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Roles of Gonadal Steroids on Exocrine Secretion of Isolated Perfused Rat Pancreas

Hyung Seo Park, Se Hoon Kim, Hyoung Jin Park[1], Mee Young Lee, and Young Hee Han

Department of Physiology, College of Medicine, Konyang University, Nonsan, Chungnam-do 320–711, Korea; [1]Department of Physiology, College of Medicine, Hallym University, Chuncheon, Gangwon-do 200–702, Korea

To clarify the roles of gonadal steroids on pancreatic exocrine secretion, effects of progesterone and estradiol-17β on spontaneous and secretagogue-induced exocrine response of isolated perfused rat pancreas were investigated. Intra-arterial infusion of progesterone resulted in significant increase of the spontaneous pancreatic fluid and amylase secretion dose-dependently. However, estradiol-17β did not exert any influence on spontaneous pancreatic exocrine secretion. Exogenous secretin, cholecystokinin (CCK), and acetylcholine markedly stimulated pancreatic fluid and amylase secretion. Progesterone initially enhanced secretin-induced amylase secretion, but this stimulatory response declined thereafter to basal value. Moreover, secretin-induced fluid secretion was not affected by infusion of progesterone. Therefore, initial increase of secretion-induced amylase secretion by progesterone seems to be a non-specific action by washout effect of secretin. Estradiol-17β failed to change the secretin-induced fluid and amylase secretion. Both progesterone and estradiol-17β did not exert any influence on CCK-induced fluid and amylase secretion. Acetylcholine-induced exocrine secretion of isolated perfused pancreas also was not affected by intra-arterial infusion of progesterone or estradiol-17β. It is concluded from the above results that progesterone could enhance the spontaneous pancreatic fluid and amylase secretion of isolated perfused rat pancreas through non-genomic short-term action, and that these effects could be masked by more potent stimulants such as secretin, CCK, and acetylcholine.

Key Words: Pancreatic secretion, Progesterone, Estradiol-17β, Secretin, CCK, Acetylcholine

INTRODUCTION

Although it has been well documented that autonomic nervous system and gastrointestinal peptide hormones regulate pancreatic exocrine secretion, the role of gonadal steroids on pancreatic exocrine function is not fully understood at present. The plasma concentration of gonadal steroids is known to fluctuate rhythmically during ovarian cycle, and various functions of gastrointestinal tract, such as gastrointestinal motility and insulin secretion, are affected by this fluctuation (El Seifi et al, 1981; Chen et al, 1995).

It has been recognized that the integrity of the exocrine pancreas may be linked to plasma concentration of steroid hormones (Grossman et al, 1969). Simultaneous ovariectomy and adrenalectomy result in marked influence on both structure and function of the exocrine pancreas, such as significant decline in pancreatic protein and amylase contents, marked depletion of zymogen granules, and widening of peri- and interlobular spaces (Grossman et al, 1983; Beaudoin et al, 1986, 1989). It has also been reported that spontaneous canine pancreatic exocrine secretion increases during pregnancy (Rosenberg et al, 1975). We have previously reported that spontaneous pancreatic exocrine secretion fluctuates during estrous cycle in anesthetized female rats (Park et al, 2000a). These results seem to suggest that ovarian steroids exert substantial influence on pancreatic exocrine function.

Recently, the interest in non-genomic effects of steroid hormones increased (Wehling 1997; Watson & Gametchu, 1999; Schmidt et al, 2000), and membrane-bound binding site for gonadal steroids has been found in the brain, sperm, oocyte and vascular smooth muscle (Blondeau & Baulieu, 1984; Alexander et al, 1996; Falkenstein et al, 1996; Ramirez et al, 1996). Although the significance of such membrane bound receptors in the exocrine pancreas is presently unknown, gonadal steroids could possibly regulate the pancreatic exocrine response through non-genomic effect like in other non-reproductive organs. Thus, the present study was aimed to investigate the short-term effects of gonadal steroids on spontaneous and secretagogue-induced exocrine secretion of isolated perfused rat pancreas.

Corresponding to: Hyung Seo Park, Department of Physiology, College of Medicine, Konyang University, Nonsan, Chungnam-do 320-711, Korea. (Tel) 041-730-5433, (Fax) 041-730-5318, (E-mail) hspark@konyang.ac.kr

ABBREVIATIONS: CCK, cholecystokinin; Ach, acetylcholine; P_4, progesterone; E_2, Estradiol-17β.

METHODS

Experimental animal preparation

Ovariectomized Sprague-Dawley female rats, weighing 200~250 g, were used for experiments. Bilateral ovariectomy was performed under anesthesia with Ketamine (0.4 mg/kg, i.p.; Yuhan, Korea) and Xylazine hydrochloride (0.2 mg/kg, i.m.; Yuhan). Before the experiment, each animal was anesthetized with an intraperitoneal injection of 25% urethane (Sigma, USA) at a dose of 0.7 ml/100 g of body weight after 24 hour-fasting with free access to water. The rats were sacrificed by an intravenous overdose of urethane after isolation of the pancreas.

Preparation of totally isolated vascularly perfused pancreas

The isolated perfused rat pancreas was prepared according to the method described previously (Park et al, 1993; Park & Park, 2000b). In brief, the abdominal aorta was carefully dissected and cannulated with PE-50 tubing (Clay Adams, USA) just above the celiac artery, and then tightly ligated below the superior mesenteric artery. The pancreatic duct was cannulated at the duodenal end with PE-10 tubing (Clay Adams). The portal vein was also cannulated with Tygon microbore tubing (Fisher Scientific, USA) to drain the perfusate. The isolated pancreas was perfused with modified Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg water) through the celiac and superior mesenteric arteries at a flow rate of 1.2 ml/min by using a multistaltic pump (Buchler, USA). The perfusate contained 0.1% bovine serum albumin (Sigma), 3% Dextran T-70 (Sigma) and 5.6 mM glucose (Sigma), and was continuously oxygenated with 95% O_2 containing 5% CO_2. The pancreas was isolated with the duodenum, but separated from other neighboring organs and tissues, and then placed in a temperature-controlled experimental chamber at 37°C. It was also continuously supplied with Krebs-Henseleit solution at a flow rate of 0.35 ml/min and oxygenated. After an equilibration period of 30 minutes, pancreatic juice secreted in 15 minutes was collected throughout the remaining period of the experiment.

Effects of gonadal steroids on spontaneous pancreatic secretion

Water-soluble progesterone (Sigma) was added to perfusate at concentrations of 0.1, 0.3, 1, 3, 10 or 30 μM during 45 minutes after the basal 30 minutes-periods. Last 30 minutes-data was used for statistical analysis. Water-soluble estradiol-17β (Sigma) was also added to perfusate at concentrations of 0.01, 0.03, 0.1, 0.3, 1 or 3 μM according to the procedure described above.

Effects of gonadal steroids on secretagogue-induced pancreatic secretion

To stimulate pancreatic exocrine secretion, synthetic secretin, cholecystokinin, or acetylcholine (Sigma) were added to the perfusates at a cencentration of 12 pM, 10 pM or 30 nM after 45 minutes of preperfusion of progesterone at a concentration of 3 μM or estradiol-17β at a concentration of 0.3 μM until the end of the experiment.

Measurements of pancreatic secretions of fluid and amylase

The volume flow of pancreatic juice was determined by measuring the length occupied by pancreatic juice, which was collected in microtube with a capacity of 3.8 μl/cm. The activity of α-amylase in pancreatic juice was measured using a previously reported method (Rick & Stegbauer, 1974).

Statistical analysis of data

All results are illustrated as means±S.E. The data were analyzed using the Student's t test. The difference was considered significant when P value was less than 0.05.

RESULTS

Spontaneous pancreatic amylase secretion

As shown in Fig. 1, isolated perfused rat pancreas spontaneously secreted a minute amount of fluid (0.98±0.23 μl/30 min) and amylase (12.78±2.14 U/30 min). However, intra-arterial infusion of progesterone resulted in dose-dependent increase of spontaneous pancreatic fluid and amylase secretion. Progesterone at a concentration of 1 μM significantly stimulated the spontaneous pancreatic fluid and amylase secretion to 2.02±0.34 μl/30 min and 21.76± 2.64 U/30 min from the basal values, respectively. Maximal effects of fluid (2.57±0.41 μl/30 min) and amylase secretion (37.72±3.93 U/30 min) were observed at 3 μM concentration of progesterone, and higher concentrations of progesterone slightly decreased these secretions, compared to the maximal effect of progesterone. In contrast, estradiol-17β did not exert any influence on spontaneous fluid and amylase secretion (Fig. 2).

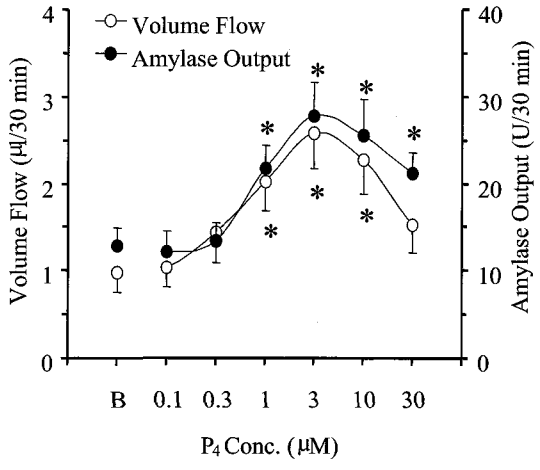

Fig. 1. Dose-response curves of progesterone (P_4) on spontaneous pancreatic fluid and amylase secretion of isolated perfused rat pancreas. Each point represents mean±SE of data from 7 experiments. Spontaneous pancreatic fluid and amylase secretion were significantly ($P<0.05$) stimulated dose-dependently by progesterone. The maximal effects were observed at 3 μM progesterone. Asterisks indicate significant difference compared to the corresponding value of basal (B) state.

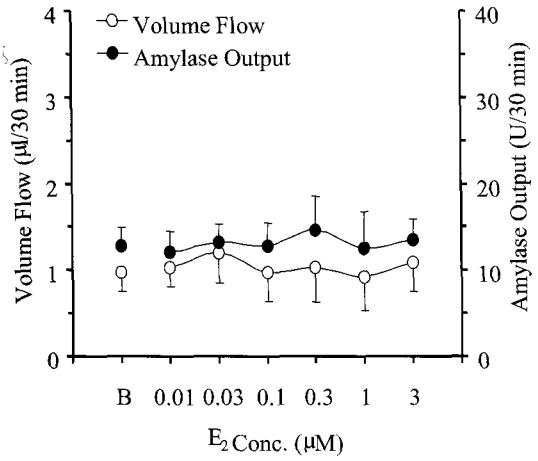

Fig. 2. Effects of estradiol-17β (E_2) on spontaneous pancreatic fluid and amylase secretion of isolated perfused rat pancreas. Each point represents mean±SE of data from 7 experiments. Spontaneous pancreatic fluid and amylase secretion were not affected by intra-arterial infusion of estradiol-17β.

Secretin-induced pancreatic amylase secretion

Although secretin induced sustained increase of fluid secretion, the positive effect on amylase secretion was only transient (see Fig. 3). Such transient secretion of amylase was most likely due to a 'washout effect' of secretion via fluid secretion. Although progesterone significantly stimulated the spontaneous fluid secretion, progesterone failed to change the fluid secretion induced by secretin. Secretin-induced amylase secretion was initially stimulated by intra-arterial infusion of progesterone, however, this response declined thereafter to basal values. Therefore, stimulatory effect of progesterone on secretin-induced amylase secretion could not be differentiated from the washout effect of secretin. Estradiol-17β did not exert any influence on secretin-induced fluid and amylase secretion.

Cholecystokinin (CCK)-induced pancreatic amylase secretion

CCK remarkably stimulated pancreatic fluid and amylase secretion, compared to the corresponding values of basal state. As shown in Fig. 4, both progesterone and estradiol-17β failed to further modulate the CCK-induced fluid and amylase secretion.

Acetylcholine-induced pancreatic amylase secretion

Acetylcholine also markedly stimulated pancreatic fluid and amylase secretion, compared to the corresponding values of basal state. As shown in Fig. 5, both progesterone and estradiol-17β also failed to change the acetylcholine-induced fluid and amylase secretion.

DISCUSSION

We have previously reported that pancreatic exocrine secretion fluctuates during the estrous cycle in anesthetized female rats, and spontaneous pancreatic exocrine secretion

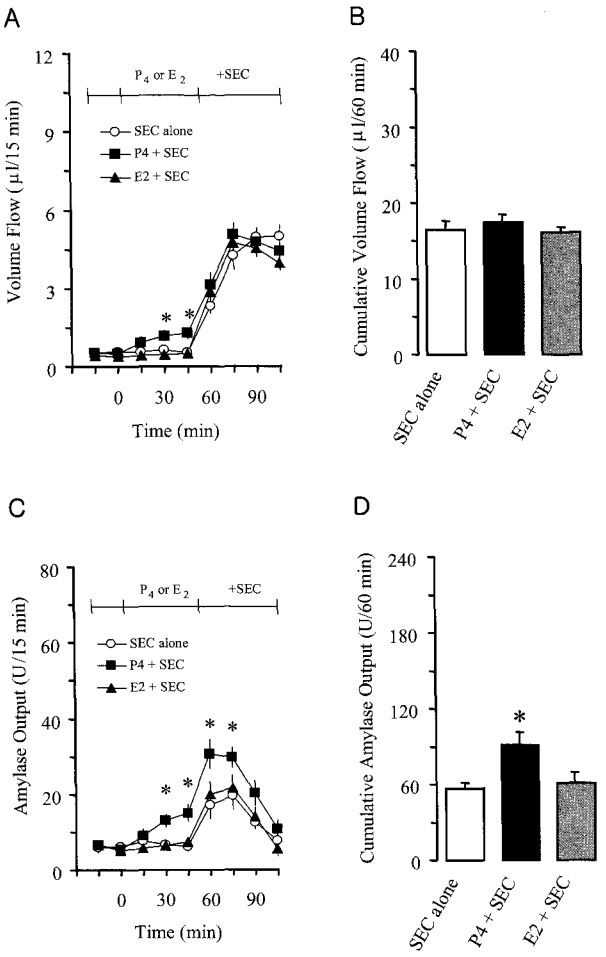

Fig. 3. Effects of progesterone (P_4) and estradiol-17β (E_2) on secretin (SEC)-induced exocrine secretion of isolated perfused rat pancreas. A, Time courses of pancreatic fluid secretion. B, Cumulative fluid secretion during 60-minutes periods. C, Time courses of pancreatic amylase secretion. D, Cumulative amylase secretion during 60-minutes periods. Each point and bar represents mean±SE of data from 7 experiments. Secretin-induced pancreatic amylase secretion was initially stimulated by progesterone ($P<0.05$), and then continuously declined to basal value. Asterisks indicate significant difference compared to the corresponding value of control.

increases during diestrus stage and decreases during estrus stage. It has been well documented that plasma concentration of progesterone and estradiol-17β increases during diestrus stage and during estrus stage, respectively (Everett, 1948). There are a few reports on the role of ovarian steroids in spontaneous exocrine pancreatic secretion. Spontaneous canine pancreatic exocrine secretion is known to increase during pregnancy, when plasma concentration of progesterone is elevated (Rosenberg et al 1975). In the present study, spontaneous pancreatic fluid and amylase secretion was dose-dependently stimulated by intra-arterial infusion of progesterone, however, estradiol-17β failed to change the spontaneous pancreatic exocrine secretion. Therefore, these results suggest that pancreatic exocrine response in the basal state may be closely linked with

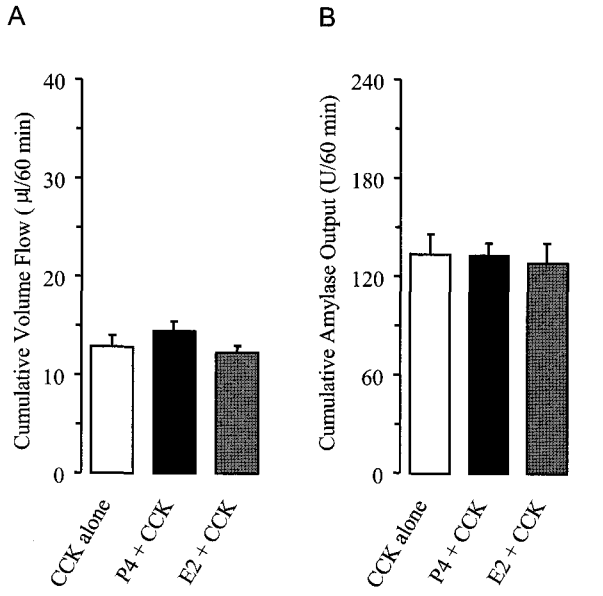

Fig. 4. Effects of progesterone (P_4) and estradiol-17β (E_2) on cholecystokinin (CCK)-induced exocrine secretion of isolated perfused rat pancreas. A, Cumulative fluid secretion during 60-minutes periods. B, Cumulative amylase secretion during 60-minutes periods. Each point and bar represents mean±SE of data from 6 experiments. Both progesterone and estradiol- 17β did not exert any influence on CCK-induced pancreatic fluid and amylase secretion.

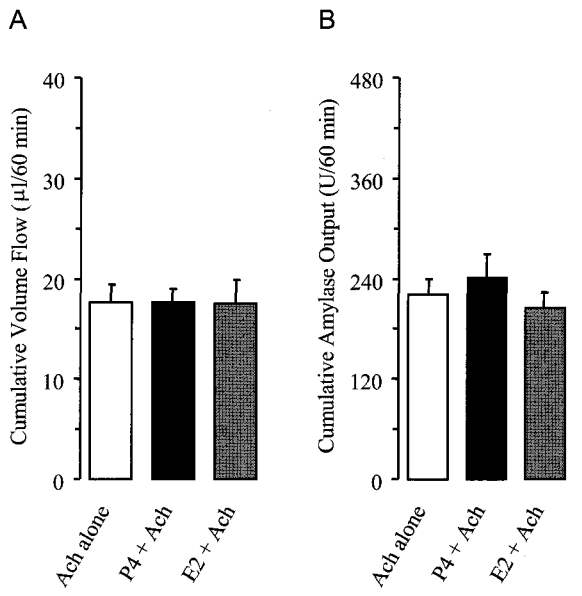

Fig. 5. Effects of progesterone (P_4) and estradiol-17β (E_2) on acetylcholine (Ach)-induced exocrine secretion of isolated perfused rat pancreas. A, Cumulative fluid secretion during 60-minutes periods. B, Cumulative amylase secretion during 60-minutes periods. Each point and bar represents mean±SE of data from 6 experiments. Both progesterone and estradiol-17β did not exert any influence on acetylcholine-induced pancreatic fluid and amylase secretion.

progesterone secreted during the diestrus stage.

Generally, steroids have been known to stimulate pancreatic enzyme synthesis through long-term gemonic action. Simultaneous ovariectomy and adrenalectomy result in decline of pancreatic amylase contents and depletion of zymogen granules (Grossman et al, 1969), and it is restored by steroids treatment (Grossman et al, 1983; Beaudoin et al, 1986; 1989). Recently, the interest in non-genomic effects of progesterone has increased (Wehling 1997; Watson & Gametchu, 1999; Schmidt et al, 2000). Membrane-bound binding sites for progesterone derivatives have been found in brain, sperm, oocyte and vascular smooth muscle (Blondeau & Baulieu, 1984; Alexander et al, 1996; Falkenstein et al, 1996; Ramirez et al, 1996). Although precise physiological significance of such membrane bound receptors in the exocrine pancreas is presently not known, progesterone could possibly regulate the pancreatic exocrine response through non-genomic effect, similar to other non-reproductive organs. In the present study, intra-arterial short-term infusion of progesterone was observed to enhance spontaneous pancreatic fluid and amylase secretion. These results suggest that progesterone not estradiol- 17β, could stimulate spontaneous pancreatic exocrine secretion through non-genomic short-term action.

In the present study, although secretin-induced fluid secretion was not affected by intra-arterial infusion of progesterone, secretin-induced amylase secretion was initially enhanced by intra-arterial infusion of progesterone. However, this stimulatory response was continuously declined to basal state, suggesting that the initial increase might have been due to a non-specific action by washout effect of secretin (Feurle et al, 1987). Moreover, progesterone did not exert any influence on CCK- and acetylcholine-induced pancreatic fluid and amylase secretion. Therefore, these results suggest that the more potent stimulants masked the stimulatory action of progesterone on spontaneous pancreatic exocrine secretion. Estradiol-17β did not have any influence on secretin-, CCK-, and acetylcholine-induced pancreatic fluid and amylase secretion. These results, has together with the data on spontaneous pancreatic response, indicate that estradiol-17β has no non-genomic effect on pancreatic exocrine secretion. The above results led us to conclude that progesterone enhances the spontaneous pancreatic fluid and amylase secretion of isolated perfused rat pancreas through non-genomic short-term action, and that these effects are masked by more potent stimulants such as secretin, CCK, and acetylcholine.

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