Sex Difference in Adrenergic Receptor-Mediated Glycogenolysis in Rat Livers

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Abstract—Catecholamine-induced stimulation of hepatic glycogenolysis in male and female rats was studied by detecting the cytosolic free Ca^{2+} concentration ([Ca^{2+}]_i), cAMP generation and adrenergic receptor function. Increase in α_{1}-adrenergic receptor-mediated [Ca^{2+}]_i and β-adrenergic receptor-mediated cAMP generation were examined using isolated hepatocytes. No difference was found in the α_{1}-adrenergic receptor-mediated response of [Ca^{2+}]_i in fura-2-loaded hepatocytes between males and females, while epinephrine-induced cAMP accumulation in hepatocytes was about 3-fold higher in females. The α_{1}- and β-adrenergic receptor properties of the plasma membrane were evaluated by ligand binding studies using [3H]prazosin (α_{1}-adrenergic antagonist) and [125I]iodocyanopindolol (β-adrenergic antagonist); and little sex difference was found in either affinity or the number of binding sites of [3H]prazosin and [125I]iodocyanopindolol. Activation of adenylate cyclase by forskolin and GTP-γ-S was also similar for both sexes. These results suggest that the sex difference of β-adrenergic response is due to a difference in the guanine nucleotide regulatory binding proteins (G proteins) and/or β-adrenergic receptor-Gs protein (the stimulatory G protein of adenylate cyclase) coupling ability.

Several distinct types of receptors for catecholamines have been defined, based on both their distinct physiological actions and pharmacological specificities. They include the α_{1-}, α_{2-}, β_{1-} and β_{2}-adrenergic subtypes (1). Moreover, the different receptor subtypes are coupled to distinct intracellular signaling systems. For example, both β_{1-} and β_{2}-adrenergic receptors stimulate the enzyme adenylate cyclase, leading to the generation of cAMP. Gs protein is the guanine nucleotide adenylate cyclase; G protein, the stimulatory G protein of adenylate cyclase, Gi protein, the inhibitory G protein of adenylate cyclase, Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [125I]ICP, [125I]iodocyanopindolol; IBMX, 3-isobutyl-1-methylxanthine; STEA solution, a solution containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, and 50 karnkline units/ml aprotinin; HEA solution, a solution containing 50 mM Hepes-NaOH (pH 7.4), 1 mM EGTA and 50 karnkline units/ml aprotinin.
glycogenolytic responses of female rats, while the response in male rats is the \( \alpha_1 \)-adrenergic receptor-mediated one (7). However, little is known about the exact mechanism leading to the sex difference in the \( \beta \)-adrenergic receptor-mediated hepatic glycogenolysis. Thus in the present study, we compared epinephrine-induced responses in male and female rat liver cells and obtained evidence that the \( \beta \)-adrenergic receptor-mediated functions increase in female rats without any changes in the number of receptors.

**Materials and Methods**

**Animals:** Male and female Slc::Wistar strain rats (9–10 weeks old), weighing between 230–260 g and 140–170 g, respectively, were obtained from Japan SLC, Inc. (Hamamatsu) and were kept in an air-condition room (25±1 °C, 50–60% humidity) lighted 12 hr a day (8:00 a.m. to 8:00 p.m.). Animals were maintained on an ordinary diet (CA-1, CLEA Japan, Inc., Tokyo) and water ad libitum.

**Materials:** [\(^3\)H]prazosin (3034 GBq/mmol) and [\(^{125}\)I]iodocyanopindolol (8140 GBq/mmol) were purchased from Du-Pont New England Nuclear (Wilmington, U.S.A.). Epinephrine bitartrate, phenylephrine hydrochloride, propranolol hydrochloride, yohimbine hydrochloride, IBMX, aprotinin, trypsin inhibitor and forskolin were obtained from Sigma Chemical Company (St. Louis, U.S.A.). Fura-2 AM was purchased from Wako Pure Chemical Industries (Osaka), the assay kit for cAMP from Yamasa Shoyu Co. (Chiba) and bovine serum albumin (Fr. V) from Seikagaku Kogyo Co. (Tokyo). Percoll and GTP-\( \gamma \)-S were obtained from Pharmacia Fine Chemicals (Upsala, Sweden) and Boehringer Mannheim (Mannheim, F.R.G.), respectively. All other chemicals used were of reagent grade.

**Preparation of isolated hepatocytes and determination of \([Ca^{2+}]_i\), in hepatocytes:** Rat liver parenchymal cells from fed rats were isolated by \textit{in situ} perfusion with collagenase, and \([Ca^{2+}]_i\), was measured with the fluorescent \(Ca^{2+}\) indicator fura-2, essentially as reported previously (10).

**Measurements of enzyme activity:** cAMP generated in isolated hepatocytes and plasma membranes fractions was measured according to the method of Itouh et al. (11). The cell suspension (3\times10^6 cells/ml) was first incubated for 15 min at 37°C in the Hepes-buffer medium (134 mM NaCl, 4.7 mM KCl, 1.2 mM \(KH_2PO_4\), 1.2 mM \(MgSO_4\), 1.3 mM \(CaCl_2\), 5 mM \(NaHCO_3\), 40 mM glucose and 10 mM Hepes, pH 7.4) containing 0.4 mM IBMX under an atmosphere of 95% \(O_2\)–5% \(CO_2\). After this preincubation, the resulting cell suspension (0.5 ml) was further incubated with 10 \(\mu\)l of a test drug dissolved in Hepes-buffer medium at 37°C for 2 min. After the
termination of the reaction by adding 50 μl of 1N HCl, the cell suspension was homogenized and centrifuged at 1,700xg for 10 min. The resulting supernatant was used for the measurement of cAMP. When purified plasma membrane was used for the assay of adenylate cyclase activity, the reaction buffer containing 5 mM MgCl₂, 1 mM EGTA, 0.4 mM IBMX, 0.5 mM ATP, 5 mM phosphocreatine, 50 units/ml creatine phosphate kinase and 25 mM Tris-HCl (pH 7.5). The assay was started by the addition of membrane pellets (20 μg of protein) to 0.2 ml of the reaction buffer. After the incubation at 30°C for 10 or 20 min with or without GTP-γ-S, respectively, the reaction was stopped by adding 20 μl of 1 N HCl. When adenylate cyclase was stimulated by forskolin, the reaction was stopped after the incubation at 30°C for 5 min. The supernatant obtained by centrifugation at 1,700xg for 10 min was used for the determination of adenylate cyclase activity. cAMP generated from ATP in isolated hepatocytes or the purified plasma membrane system was measured with a Yamasa cAMP assay kit (12). Phosphorylase a activity in hepatocytes was determined as described previously (10).

5′-Nucleotidase activities in the liver homogenates and the membrane fractions were measured according to the method of Aronson and Touster (13).

Assay of [³H]prazosin binding: [³H]Prazosin binding with liver plasma membrane was carried out by a modification of the method of Nakamura et al. (14). Rough liver plasma membrane (500 μg protein) in 500 μl of HM solution containing 1 mM sodium ascorbate and 1 mM pyrocatechol was incubated at 25°C for 20 min with various concentrations of [³H]prazosin. Incubation was terminated by rapidly diluting with 5 ml of ice-cold 20 mM Tris-HCl (pH 7.5) containing 5 mM MgCl₂ and 100 mM NaCl. The diluted samples were subsequently treated as described for [³H]prazosin binding experiments and their radioactivities were measured in an Aloka ARC 600. Non-specific binding was determined in the presence of 1 μM prazosin, which was routinely less than 20%.

Assay of [¹²⁵I]ICP binding: [¹²⁵I]ICP binding was performed according to the method of Nakamura et al. (14) with a minor modification. Liver plasma membranes (100 μg protein) in 250 μl of HEA solution (pH 7.4) containing 100 mM NaCl, 5 mM MgCl₂, 1 mM sodium ascorbate and 1 mM pyrocatechol were incubated at 25°C for 40 min with various concentrations of [¹²⁵I]ICP. Incubation was terminated by rapidly diluting with 5 ml of ice-cold 20 mM Tris-HCl (pH 7.5) containing 5 mM MgCl₂ and 100 mM NaCl. The diluted samples were subsequently treated as described for [³H]prazosin binding experiments and their radioactivities were measured in an Aloka ARC 600. Non-specific binding was determined in the presence of 10 μM d,l-propranolol, which was routinely less than 20%.

Results

Adrenergic agonist-induced increase of [Ca²⁺], in fura-2-loaded hepatocytes: In liver cells, α₁-adrenergic agonists are known to cause an increase of [Ca²⁺],. We examined the receptor-mediated increase of [Ca²⁺], using fura-2-loaded hepatocytes and found that it was increased by epinephrine concentration-dependently in the range of 1x10⁻⁸ - 1x10⁻⁴ M, and the maximal increases of [Ca²⁺], were 0.8 μM for males and 1.1 μM for females (Fig. 1). In both males and females, phenylephrine, an α₁-adrenergic agonist, was more effective than isoproterenol, a β-adrenergic agonist. The [Ca²⁺], increase induced by epinephrine was antagonized most effectively by prazosin, an α₁-adrenergic antagonist. The α₂-adrenergic antagonist yohimbine and the β-adrenergic antagonist propranolol also were inhibitory, but their actions were much weaker than that of prazosin (Fig. 2). No male-female difference was found in the action of these antagonists. The results indicated that the intracellular Ca²⁺ mobilization in hepatocytes is functionally coupled to the α₁-adrenergic receptor in both male and female rats.

Adrenergic agonist-induced cAMP gener-
cation in IBMX-treated hepatocytes: β-Adrenergic receptor-mediated cAMP formation was examined using isolated hepatocytes from male and female rats. The basal level of cAMP accumulation in female hepatocytes was not so different from that of male, but epinephrine markedly stimulated cAMP accumulation in female hepatocytes (Fig. 3). By the addition of $10^{-5}$ M epinephrine, cAMP accumulation in female hepatocytes increased to 6.0-fold that of the basal level, while cAMP accumulation in male hepatocytes increased about 2-fold. The β-adrenergic agonist isoproterenol was more effective in females for stimulating cAMP accumulation than the α₁-adrenergic agonist phenylephrine. On the other hand, in the males, phenylephrine and isoproterenol exhibited a slight or no stimulatory effect as shown in Fig. 3. The stimulation of the cAMP generation system by epinephrine was antagonized most effectively by the β-adrenergic antagonist propranolol in both males and females (Fig. 4). These results indicated that the cAMP generation system is functionally coupled to the β-adrenergic receptor, and the β-adrenergic response is more strongly expressed in the females than in the males.

Effects of adrenergic agonist on phos-

![Fig. 1. Effects of adrenergic agonists on $[Ca^{2+}]_i$ in fura-2-loaded hepatocytes. Hepatocytes isolated from male (panel A) or female (panel B) rats were loaded with fura-2 (5 μM) as described in "Materials and Methods", and then they were incubated with the indicated concentrations of epinephrine (Epi, open circles), phenylephrine (Phe, open triangles), or isoproterenol (Iso, open squares). Other conditions are described in "Materials and Methods." The resting levels of $[Ca^{2+}]_i$ in male and female hepatocytes were 1.8 μM and 1.6 μM, respectively. The maximal level of $[Ca^{2+}]_i$ in male and female hepatocytes were 2.6 μM and 2.7 μM, respectively. The maximal increase of $[Ca^{2+}]_i$ in male and female hepatocytes were 0.8 μM and 1.1 μM, respectively. $[Ca^{2+}]_i$ is represented as a relative value to the maximal response induced by epinephrine.]

![Fig. 2. Effects of adrenergic antagonists on the epinephrine-induced increase of $[Ca^{2+}]_i$ in fura-2-loaded hepatocytes. Hepatocytes isolated from male (panel A) and female (panel B) rats were incubated with 1 μM epinephrine, in the presence of the indicated concentrations of prazosin (Pra, open circles), yohimbine (Yoh, open triangles) or propranolol (Pro, open squares). Other conditions are as given in the legend to Fig. 1. Control values for $J[Ca^{2+}]_i$ are 0.8 μM (male) and 1.1 μM (female). The relative value of $J[Ca^{2+}]_i$ against the control is plotted in the figure as a function of the antagonist concentration.]

Phosphorylase activity in isolated hepatocytes: Phosphorylase activity in isolated hepatocytes was determined after the addition of various concentrations of adrenergic agonists. Although the basal enzyme activity was higher in males than in females, epinephrine stimulated the enzyme activity concentration-dependently in the range of $10^{-7}$ to $10^{-4}$ M in hepatocytes of both sexes; no sex difference was observed (Fig. 5). Stimulation of phosphorylase activity in hepatocytes by the α-adrenergic agonist phenylephrine was also similar for both sexes. Interestingly, the β-adrenergic agonist isoproterenol activated phosphorylase activity more pronouncedly in females. The results indicated that the activation of phosphorylase is more responsive to β-adrenergic agonist in the females than in the males.

Distribution of adrenergic receptors in hepatic membranes: To clarify the sex difference in the adrenergic receptor mediated response of hepatocytes, the distribution of adrenergic receptors in liver plasma membranes was examined by the binding assay procedure. We show here the representative data of two experimental results, because we obtained similar results in two experiments. The rough plasma membrane fractions were prepared as described in "Materials and Methods" from livers and measuring 5'-nucleotidase activity as a marker of plasma membrane. The distribution of α₁-adrenergic
Fig. 5. Effect of adrenergic agonists on phosphorylase a activity in isolated hepatocytes. Hepatocytes isolated from male (panel A) and female (panel B) rats were incubated with the indicated concentrations of epinephrine (Epi, open circles), phenylephrine (Phe, open triangles) or isoproterenol (Iso, open squares). Other conditions are described in "Materials and Methods."

Fig. 6. Scatchard analysis of [3H]prazosin binding to rough plasma membrane fraction of liver cells. Rough plasma membranes (500 µg protein) prepared from male (open circles) and female (open triangles) rats were incubated with various concentrations of [3H]prazosin (0–360 pM). After the incubation at 25°C for 20 min, free and bound forms of [3H]prazosin were determined as described in "Materials and Methods." Non-specific binding of [3H]prazosin was measured in the presence of 1 µM prazosin.

Fig. 7. Scatchard analysis of [125I]ICP binding to purified plasma membrane fraction of liver cells. Purified plasma membranes (100 µg protein) prepared from male (open circles) and female (open triangles) rats were incubated with various concentrations of [125I]ICP (0–1600 pM). After the incubation at 25°C for 40 min, free and bound forms of [125I]ICP were determined as described in "Materials and Methods." Non-specific binding was measured in the presence of 10 µM d,l-propranolol.

receptors was examined using [3H]prazosin. Figure 6 shows Scatchard plots of [3H]prazosin binding to the membrane. The dissociation constant ($K_d$) and the apparent maximum number ($B_{max}$) of [3H]prazosin binding sites were also calculated for both male and female rat membranes. After the correction for plasma membrane recovery,
the K_d and B_{max} of the [3H]prazosin binding sites were estimated as shown in Table 1. Although it was reported that the K_d value of the [3H]prazosin binding sites in the purified plasma membranes was about 200 pM (14), the K_d value of our result was about 50 pM. The K_d value may be influenced by the plasma membrane preparation method, because it was reported that the K_d value was 117 ± 21 pM in the binding assay to intact hepatocytes. Therefore, we think that the K_d value of [3H]prazosin binding sites in our results was lower than previous reported results because we used the microsomal fraction but not purified plasma membrane in the binding assay. As the result of the inhibition of [3H]prazosin binding by adrenergic antagonists, pK_{1} values of prazosin, d,l-propranolol, and yohimbine in the rough plasma membranes from male rats were 5.4 x 10^{-11} M, 1.1 x 10^{-5} M, and 3.4 x 10^{-7} M, respectively. These values from female rats were 5.9 x 10^{-11} M, 7.4 x 10^{-6} M, and 7.4 x 10^{-7} M, respectively. Because [3H]prazosin binding to rough plasma membranes was inhibited the most strongly by an α₁-adrenergic antagonist, it is considered that [3H]prazosin binds specifically to α₁-adrenergic receptors. Next, the distribution of β-adrenergic receptor in the liver plasma membrane was examined using [125I]ICP. The plasma membrane for the β-adrenergic receptor binding assay was purified from the crude nucleus fraction of male and female livers, because there was only a small number of β-adrenergic receptors in the plasma membranes (14). Judging from the specific activity of 5'-nucleotidase, the plasma membranes were purified 14.6-fold in males and 8.0-fold in females. Figure 7 shows Scatchard plots of [125I]ICP binding to the purified plasma membranes. From the data of Scatchard analysis, the K_d and B_{max} of the [125I]ICP binding sites were calculated after correction for membrane recovery (Table 1). Data on the inhibition of [125I]ICP binding by adrenergic antagonists indicated that the pK_{1} value of phentolamine (α-adrenergic antagonist) or d,l-propranolol in the purified plasma membranes from male rats were 1.3 x 10^{-4} M or 3.6 x 10^{-9} M, respectively. These values from female rats were 9 x 10^{-5} M or 9 x 10^{-9} M, respectively. Because [125I]ICP binding was inhibited more strongly by β-adrenergic antagonists, it is considered that [125I]ICP binds specifically to β-adrenergic receptors. These results for α₁- and β-adrenergic receptors in liver plasma membranes indicated that there is little difference between male and female rats.

**Activation of adenylate cyclase in purified plasma membranes:** β-Adrenergic receptor-mediated stimulation of cAMP accumulation in hepatocytes was much greater in females than in males (Fig. 3). However, it was very difficult to observe the sex difference in β-adrenergic receptor-mediated cAMP generation in purified plasma membranes, because GTP·γ·S which was contained in the reaction mixture enhanced adenylate cyclase activity via mediation by activation of G proteins. Next, in order to study the sexual dimorphism in the post-receptor metabolic difference, we estimated the GTP·γ·S- and forskolin-induced adenylate cyclase activity in purified plasma membranes. Although the basal activity of adenylate cyclase was slightly different between male and female rats, forskolin stimulated adenylate cyclase activity concentration-dependently in the range of 10^{-9}-10^{-5} M similarly in both sexes (data

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**Table 1.** B_{max} and K_d values of adrenergic receptors on plasma membranes from male and female rats

| α₁-Adrenergic receptor | B_{max} (fmol/mg protein) | K_d (pM) | β-Adrenergic receptor | B_{max} (fmol/mg protein) | K_d (pM) |
|------------------------|---------------------------|----------|-----------------------|---------------------------|----------|
| Male                   | 115                       | 58       | 105 (6.8)             | 130                       |
| Female                 | 93                        | 39       | 75 (9.4)              | 127                       |

B_{max} and K_d values were determined from the Scatchard analysis data of [3H]prazosin and [125I]ICP binding to liver plasma membrane shown in Figs. 6 and 7. Values in parentheses indicate the extent of B_{max}/membrane purification.
not shown), and the activity stimulated by 10 μM forskolin is shown in Table 2. On the other hand, the GTP-γ-S-induced adenylate cyclase activity was slightly higher in the females than in the males, but this difference was not sufficient to account for the sex difference of the β-adrenergic response in hepatocytes. These results indicated that the adenylate cyclase activity and G protein-adenylate cyclase coupling ability were similar between males and females.

**Table 2. Effect of GTP-γ-S and forskolin on adenylate cyclase activity in liver plasma membranes**

|               | cAMP formed (pmol/min/mg protein) |
|---------------|-----------------------------------|
|               | male                          | female                      |
| Control       | 2.6±0.1 (0.18±0.01)            | 2.4±0.1 (0.3±0.01)          |
| GTP-γ-S (10 μM)| 16.4±0.9 (1.1±0.06)           | 13.8±1.4 (1.7±0.18)        |
| Forskolin (10 μM)| 96.7±8.7 (6.6±0.6)     | 56.5±1.5 (7.1±0.19)        |

Purified plasma membranes from male and female rat livers were used for the assay of adenylate cyclase. Assay conditions are described in "Materials and Methods". Values are means±S.E. of 3 experiments. Values in parentheses indicate the extent of cyclic AMP formed/membrane purification.

**Discussion**

Many physiological and pathological conditions are associated with an inverse change in the α1-adrenergic receptor-mediated response in livers. The β-adrenergic response was found to increase in adult male rats under the conditions of hypothyroidism (15, 16), cholestasis (17), and other diseases. Katz et al. reported an age-associated decrease in β-adrenergic receptors and in the β-adrenergic response in male rat livers (18). In female rats, the β-adrenergic response decreased similarly with aging, but is more strongly expressed in adult male rats at all ages (19).

Using isolated hepatocytes prepared from adult male and female rats, we examined the α1-adrenergic receptor-mediated intracellular Ca2+ mobilization and the β-adrenergic receptor-mediated cAMP generation which seemed to display a sex difference in the β-adrenergic response, (Figs. 1–4). The increase of [Ca2+]i, intracellular Ca2+ concentration, of fura-2-loaded hepatocytes indicated functional coupling of the intracellular Ca2+ mobilizing system to the α1-adrenergic receptor, with the maximal response to epinephrine being similar between male and female rats (Figs. 1 and 2). On the other hand, the results obtained using IBMX-treated hepatocytes indicated that the cAMP generation system is functionally coupled by the β-adrenergic receptor in both male and female rats, but the maximal stimulation of cAMP formation by epinephrine is more effective in the females than in the males (Figs. 3 and 4). We also examined the effect of adrenergic agonists on the activity of phosphorylase a, which is activated by phosphorylase b by both the α1- and β-adrenergic receptor mediated processes. Interestingly, phosphorylase was activated more strongly in the females than in the males by isoproterenol, but not in the case of epinephrine and phenylephrine (Fig. 5). Although cAMP accumulation was not stimulated by isoproterenol in the male hepatocytes, the increase of [Ca2+]i (Fig. 3) and the activation of phosphorylase (Fig. 5) were caused by isoproterenol. It is known that α-adrenergic agonist isoproterenol binds to not only β-adrenergic receptors but also α1-adrenergic receptor at high concentration. Therefore, it is considered that the responses to isoproterenol in male hepatocytes were mediated by α1-adrenergic receptors rather than β-adrenergic receptors. These results indicated that the β-adrenergic response is expressed more sensitively in the females, although the α1-adrenergic response is expressed to the same extent in both sexes. The pronounced β-adrenergic responsiveness in female rats is consistent with the previous results (7).

The question arises of whether this difference is due to a difference in the number of β-adrenergic receptors or in the post-receptor metabolic processes. Thus, we studied the distribution of adrenergic receptors in liver plasma membranes using [3H]prazosin and [125I]ICP as a ligand of α1- and β-adrenergic
receptors, respectively. As shown in Figs. 6 and 7 and Table 1, the $K_d$ and $B_{max}$ values of $[3H]prazosin$ and $[125I]ICP$ bindings to $\alpha_1$ and $\beta$-adrenergic receptors, respectively, were similar in hepatocytes from both males and females. The recent studies of Studer and Ganas also have demonstrated no sex difference in the distribution of the adrenergic receptors in rat liver plasma membranes by in vitro radioligand binding studies (20). These results suggest that the receptor function are not different between male and female rats, although the $\beta$-adrenergic response is more markedly activated in females. Therefore, to test the sexual dimorphism in the post receptor metabolic difference, we estimated the stimulation of adenylate cyclase activity by GTP-$\gamma$-S and forskolin (Table 2). The results show that adenylate cyclase activity and G protein-adenylate cyclase coupling ability were similar for males and females.

We could not demonstrate here the involvement of a cellular site(s) in the sex difference in the $\beta$-adrenergic receptor-mediated reactions in rat livers. However, several recent studies have suggested that the receptor-mediated functions are modified due to alteration of the $\beta$-adrenergic receptor-Gs protein coupling ability (21), alteration of the composition of Gs protein (22, 23), and redistribution of the $\beta$-adrenergic receptor in the intact cell surface (24, 25). Another explanation for the development of $\beta$-adrenergic receptor function in the female hepatocytes is that the pathway of $\alpha_2$-adrenergic receptor-Gi protein is inhibited in the females (11). Further study is needed to determine the cellular sites at which the male-female difference occurs in $\beta$-adrenergic receptor function.

In conclusion, our results confirm no sex difference in the $\alpha_1$-adrenergic receptor-mediated intracellular Ca$^{2+}$ mobilization in isolated hepatocytes. On the other hand, female hepatocytes displayed stronger $\beta$-adrenergic receptor-mediated cAMP generation than male hepatocytes. In purified plasma membranes from male and female rat livers, it was demonstrated that there was little difference in the distribution of adrenergic receptors and the functions of the post-G proteins. These results suggest that the cellular site(s) where the sex difference occurs in the G proteins and/or $\beta$-adrenergic receptor-Gs protein coupling. Judging from the preliminary experiment of the epinephrine competition curves for $[125I]ICP$ binding in purified plasma membranes, it is suggested that $\beta$-adrenergic receptor-Gs protein is coupled more efficiently in females than in males.

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