Rapid insulinotropic effect of 17β-estradiol via a plasma membrane receptor

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ABSTRACT **Impaired insulin secretion is a hallmark in both type I and type II diabetic individuals. Whereas type I (insulin-dependent diabetes mellitus) implies β-cell destruction, type II (non-insulin dependent diabetes mellitus), responsible for 75% of diabetic syndromes, involves diminished glucose-dependent secretion of insulin from pancreatic β-cells. Although a clear demonstration of a direct effect of 17β-estradiol on the pancreatic β-cell is lacking, an *in vivo* insulinotropic effect has been suggested. In this report we describe the effects of 17β-estradiol in mouse pancreatic β-cells. 17β-Estradiol, at physiological concentrations, closes K_{ATP} channels, which are also targets for antidiabetic sulfonylureas, in a rapid and reversible manner. Furthermore, in synergy with glucose, 17β-estradiol depolarizes the plasma membrane, eliciting electrical activity and intracellular calcium signals, which in turn enhance insulin secretion. These effects occur through a receptor located at the plasma membrane, distinct from the classic cytosolic estrogen receptor. Specific competitive binding and localization of 17β-estradiol receptors at the plasma membrane was demonstrated using confocal reflective microscopy and immunocytochemistry. Gaining deeper knowledge of the effect induced by 17β-estradiol may be important in order to better understand the hormonal regulation of insulin secretion and for the treatment of NIDDM.—Nadal, A., Rovira, J. M., Laribi, O., Leon-Quinto, T., Andreu, E., Ripoll, C., Soria, B. Rapid insulinotropic effect of 17β-estradiol via a plasma membrane receptor. *FASEB J.* 12, 1341–1348 (1998)**

Key Words: intracellular calcium · K_{ATP} channels · insulin secretion · confocal microscopy · membrane estrogen receptor.

Classically, estrogen effects are mediated through genomic mechanisms that involve the diffusion of estrogen across the plasma membrane and activation of specific intracellular receptors. In the last few years, evidence has accumulated in favor of nongenomic actions of estrogen via a putative membrane receptor. Steroid sex hormone receptors at the plasma membrane are proposed to be involved in rapid nongenomic effects of estrogens in several types of cells (1, 2). For instance, sex hormones modulate $[Ca^{2+}]_i$ signals in sperm (3), granulosa cells (4), and male osteoblast (5) whereas the modulation of ion channels has been described in vascular smooth muscle (6, 7), hippocampal neurons (8), and fibroblasts (9), among others. The rapid action of 17β-estradiol could conceivably be due either to a change in properties of the membrane or to a direct modulation of a membrane protein. The latter would include binding to ionic channels, enzyme systems, and the existence of a receptor for 17β-estradiol (2).

Some recent observations strongly suggest that estrogens may have an *in vivo* insulinotropic effect. For instance, estradiol has been shown to reverse the effects of menopause on glucose and insulin metabolism, resulting in an increase in pancreatic insulin secretion and a decrease in insulin resistance (10, 11). Treatment of male and ovariectomized female mice with estrogen prevents the development of diabetes (12). Moreover, development of diabetes mellitus after major resection of the pancreas can be markedly prevented with estrogen administration due to improvement in insulin secretion and glucose tolerance (13). In spite of this circumstantial evidence, a direct effect of 17β-estradiol upon β-cells has yet to be demonstrated.

We demonstrate here a rapid insulinotropic effect of 17β-estradiol due to regulation of K_{ATP} channel activity and calcium signals through a membrane receptor.

METHODS

Islets were obtained from the pancreas of male mice 2–3 months of age and treated as described below. The dimethyl sulfoxide (DMSO)[2] concentration normally used was 10^{-5}% and had no effect on records with the techniques used. In any

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[2] Abbreviations: TMR, tetramethylrhodamine; DMSO, dimethyl sulfoxide; BSA, bovine serum albumin; NIDDM, non-insulin-dependent diabetes mellitus; DAB, diaminobenzidine.

case, control solutions had DMSO in the same proportion as 17β-estradiol-containing solutions. All agents were purchased from Sigma (Madrid, Spain) unless stated otherwise.

Membrane potential measurement

The β-cell membrane potential was recorded using high-resistance microelectrodes, essentially as described (14). Once microdissected, islets were placed in a 50 μl chamber perfused with fresh modified Krebs medium and constantly gassed with a mixture of 95% O_2 and 5% CO_2 for a final pH of 7.4. Temperature was maintained at 36°C. β-Cells were impaled using thick-walled, high-resistance microelectrodes and recordings were made with an Axoclamp 2B amplifier (bridge mode). Data acquisition was performed with Axoscope1.1.

Intracellular calcium measurement

Whole islet of Langerhans isolated with collagenase were loaded with Indo-1 by incubation with the AM ester (5 μM, Molecular Probes, Eugene, Oreg.) for 45–90 min at room temperature before measuring intracellular calcium using a double emission microfluorescence system (15).

Insulin secretion

Static incubation was conducted using batches of three islets each and incubated for 20 min at 37°C, pH 7.4, in 1 ml Krebs-Ringer buffer containing 1% bovine serum albumin (BSA) plus appropriate stimuli. At the end of each incubation, insulin was determined by radioimmunoassay (Diagnostic Products, Los Angeles, Calif.). Intraassay variation coefficient oscillated between 13% at 5 μIU/ml and 5% at insulin concentrations between 15 and 400 μIU/ml. Interassay variation coefficient was 7% at a detection limit of 1.2 μIU/ml.

Patch-clamp recording

Islets were dispersed into single cells and cultured as previously described (16). Patch pipettes were filled with a standard solution (in mM): KCl 140, HEPES 10, $CaCl_2$ 2, $MgCl_2$ 2; pH 7.4. Bath solution contained (in mM): KCl 5, NaCl 135, $CaCl_2$ 2.5, HEPES 10, $MgCl_2$ 1.1; pH 7.4. Solutions used for the inside-out experiments were (in mM): KCl 5, NaCl 135, HEPES 10, $CaCl_2$ 2.5, $MgCl_2$ 2.5, pH 7.4; for pipette, the solution contained (in mM): KCl 140, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10, EGTA 1; pH 7.2. K_{ATP} channel activity was quantified by digitizing 30 s sections of the current record, filtered at 3 kHz and sampled at 10 kHz by a Digidata 1200 (Axon Instruments), and calculating the mean NP_o during the sweep (n=5). Experiments were carried out at room temperature (20–24°C). Data are expressed as mean ± SD.

Assay for estradiol-peroxidase binding

Pancreatic islets cells cultured on polylysine-coated coverslips for 5 h were fixed in 4% (wt/vol) paraformaldehyde for 30 s and exposed overnight to 4.5 μg/ml estradiol-peroxidase. Cells were then washed and peroxidase was developed using 0.5 mg/ml 3,3′-diaminobenzidine (DAB) in the presence of 0.075% (vol/vol) H_2O_2 for 5 min. Cells were visualized using a Leica DMRB microscope.

Plasma membrane permeability assay

Cells were incubated with 2.5 mg/ml dextran-conjugated tetramethylrhodamine (dextran-TMR) and immediately visualized using a Zeiss LSM510 confocal microscope with a Zeiss 63× oil immersion lens, numerical aperture 1.3. The optical section was always lower than 600 nm. Results are representative of at least 50 cells in 3 different coverslips from 2 different experiments.

Location of estradiol-peroxidase binding

Confocal reflective microscopy

Once peroxidase assay was completed as described above, DAB precipitate was visualized using the reflective mode of conventional confocal microscopy.

Immunofluorescence

Peroxidase was labeled with rabbit anti-horseradish peroxidase (HPX) antiserum [1:50 dilution in phosphate-buffered saline with 2% (vol/vol) goat serum, 2 h at 4°] and followed, after washing, with fluorescein-labeled goat anti-rabbit IgG (1:100 dilution with 2% goat serum, 1 h at 4°C). Stained cells were visualized using a Zeiss LSM510 confocal microscope equipped with a Zeiss 63× oil immersion lens, numerical aperture 1.3. Results are representative from at least 80 cells from 3 different coverslips in 3 different experiments.

RESULTS

To investigate the role of 17β-estradiol as a modulator of pancreatic β-cell signaling, electrical activity recordings were obtained from a cell within an islet of Langerhans isolated from male mice (17). When 100 pM 17β-estradiol was applied in the absence of glucose, a small depolarization lower than 10 mV was elicited (not shown), which in itself is insufficient to initiate an oscillatory electrical activity. Nonetheless, when 17β-estradiol was applied in the presence of a stimulatory concentration of glucose such as 8.3 mM, an increase of 40 ± 13% in the frequency of bursts of electrical activity was observed (**Fig. 1*A***).

It is well known that the bursting pattern of electrical activity generates simultaneous $[Ca^{2+}]_i$ oscillations in the whole islet of Langerhans (18, 19). Accordingly, when intracellular calcium was measured in intact islet, 17β-estradiol did not induce any change in $[Ca^{2+}]_i$ at substimulatory glucose concentrations. In marked contrast, when 17β-estradiol was applied in the presence of a stimulatory concentration of glucose, a transitory peak of calcium followed by a repetitive pattern of $[Ca^{2+}]_i$ oscillations on a plateau phase was elicited (Fig. 1*B*). The frequency of $[Ca^{2+}]_i$ oscillations was enhanced by 138 ± 33% (Fig. 1*C*). Remarkably, in islets in which no $[Ca^{2+}]_i$ oscillations were observed in the presence of 8.3 mM glucose, 17β-estradiol triggered a repetitive oscillatory pattern (not shown). As a general rule, the lower the frequency of oscillations elicited by glucose, the higher the effect on both frequency and amplitude produced by 17β-estradiol. Modulation of the calcium signals was not a sex-dependent effect, since it

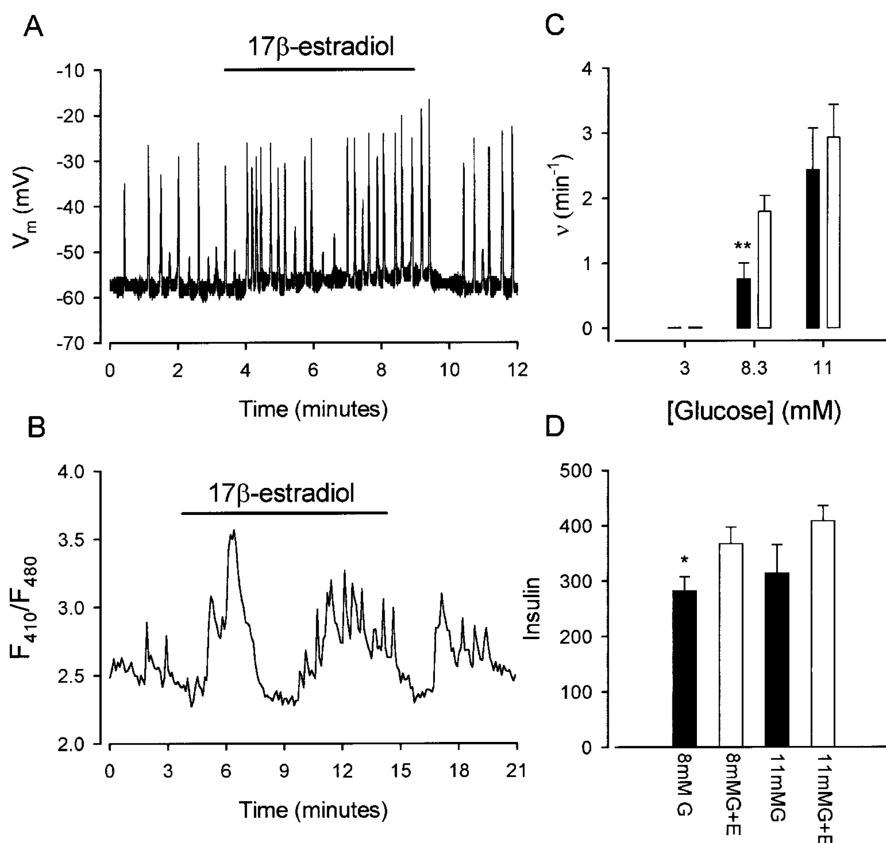

Figure 1. 17β-Estradiol modulates β-cell signaling system and insulin secretion. *A)* Membrane potential recording from a β-cell within an islet of Langerhans. Islet is perfused with 8.3 mM glucose, and 100 pM 17β-estradiol is applied during the bar. Change in frequency of bursts was from 3.4 ± 0.5 to 4.3 ± 0.6 min^{-1}, measured in a total of 11 cells. The effect was partially reversible after 10 min. *B)* Change in the fluorescence ratio of Indo-1 in a whole islet of Langerhans. Islet exhibited a frequency of oscillations in the presence of 8.3 mM glucose of 0.75 ± 0.24 min^{-1}. When 100 pM 17β-estradiol was applied, a first transient of $[Ca^{2+}]_i$ of ΔR = 0.77 ± 0.15 and a duration of 1.26 ± 0.23 min was elicited. Such a transient was followed by a silent period of 0.46 ± 0.28 min, which became shorter when estradiol concentration was increased. After the silent phase, a plateau of ΔR = 0.15 ± 0.03 was generated with $[Ca^{2+}]_i$ oscillations on it with a frequency of 1.79 ± 0.29. Results are representative of 10 experiments. *C)* Analysis of frequency of $[Ca^{2+}]_i$ oscillations at different concentrations of glucose in the absence (filled bars) and presence (white bars) of 100 pM 17β-estradiol. Estradiol was inactive when a nonstimulatory glucose concentration was used. Maximal effect was elicited when 17β-estradiol was applied in the presence of 8.3 mM glucose. Points are the mean of at least five different experiments. The analysis was done on 2 min periods, always taken when a steady state was reached, after the initial transient for 17β-estradiol. *D)* Insulin secretion in pg (ml·20 min) obtained by radioimmunoassay in the absence (filled bars) and presence (white bars) of a maximal dose of 17β-estradiol 10 nM at different stimulatory glucose concentration. The effect is only significant at 8.3 mM glucose. Results are from three or four separate static incubation per mouse, and eight different mice were used. Data are taken from eight unpaired experiments with triplicate or quadruplicate of each point. Data are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.005$ Student's *t* test.

was observed in both male and female mice. Insulin secretion is a calcium-dependent process (20, 21); indeed, intracellular calcium oscillations generate an oscillatory pattern of insulin secretion (22, 23). In our case, insulin secretion was enhanced by 30 ± 10% when 17β-estradiol was applied synergistically with 8.3 mM glucose. No significant effect was observed at higher glucose concentrations (Fig. 1*D*).

As expected for a physiological modulator, the 17β-estradiol potentiation of glucose effects was concentration dependent (**Fig. 2*A, B***), with a half-maximal stimulatory concentration of about 500 pM, well within the physiological range. The response exhibited some pharmacological specificity, since the stereoisomer 17α-estradiol presented half the activity of 17β-estradiol. Further selectivity of the system is manifested by the fact that other hormones with similar structures, such as testosterone and the 17β-estradiol metabolite estriol, had a very reduced effect (Fig. 2*C*). In summary, the relative potency for enhancing glucose-induced $[Ca^{2+}]_i$ oscillations was in the following order: 17β-estradiol, estrone > α-estradiol ≫ testosterone, estriol.

We have also demonstrated that fast modulation of the glucose response by 17β-estradiol is a nongenomic effect. Pretreatment for at least 3 h with actinomycin-D, an inhibitor of RNA synthesis, did not prevent the effect of 17β-estradiol on $[Ca^{2+}]_i$ (Fig. 2*D*). When islets were incubated for 4 h in the presence of cycloheximide, a potent inhibitor of protein

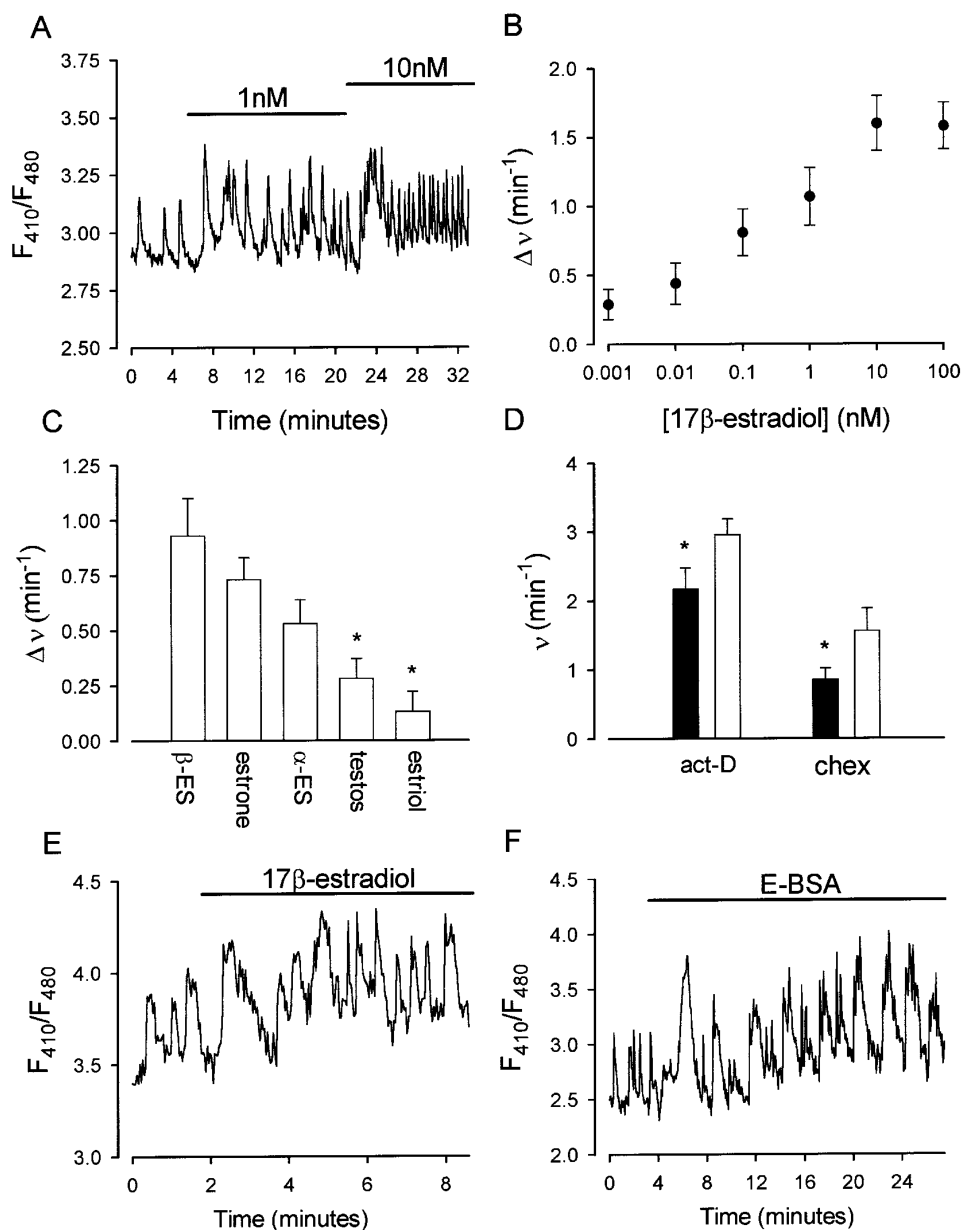

Figure 2. Characterization of nongenomic effects of 17β-estradiol. *A)* Changes in $[Ca^{2+}]_i$ observed using increasing concentrations of 17β-estradiol. 8.3 mM glucose was applied during the entire record. *B)* Change in the frequency of $[Ca^{2+}]_i$ oscillation as a function of 17β-estradiol concentration. *C)* Change in the frequency of oscillation due to 17β-estradiol and different structurally related compounds. All the stimuli were applied at 1 nM concentration in the continuous presence of 8.3 mM glucose. *Significantly different from 17β-estradiol. *D)* Comparison between frequency of oscillations in the absence (filled bars) and presence (white bars) of 17β-estradiol 100 pM on an islet of Langerhans stimulated by 11 mM glucose. Islets were incubated previously with actinomycin-D 4 M or cycloheximide 100 μM, between 3 and 4 h. *E)* Tamoxifen 5 μM does not counteract changes in $[Ca^{2+}]_i$ elicited by 17β-estradiol 1 nM. Tamoxifen was applied 15 min before and during 17β-estradiol application. *F)* Changes in $[Ca^{2+}]_i$ elicited by BSA–estradiol complex are equivalent to those produced by 17β-estradiol alone 1 nM. The conjugate linker is β-estradiol 17-hemisuccinate:BSA. Records in panels *E* and *F* are representative of three different experiments. Each point is the mean of at least four different experiments. Error bars shown are mean ± SEM. Records are representative of a least four different experiments. $^*P < 0.05$, $^{**}P < 0.005$ Student's *t* test.

synthesis, 17β-estradiol effects on intracellular calcium oscillations were unchanged (Fig. 2*D*). Furthermore, tamoxifen, a classic inhibitor of the cytosolic/nuclear receptor, was without effect on 17β-estradiol regulation of intracellular calcium, further excluding the involvement of the intracellular estrogen receptor (Fig. 2*E*). Nonetheless, tamoxifen elicited a small change in the glucose-induced oscillatory pattern, acting as a weak agonist, although such a response was never large enough to mask 17β-estradiol effects. Estradiol conjugated to BSA, which is not membrane permeable, was able to reproduce the effect of 17β-estradiol on $[Ca^{2+}]_i$ in the presence of stimulating glucose (Fig. 2*F*).

The foregoing results strongly suggest the existence of a signal-generating receptor for 17β-estradiol on the cell surface distinct from the classic cytosolic/nuclear steroid-receptor. Since the resting membrane potential in pancreatic β-cells is governed mainly by K_{ATP} channels, the small depolarization elicited by 17β-estradiol in the absence of glucose suggests an effect of 17β-estradiol on K_{ATP} channel activity. To investigate this possibility, cell-attached and excised patch recordings were obtained from isolated β-cells from mouse islet of Langerhans and maintained in cell culture for 1 to 3 days. When cell-attached recordings were obtained from isolated cells under conditions in which the bathing solution contained no glucose, physiological concentrations of 17β-estradiol induced a substantial decrease in K_{ATP} channel activity (**Fig. 3*A***). This response was gradual and completely reversible. Figure 3*B* shows the time course of K_{ATP} mean open probability (NP_o) when applying 100 pM 17β-estradiol. The time to reach the maximum current inhibition after 17β-estradiol application was 3 to 7 min, which was transitory: channel activity returned to control values in about 30 min even in the presence of 17β-estradiol (not shown).

To further substantiate that these effects were mediated through a plasma membrane receptor, estradiol linked to BSA was tested under the same conditions. As with calcium signals, no significant differences were found between channel activity inhibition by 17β-estradiol and estradiol conjugated to BSA. Moreover, application of 17β-estradiol to the inner side of an excised membrane patch did not affect K_{ATP} channel activity, even at the highest dose examined (10 nM) (Fig. 3*C*). These experiments indicate that 17β-estradiol effects are initiated at the plasma membrane. Moreover, exposure to 17β-estradiol induced a prolonged period of refractoriness to further estradiol stimulation (not shown). The existence of desensitization of the response after 20 min application of 17β-estradiol is in accordance with the existence of a receptor responsible of the decrease in K_{ATP} channel activity. Such a receptor must be lo-

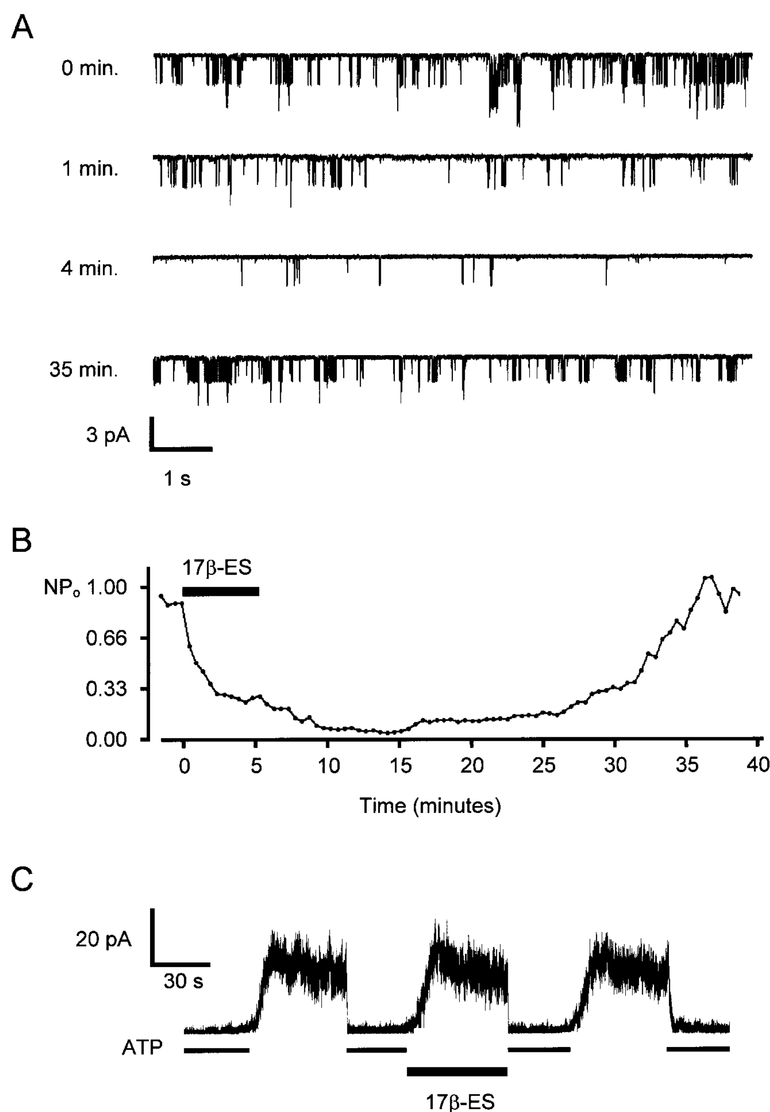

Figure 3. 17β-Estradiol effect on K_{ATP} channels. *A)* 17β-estradiol attenuates K_{ATP} channel activity in intact pancreatic islet cells. Single K_{ATP} currents were recorded from cell-attached patches on cultured islet cells at a 0 mV pipette potential. Cells were exposed to 100 pM 17β-estradiol. The record, representative of five, shows K_{ATP} channel activity before application of 17β-estradiol 1 min after application, 4 min after application, and 35 min after washing. *B)* Time course of K_{ATP} channel mean open probability (NP_o) on application of 100 pM 17β-estradiol. 17β-Estradiol was applied during the bar. *C)* Lack of effect of 10 nM 17β-estradiol on K_{ATP} channel activity in inside-out cell membrane patches (n=3). Patch potential was held at 0 mV. 17β-Estradiol was applied during the period indicated below the record. Nonetheless, longer records up to 5 min have been performed with the same result. Complete blockade of channel activity in the presence of 2 mM ATP assured K_{ATP} channel responsiveness.

cated at the plasma membrane because estradiol conjugated to BSA not only reproduces 17β-estradiol signals, but also decreases K_{ATP} channel activity recorded in the cell-attached mode.

To further investigate the existence of an estrogen receptor at the membrane of pancreatic β-cells, the experiment shown in **Fig. 4** was performed. Figure 4*A* shows labeling of estradiol conjugated to HPX developed using diaminobenzidine. Binding of estradiol–HPX appeared to be competitive, since it could be blocked by incubating in the presence 300-fold excess of 17β-estradiol (Fig. 4*B*). Nonetheless, it could be argued that estradiol–HPX might enter through pores at the plasma membrane caused by paraformaldehyde fixation and binding to cytosolic/nuclear receptors. Figure 4*C, D* illustrates that this is not the case. A dextran-TMR was used as a dye to demonstrate permeability of the plasma membrane when visualized using confocal microscopy. The molecular weight of this compound is 40,000, very similar to the 44,000 of estradiol–HPX complex. In our hands, 98% of the cells were impermeable to dextran-TMR after paraformaldehyde treatment, which clearly demonstrates that estradiol–HPX binds competitively to the plasma membrane. Furthermore, when confocal reflective microscopy was used, peroxidase labeling was visualized as a ring when a section through the middle of the cell was imaged (Fig. 4*E, F*). Such a result clearly indicates that the DAB precipitate was located at the membrane. The experiment in Fig. 4*G* shows the location of estradiol–HPX via a double immunocytochemistry, where cells incubated with estradiol–HPX as in Fig. 4*A* were exposed to an antibody anti-HPX. A secondary antibody anti-IgG fluorescein conjugated was applied later. When cells were imaged through the center of the cell, staining was clearly seen only at the plasma membrane. These experiments clearly demonstrate that estradiol–HPX has a high affinity binding site at the plasma membrane of pancreatic islet cells that can only be explained by the existence of an estrogen receptor.

DISCUSSION

The findings described in this work demonstrate that 17β-estradiol, a circulating hormone, synergizes with glucose to inhibit K_{ATP} channel activity. Consequently, the increased excitability of the plasma membrane fa-

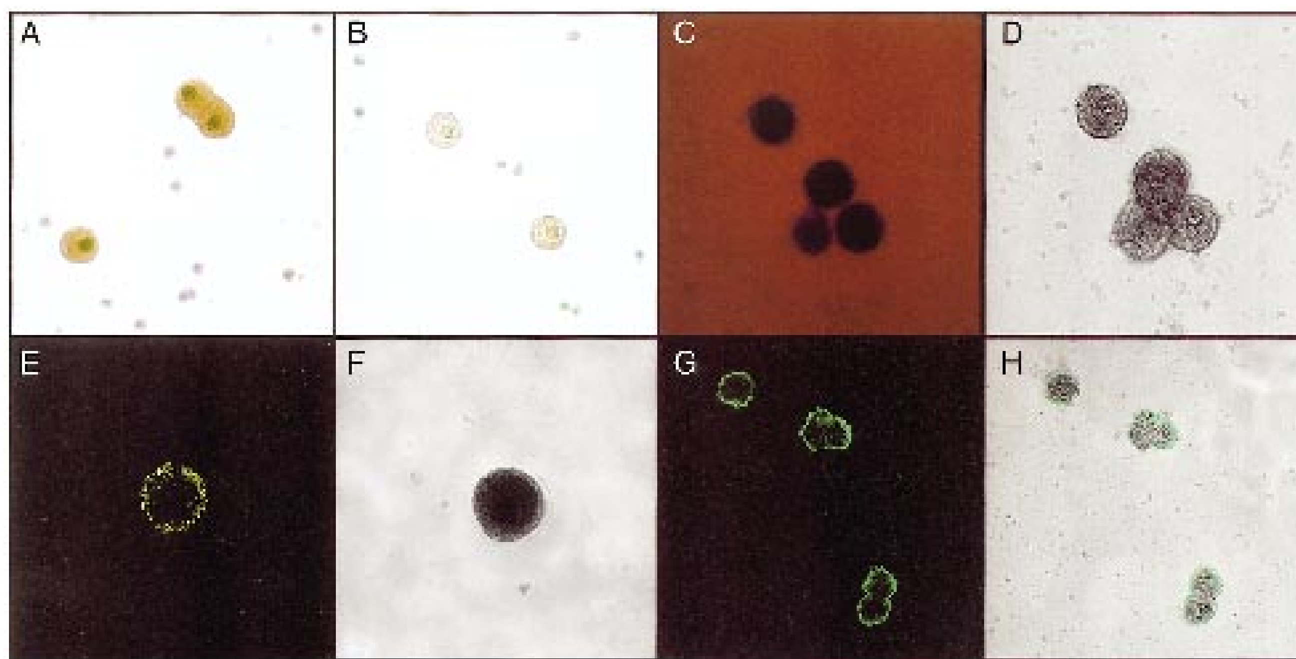

Figure 4. 17β-Estradiol has a high-affinity binding site at the plasma membrane of pancreatic islet cells. *A)* Binding of estradiol–HPX to pancreatic islet cells in primary culture. Labeling was obtained by using DAB as a substrate of HPX in the presence of H_2O_2. Results are representative of six different coverslips in three different experiments. *B)* Binding of estradiol–HPX 100 nM is competed using 300-fold excess 17β-estradiol. *C)* Dextran-TMR (40,000 mol wt) does not cross the plasma membrane of paraformaldehyde-fixed cells. Dextran-TMR labeling is shown in red and impermeable cells in in black. Image was taken using confocal microscopy with an optical section lower than 600 nm. *D)* Transmission image of cells in panel *C.* Cells are stained in black due to peroxidase labeling. *E)* Location of estradiol–HPX at the plasma membrane by using confocal reflective microscopy. Optical section used was lower than 700 nm. *F)* Transmission image of cells in panel *E. G)* Location of estradiol–HPX at the plasma membrane by using immunofluorescence. Fluorescein staining was visualized at the plasma membrane using a confocal microscope and an optical section lower than 1 μM. *H)* Transmitted image of cells shown in panel *F* with fluorescein staining showed simultaneously.

cilitates action potential firing and hence an increased frequency of $[Ca^{2+}]_i$ oscillations. As a result of the latter, insulin secretion is augmented. Regulation of the glucose effect by 17β-estradiol is a receptor-mediated, nongenomic process. Results in Fig. 2 illustrate that 17β-estradiol enhances frequency of $[Ca^{2+}]_i$ oscillations in a concentration-dependent manner. 17β-Estradiol concentrations used were well within the physiological range (from 100 pM to 1 nM) but even low concentrations, such as 10 pM, had a small but significant effect on intracellular calcium.

The effect is partially stereospecific and is not reproduced by other estrogens of similar chemical structure such as estriol or testosterone. These results strongly suggest the existence of a receptor for 17β-estradiol in pancreatic β-cell. Such a receptor must be different from the classic cytosolic/nuclear receptor since the effect is nongenomic, as demonstrated by the lack of efficacy of RNA/protein synthesis inhibitors. Furthermore, steroid responses mediated by intracellular receptor occur with a latency of 1–2 h. The fast time course of the results presented here indicates that they are not mediated by a classical genomic mechanism. Moreover, tamoxifen, a classic antiestrogen, was without action in changing 17β-estradiol responses.

The receptor responsible for the 17β-estradiol effect should be located at the plasma membrane. E-BSA, which does not cross plasma membrane, mimics 17β-estradiol actions both on calcium signals and K_{ATP} channel activity. A crucial issue is the mechanism by which 17β-estradiol is exerting its effect, and several lines of evidence are consistent with the generation of an intracellular second messenger. First, there is a time delay between application of the steroid and changes in $[Ca^{2+}]_i$ or channel activity. Second, 17β-estradiol has no effect in excised patches. The reduction of K_{ATP} channel activity elicited by 17β-estradiol is also a receptor-mediated process distinct from the more direct inhibitory action of sulfonylureas on K_{ATP} channels. The latter rules out a direct binding of 17β-estradiol to the K_{ATP} channel or to the sulfonylurea receptor, since sulfonylureas are able to block K_{ATP} channels in inside-out patches (24). We can conclude from these experiments that binding of 17β-estradiol to its receptor will activate a second-messenger system, which regulates K_{ATP} channels. This second messenger acts in synergy with other intracellular channel regulators generated by glucose metabolism, as summarized in **Fig. 5**.

The existence of a membrane receptor for estrogens is still a matter of controversy, but several works have demonstrated specific binding of 17β-estradiol (26, 27) or have detected proteins in the plasma membrane that cross-react with an antibody raised against cytosolic classical estrogen receptor (28). One of the most powerful tools of receptor identification is specific antibody recognition. Due to the absence of antibodies to membrane estrogen receptors, we have used a new and simple ligand binding assay, combined with confocal reflective microscopy and immunofluorescence. We have been able to demonstrate with these techniques a specific binding site for 17β-estradiol at the plasma membrane of acutely isolated cells.

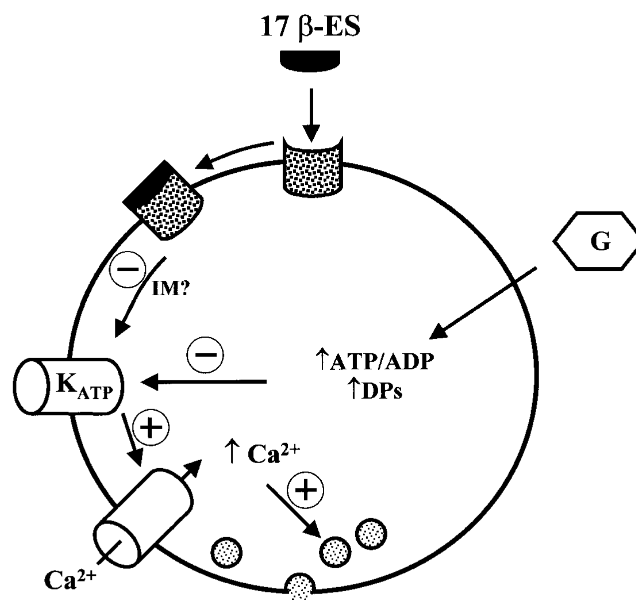

Figure 5. Proposed model for the action of 17β-estradiol on β-cell signaling system. Glucose (G) enters the pancreatic β-cell when plasma concentration is increased. Intracellular glucose metabolism raises ATP/ADP ratio and diadenosine polyphosphates (DPs) (25), which close K_{ATP} channels. The subsequent depolarization opens calcium channels, increasing $[Ca^{2+}]_i$ and promoting insulin secretion. In synergy with glucose, when 17β-estradiol (17β-ES) binds to a membrane receptor, it closes K_{ATP} channels through an unknown intracellular messenger (IM). As a consequence, calcium signal is enhanced, potentiating insulin secretion.

In the endocrine pancreas (classically a nontarget tissue), the effect of estradiol was observed only during glucose stimulation, and not in basal conditions. Such a synergistic interaction indicates that 17β-estradiol acts as a modulatory hormone: it enhances glucose responsiveness of pancreatic islet of Langerhans, yet is without effect in the absence of glucose. The effects presented on electrical activity, intracellular calcium, and insulin secretion suggest a potential role for 17β-estradiol or some of its analogs as a treatment for non-insulin-dependent diabetes mellitus (NIDDM). Since 17β-estradiol will enhance insulin secretion synergistically with a stimulatory glucose concentration, it would avoid hypoglycemia caused by sulfonylureas when taken in the absence of stimulatory glucose concentration. The insulinotropic action induced by 17β-estradiol resembles that described for other modulators such as GLP-1 (29) and is in the range of other insulin secretion modulators such as vasoactive intestinal polypeptide (30) or gastrin-releasing peptide (31). GLP-1 has been proposed

as an alternative treatment to sulfonylureas for NIDDM due to its synergistic effect with glucose. The synergistic effect of 17β-estradiol with glucose described in this work may open up the possibility of a new treatment for NIDDM based on the use of estrogens as modulators of insulin secretion via a plasma membrane receptor.

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