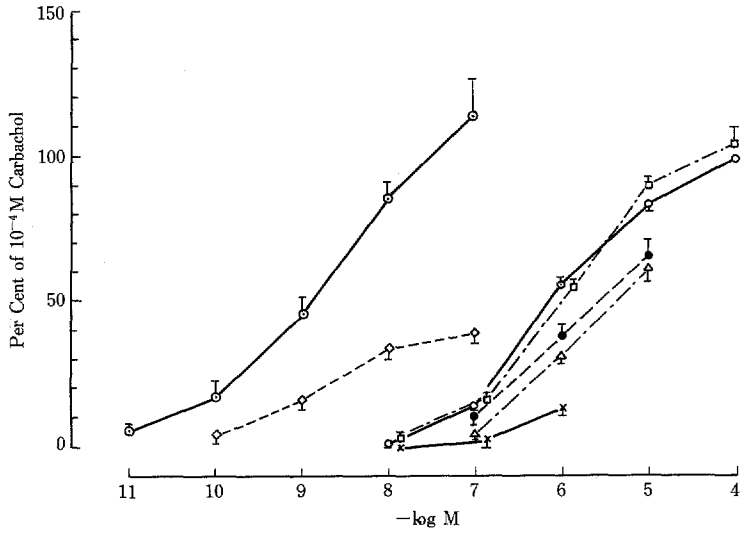

Fig. 3. Effects of various drugs on the contractile motility of the guinea pig gallbladder. Contractions induced by 10^{-4} M carbachol were taken as 100 per cent. Figures in parentheses indicate the numbers of strips used.
○—○ Carbachol (5); □--□ Histamine (5); ⊙—⊙ CCK-OP (5); △--△ PGE_2 (5); ●—● $PGF_{2\alpha}$ (5); ×—× Pentagastrin (3); ◇—◇ Bombesin (4)

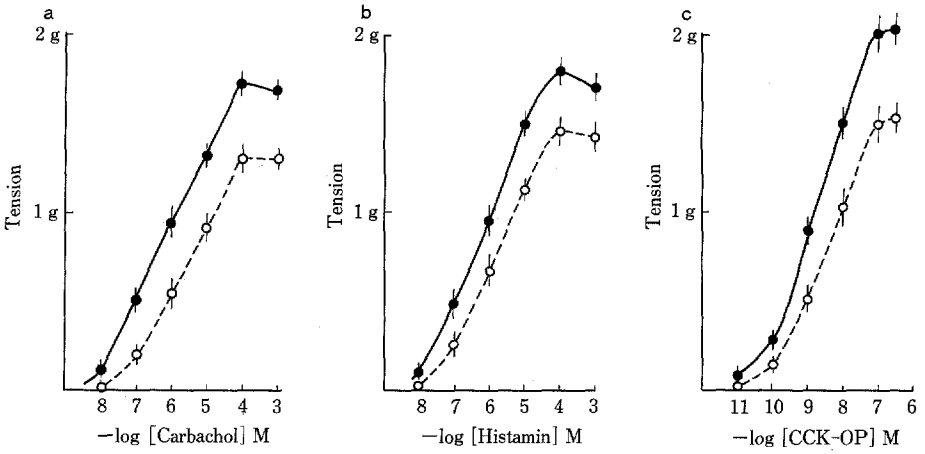

Fig. 4. **a.** Effects of 5×10^{-7} M progesterone on carbachol (10^{-8}–10^{-3} M) induced contractions. Results were obtained on 8 determinations. Solid circles represent the control response. Open circles represent the response with the pretreatment of progesterone. **b.** Effects of 5×10^{-7} M progesterone on histamine (10^{-8} M–10^{-3} M) induced contractions. Results were obtained on 8 determinations. **c.** Effects of 5×10^{-7} M progesterone on CCK-OP (10^{-11} M–3×10^{-7} M) induced contractions. Results were obtained on 8 determinations.

resulted in a concentration-dependent increase in tension that was maximal at 60 mM. Progesterone, 5×10^{-7} M, had no effect on the contractile responses to potassium stimulation (Fig. 5a).

By the addition of 30 mM KCl once or twice to the organ bath containing calcium-free Krebs solution, the contractions evoked by high-potassium induced depolarization were completely abolished. One minute after the addition of 30 mM KCl, 0.2 mM–3.2 mM $CaCl_2$ was then added to the organ bath. The dose response curves for the contractions evoked by $CaCl_2$ were compared with the data obtained from similar experiments with the pretreatment of progesterone for 20 mins. There were no significant differences between the control experiments and those with progesterone pretreatment (Fig. 5b).

Discussion

It has been demonstrated that pregnancy increases the risk of cholesterol gallstones,[7–11] but little is known of the mechanism responsible for the increased risk of gallstone formation in pregnancy. Biliary bile acids and lipids in pregnant women[11,16] and contractile motility of the gallbladder in pregnancy[12,14] have been studied in an attempt to clarify this mechanism.

Everson et al.[12] demonstrated that the volume of the gallbladder was increased and its contraction was sluggish in all trimesters of pregnancy. They also showed a direct correlation of fasting and residual volumes of the gallbladder with serum progesterone concentrations. Ryan and Pellecchia[13] showed that chronic pretreatment with progesterone produced a rightward shift in the acetylcholine and CCK-OP curves of the guinea pig gallbladder. These data led to the hypothesis that progesterone has an inhibitory effect on gallbladder smooth muscle.

In the present experiments, the addition of progesterone to an organ bath containing isolated gallbladder muscle strips reduced the contractile responses evoked by 10^{-6} M carbachol, and this reduction was dose-dependent in the concentration range of 10^{-8}

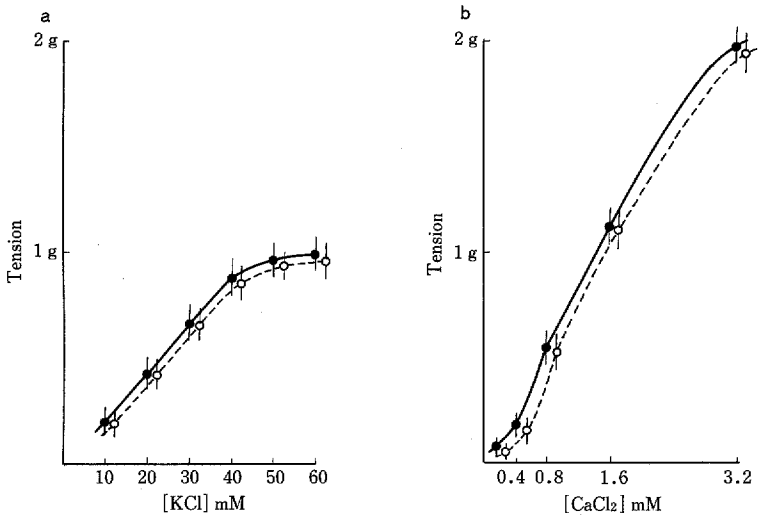

Fig. 5. **a.** Effects of 5×10^{-7} M progesterone on potassium (10–60 mM) induced contractions. Results were obtained on 8 determinations. The symbols are the same as in the previous figures. **b.** Effects of 5×10^{-7} M progesterone on $CaCl_2$ stimulation. $CaCl_2$ (0.2 mM–3.2 mM) was added to calcium-free Krebs solution containing 1 mM EDTA after the contractions evoked by 30 mM KCl were completely abolished. Results were compared with the data obtained from similar experiments with the pretreatment of progesterone for 20 mins on the same muscle strip. Results were obtained on 15 determinations.

M–10^{-6} M. Challis et al.[15] reported that plasma progesterone concentrations rose to a peak value of 329 ± 14 ng/ml ($\fallingdotseq 10^{-6}$ M) in pregnant guinea pigs. In the present work, progesterone concentrations, comparable to those observed by Challis et al. in the plasma during pregnancy, inhibited contractions evoked by 10^{-6} M carbachol. In addition, 5×10^{-7} M progesterone inhibited contractile responses evoked by carbachol, histamine, and CCK-OP. These inhibitory effects of progesterone did not change in the presence of α-, β-adrenergic antagonists. It is accepted that carbachol and histamine produced direct muscular stimulations by specific membrane muscarinic and H_1 receptors, respectively, in the guinea pig gallbladder.[21] It still remains unclear, on the other hand, whether the effects of CCK-OP on gallbladder contractility are directly on the smooth muscle cells[22] or whether the effect is caused indirectly through the intramural cholinergic neurons.[22] Progesterone however, had no effect on the ED_{50} dose of carbachol, histamine and CCK-OP, thereby suggesting no change in the sensitivity of the smooth muscle of the gallbladder to these agonists. A decrease in the efficacy of agonists with no apparent changes in tissue sensitivity suggests that progesterone evokes alterations in the contractile process that is independent of the stimulus applied. It was also suggested, therefore, that progesterone had direct inhibitory effects on gallbladder smooth muscle.

Although the mechanism by which progesterone exerts its antagonistic effect on smooth muscle is unknown, reduced availability of calcium has been suggested.[17] Carsten[17] demonstrated that progesterone increased ATP-dependent calcium binding of the isolated sarcoplasmic reticulum of the bovine uterus and antagonized the effect of prostaglandin $F_{2\alpha}$ and oxytocin. He sug-

gested that progesterone exerts its inhibitory effect on uterine contractions by blocking calcium release from the sarcoplasmic reticulum. With respect to smooth muscle contraction however, not only calcium release from the intracellular calcium storage sites, but also calcium influx from the extracellular sites may be important.[18] A recent study in fact, demonstrated that acetylcholine and CCK-OP induced contractions of the guinea pig gallbladder, involved both extracellular and intracellular calcium stores.[20] It is therefore necessary to consider the possible effects of progesterone on both these calcium channels.

As the contractions evoked by high potassium depolarization in calcium-free medium are believed to be caused by calcium released from the intracellular storage sites, it is considered that intracellular calcium ions may be absent after the contractions evoked by 30 mM KCl have been completely abolished. Under these conditions, the contractions evoked by extracellular calcium, 0.2 mM–3.2 mM, which occurred presumably due to the calcium influx, were not affected by 5×10^{-7} M progesterone.

In addition, progesterone had no effect on the contractile responses to potassium, which was thought to activate the contractile machinery by increasing the influx of extracellular calcium.[18] Because progesterone had no influence on the stimulatory effect of extracellular calcium, its action might be explained by the inhibition of calcium release from the intracellular storage sites as previously suggested by Carsten.[17] How the hormone might interfere with the release of bound intracellular calcium however, must remain speculative.

Although the possible future value of conducting experiments on the gallbladders from ovariectomized females, or from females at different phases of their reproductive cycles, remains to be determined, it is evident that progesterone has a direct inhibitory effect on the contractility of the gallbladder smooth muscle to a variety of stimuli. These findings may be of significance in relation to the pathogenesis of cholesterol cholelithiasis in pregnancy.

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