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Androgens Induce Increases in Intracellular Calcium Via a G Protein–Coupled Receptor in LNCaP Prostate Cancer Cells

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Abstract

The receptor mechanism of testosterone-induced nongenomic Ca^{2+} signaling in prostate cancer cells is poorly understood. In this study we investigated androgen-induced intracellular Ca^{2+} increases in LNCaP human prostate cancer cells with Fura-2 as a Ca^{2+} probe. 5α-dihydrotestosterone (DHT) produced fast and transient increases in intracellular Ca^{2+} in LNCaP cells in a concentration-dependent manner. These effects were abolished by extracellular Ca^{2+} removal or pretreatment with L-type Ca^{2+} channel inhibitors (nifedipine, verapamil, and diltiazem). Pretreatment with endoplasmic reticulum ryanodine receptor blocker (procaine) or phospholipase C inhibitor (neomycin sulfate) did not alter DHT-induced Ca^{2+} influx. The concentration of Ca^{2+} was also increased by impermeable testosterone conjugated to bovine serum albumin. Neither an antagonist of intracellular androgen receptors (cyproterone acetate) nor a protein synthesis inhibitor (cycloheximide) affected this fast Ca^{2+} influx. Furthermore, the effect of DHT was abolished in cells incubated with a G protein inhibitor (pertussis toxin) and a nonhydrolyzable analog of guanosine triphosphate (guanosine 5-[b-thio]disphosphate) but not in cells incubated with the tyrosine kinase inhibitor genistein. These results indicate that androgens induced an L-type calcium channel–dependent intracellular Ca^{2+} increase in LNCaP prostate cancer cells. The rapid responses triggered by DHT did not appear to be mediated through classic intracellular androgen receptors, c-Src kinase-

androgen receptor complex, or sex hormone–binding globulin but through a G protein–coupled receptor in LNCaP prostate cancer cells. These results may provide a new explanation for progression of prostate cancer.

Key words: 5α-Dihydrostestosterone, Ca^{2+}, GPCR

Appropriate binding of androgen to its receptor is necessary for the development and progression of prostate cancer. Testosterone, the principal steroidal androgen, and its metabolite 5α-dihydrotestosterone (DHT), are thought to mediate their biologic effects through binding to the intracellular androgen receptor (AR). AR, like other members of the nuclear receptor superfamily, functions as a ligand-inducible transcription factor. Binding of testosterone or DHT to AR induces receptor dimerization, facilitating the ability of AR to bind to its cognate response element and recruit coregulators to promote the expression of target genes.

In addition, the effect of androgens, as reported in a number of studies, is very rapid, occurring in minutes, a time lag noncompatible with the classic action of a nuclear receptor (Wehling, 1997). These findings led to the identification of nongenomic actions of testosterone through membrane androgen receptors (mAR) on cell surfaces. The nongenomic effects of testosterone include Ca^{2+} mobilization and secretion and cytoskeleton modifications (Kampa et al, 2002), regulated by the activation of signaling molecules. Androgens can induce rapid Ca^{2+} flux in a variety of cell types, including human prostate cancer cells (Steinsapir et al, 1991), rat heart myocytes (Koenig et al, 1989), male (but not female) rat osteoblasts (Lieberherr and Grosse, 1994), and mouse T cells, in which the presence of a functional classic AR could not be demonstrated (Benten et al, 1999). Also, human granulosa cells have been shown to make a Ca^{2+} response to androstenedione but not to testosterone (Machelon et al, 1998). Furthermore, a rapid Ca^{2+} influx was observed after adding high concentrations of androgens to freshly isolated immature rat Sertoli cells (Gorczynska and Handelsman, 1995).

The mechanism of the nongenomic effects of androgen varies with cell type. In murine T-cells, for example, mAR mediates ligand-induced Ca^{2+} influx through nonvoltage-gated, Ni^{2+}-blockable Ca^{2+} channels (Benten et al, 1999). In rat osteoblasts, testosterone induces both extracellular Ca^{2+} influx via voltage-gated Ca^{2+} channels and Ca^{2+} release from intracellular stores through G protein–coupled receptors (GPCR), activating phospholipase C via a pertussis toxin (PTX)–sensitive G protein (Lieberherr and Grosse, 1994). Murine macrophages of the cell line IC-21 respond to testosterone with predominantly intracellular Ca^{2+} mobilization mediated through GPCR for testosterone (Wunderlich et al, 2002). However, the receptor mechanism of testosterone-induced nongenomic Ca^{2+} signaling in prostate cancer cells is poorly understood.

In the present study we investigated the mechanism of the effect of DHT on the intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) in LNCaP human prostate cancer cells. With Fura-2 as a Ca^{2+} probe, we found that androgen caused a significant increase in $[Ca^{2+}]i$. The concentration-response relationship has been established, and the sources and mechanisms of the $[Ca^{2+}]i$ increase have been explored. We report that androgens induce Ca^{2+} influx via GPCR in LNCaP prostate cancer cells.

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Cell Culture

The LNCaP cell line was purchased from the American Type Culture Collection. Cells were grown in normal RPMI 1640 medium without phenol red supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. Cells were subcultured once a week and incubated in serum-free medium for 24 hours before experiments.

Solutions

Krebs-HEPES (pH 7.4) contained 125 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 10 mM HEPES, and 10 mM glucose. Ca^{2+}-free Krebs-HEPES contained no added Ca^{2+} plus 1 mM EGTA to chelate residual Ca^{2+}. The experimental solution contained less than 0.1% of solvent (dimethyl sulfoxide or ethanol) which did not affect $[Ca^{2+}]i$ (n = 3).

Measurement of Free $[Ca^{2+}]i$

$[Ca^{2+}]i$ was measured as described previously (Huang and Jan, 2001). Cells (10^6/mL) were planted on a cover slip, loaded with the ester form of Fura-2 (Fura-2/AM; 10 µM) for 30 minutes at 37 °C in Krebs-HEPES, and washed with Krebs-HEPES before use. Fura-2 fluorescence was imaged on an Olympus IX-70 inverted microscope with a 75-W xenon arc lamp equipped with a rotating filter wheel (Lambda 10—2; Sutter Instruments, Novato, Calif) and a cooled CCD camera, CoolSNAP-HQ, controlled by MetaFluor (Roper Scientific, Trenton, NJ). The excitation signals at 340 and 380 nm and emission signal at 510 nm were recorded at 1-second intervals. The Fura-2 signal was converted to $[Ca^{2+}]i$ using an in vitro calibration method. The relationship between $[Ca^{2+}]i$ and the ratio (R) of fluorescence intensity at 340 and 380 nm excitation is

$$[Ca^{2+}]i = K_d\beta[(R - R_{min})/(R_{max} - R)].$$

R_{min} and $_{max}$ refer to ratio values at zero $[Ca^{2+}]i$ and saturating $[Ca^{2+}]i$, respectively. Data of maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 and 20 mM EGTA sequentially at the end of each experiment. ß is the emission intensity ratio at zero $[Ca^{2+}]i$ and saturating $[Ca^{2+}]i$ at 380 nm excitation; K_d is the dissociation constant of Fura-2 for Ca^{2+} (a value of 155 nM was previously determined [Grynkiewicz et al, 1985]).

Chemical Reagents

Testosterone, verapamil, diltiazem, nifedipine, neomycin sulfate, procaine, cycloheximide, cyproterone acetate (CPA), EGTA, testosterone-bovine serum albumin (T-BSA) (29 mol steroid/mol BSA), and genistein were obtained from the Sigma Chemical Company (St Louis, Mo). PTX, guanosine 5-[ß-thio]disphosphate (GDPßS), and saponin were obtained from Calbiochem (San Diego, Calif). Dihydrotestosterone was a gift from Professor Jung (Charite Medical School, Germany). All compounds were dissolved in ethanol. Fura-2/AM (from Molecular Probes, Leiden, The Netherlands) was dissolved in DMSO.

Statistical Analyses

Data were reported as the mean ± SEM (n = 40—60). Statistical analysis was performed with SPSS 11.5 software (SPSS Inc, Chicago, Ill) and the one-way analysis of variance with the least significant difference or Student-Newman-Keuls method to evaluate the possible differences across groups. Significance was accepted when $P < .05$.

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Effect of Androgens on [Ca^{2+}]i

In normal Krebs-HEPES, DHT (1–1000 nM) increased [Ca^{2+}]i (Figure 1A and B). The basal [Ca^{2+}]i was approximately 28 to 30 nM. At a concentration of 1 nM, DHT had no effect. At a concentration of 10 nM, DHT induced a [Ca^{2+}]i increase that reached a maximum value at 25 seconds ($n = 40$; $P < .05$). At a concentration of 100 nM, DHT induced a [Ca^{2+}]i increase that reached a maximum value of 193 ± 33 nM ($n = 40$; $P < .001$) after at least 3 minutes. The response induced by 1 µM DHT was similar to that induced by 100 nM DHT. The effects of DHT (100 nM) on [Ca^{2+}]i in LNCaP cells are shown in Figure 1C, which represents a sequence of fluorescence images acquired at the times indicated. In this experiment a fast increase in the fluorescence of LNCaP cells preloaded with Fura-2/AM after hormone exposition was observed.

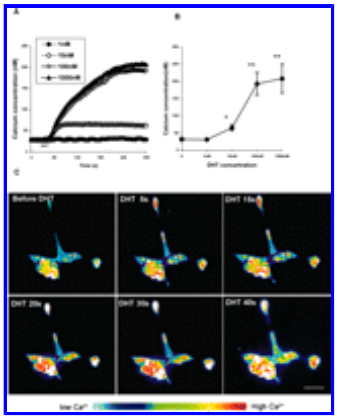

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Figure 1. Effects of 5α-dihydrotestosterone (DHT) on the intracellular concentration of Ca^{2+} ([Ca^{2+}]i) in Fura-2–loaded LNCaP cells. Concentrations of DHT were 1–1000 nM. **(A)** DHT-induced [Ca^{2+}]i increase in Ca^{2+} medium. Concentrations of DHT were 1 nM (•), 10 nM (○), 100 nM (◇), and 1 mM (▲). The black bar indicates the time of addition of DHT. **(B)** Concentration-response plots of DHT-induced [Ca^{2+}]i increase. Control is the basal [Ca^{2+}]i. The y-axis is the net maximum [Ca^{2+}]i induced by DHT at different concentrations. Data are mean ± SEM. *P < .05, **P < .001. Data are representative of at least 6 individual experiments. **(C)** Series of fluorescence ratio images, in pseudocolor, from LNCaP cells preloaded with Fura-2/AM dye. The sequence shows a fast and transient fluorescence increase after testosterone addition. Scale bar = 200 µm.

Effect of Extracellular Ca^{2+} Removal and L-Type Ca^{2+} Channel Inhibitors on Androgen Responses

The extracellular or intracellular origin of the Ca^{2+} involved in these signals was investigated using a variety of experimental conditions. First, cells were incubated in a virtually Ca^{2+}-free medium (1 mM EGTA) prior to androgen stimulation. Extracellular Ca^{2+} removal inhibited the DHT-induced [Ca^{2+}]i increase ($n = 50$; $P < .05$). The basal [Ca^{2+}]i was 9 ± 2 nM. The concentration-response relationships of DHT-induced [Ca^{2+}]i increases in the presence and absence of Ca^{2+} are shown in Figure 2A.

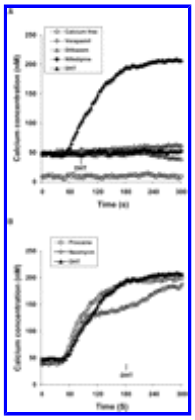

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Figure 2. Source of intracellular Ca^{2+} signal induced by androgens. **(A)** Effect of extracellular Ca^{2+} removal and inhibitors of L-type Ca^{2+} channel on 5α-dihydrotestosterone (DHT)-induced intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) increase. Preincubation of LNCaP cells in Ca^{2+}-free medium (○) for 30 seconds abolished the $[Ca^{2+}]i$ increase after stimulation with 1 mM DHT. Pretreatment with the L-type calcium channel inhibitors, ^{50} µM verapamil (♢), 100 µM diltiazem (△), or 1 mM nifedipine (•), for 5 minutes also blocked $[Ca^{2+}]i$ increases. The black bar indicates the time of addition of DHT. **(B)** Incubation of cells with 1000 nM neomycin sulfate (♢; phospholipase C inhibitor) for 5 minutes or 50 mM procaine (○; ryanodine receptor blocker) for 3 minutes did not affect the Ca^{2+} signal. The inhibitors remained present in the superfusion medium during the stimulation phase. The black bar indicates the time of addition of DHT.

Furthermore, pretreatment with nifedipine (5 mM), verapamil (50 µM), and diltiazem (100 µM), inhibitors of L-type voltage-gated Ca^{2+} channels for 5 minutes and addition of 1000 nM DHT did not increase $[Ca^{2+}]i$ (Figure 2A), indicating that androgen-induced Ca^{2+} increases originated from an extracellular source through L-type voltage-gated calcium channels.

Intracellular Ca^{2+} Stores Are Not Involved in Androgen Responses

The contribution of the Ca^{2+} stores in the endoplasmic reticulum was examined. Procaine is an inhibitor of ryanodine receptor, which was shown to release endoplasmic reticulum Ca^{2+} in cells. Pretreatment with this drug did not modify the Ca^{2+} increase induced by DHT. Figure 2B shows that pretreatment with 50 mM procaine for 3 minutes and addition of DHT induced an immediate increase in $[Ca^{2+}]i$ with a peak value of 205 ± 44 nM (n = 45; P < .001).

Experiments were performed to examine whether androgens released Ca^{2+} via stimulating inositol 1,4,5-trisphosphate formation by exploring the inhibitory effect of phospholipase C on androgen-induced $[Ca^{2+}]i$ increases. Figure 2B shows that pretreatment with 1 mM neomycin sulfate, a phospholipase C inhibitor, for 5 minutes and addition of DHT (1000 nM) induced an immediate $[Ca^{2+}]i$ increase with a peak value of 201 ± 91 nM indistinguishable from controls shown in Figure 1A (n = 55, P < .001), indicating that the androgen-induced Ca^{2+} increase did not originate from intracellular Ca^{2+} stores.

Androgen-Induced $[Ca^{2+}]i$ Is Mediated by a Nongenomic Mechanism

The possibility that androgens induce $[Ca^{2+}]i$ increases in a receptor-dependent manner was examined. If the intracellular AR is responsible for the androgen-triggered Ca^{2+} increase in LNCaP cells, this increase should be blocked by CPA, an antagonist of the intracellular AR. CPA has been shown to block genomic activation in a number of cell systems. However, the $[Ca^{2+}]i$ transiently triggered by DHT (n = 60) was not affected by pretreating the cells with a high concentration (1 µM) of CPA for 30 minutes (Figure 3A). Furthermore, pretreatment with 10 µM cycloheximide, an inhibitor of protein synthesis, for 3 hours did not affect $[Ca^{2+}]i$ transiently triggered by DHT, suggesting that the effect of the hormone was mediated by a nongenomic mechanism.

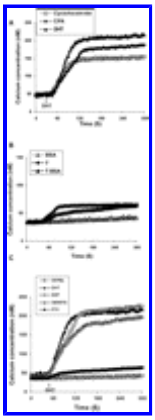

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Figure 3. The mechanism of androgen-induced Ca^{2+} release. **(A)** The effect of cyproterone acetate (CPA), an antagonist of intracellular androgen receptor, and cycloheximide, a protein synthesis inhibitor, on the response of LNCaP cells to 1 µM 5α-dihydrotestosterone (DHT). Cells were incubated for 3 hours with 10 µM cycloheximide and then stimulated with DHT (□; 1 µM). Cycloheximide did not affect the Ca^{2+} increases induced by the hormone. Cells were incubated for 20 minutes with 1 µM CPA and then stimulated with DHT (♦). The use of CPA did not modify the Ca^{2+} increases produced by the hormone. The black bar indicates the time of addition of DHT. **(B)** Testosterone-induced rise in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) of LNCaP cells. 10 nM testosterone (♦) elicited an immediate $[Ca^{2+}]i$ increase in LNCaP cells. 10 nM T-BSA also increased $[Ca^{2+}]i$ (▲), while BSA alone (△) was ineffective. **(C)** Effects of genistein (a tyrosine kinase inhibitor), GDPßS, and PTX on testosterone-induced $[Ca^{2+}]i$ increases. Cells were incubated with 100 ng/mL PTX for 24 hours and then stimulated with DHT (•; 1 µM). PTX blocked the Ca^{2+} increases induced by the hormone. Cells were permeabilized with saponin and stimulated with DHT (○). It is noteworthy that under these conditions the cells did not lose the capacity to respond to the hormone. Nevertheless, permeabilization in the presence of GDPßS (500 nM) blocked the DHT-induced Ca^{2+} increases (□). Cells were incubated for 20 minutes with 100 µM genistein and then stimulated with DHT (△). The use of genistein did not modify the Ca^{2+} increases produced by the hormone. The black bar indicates the time of addition of DHT.

Rapid testosterone effects (within the first minute) involving second messengers have been reported in other cell types, and nongenomic mechanisms of signal transduction have been proposed. Testosterone is an analog of DHT; it mimics the effects of DHT, suggesting that $[Ca^{2+}]i$ increase is a common pathway for androgen steroid action in LNCaP prostate cancer cells. To evaluate whether the effect of the hormone was mediated by extracellular membrane receptors, we tested the effect of testosterone covalently bound to bovine serum albumin (T-BSA). This compound does not cross the plasma membrane nor has it been reported to act on intracellular ARs. T-BSA produced $[Ca^{2+}]i$ increases in LNCaP cells. BSA (0.1%) did not produce any change in the $[Ca^{2+}]i$ (Figure 3B).

Androgen Stimulates Intracellular Ca^{2+} Release via GPCR in LNCaP Prostate Cancer Cells

To determine the early events involved in the Ca^{2+} signal produced by DHT, LNCaP cells were incubated for 20 minutes with 100 µM genistein, a tyrosine kinase inhibitor, prior to hormone stimulation. This inhibitor did not modify the DHT-induced $[Ca^{2+}]i$ increases (Figure 3C). A role for a G protein in this effect was evaluated. LNCaP cells were permeabilized for 5 minutes with saponin in the presence of 500 µM GDPßS, a nonhydrolyzable analog of guanosine triphosphate. Permeabilization did not modify DHT-induced responses, while GDPßS suppressed the Ca^{2+} increases induced by the hormone. Furthermore, cells were incubated with 100 ng/mL PTX before DHT stimulation. Figure 3C shows that PTX inhibited the Ca^{2+} signals produced by the hormone. These results suggest that androgen action requires a PTX-sensitive G protein to produce Ca^{2+} increases in LNCaP prostate cancer cells.

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This study examined the mechanism of androgen-induced rapid Ca^{2+} increase in LNCaP human prostate cancer cells. The results suggest that DHT and testosterone induced rapid $[Ca^{2+}]i$ increases at 10 nM. Since the plasma concentration of testosterone was between 12 and 27 nM, our results suggest that the clinical plasma level of testosterone may alter Ca^{2+} signaling in patients' prostate cancer cells. We show here suggestive evidence of a G protein–linked membrane receptor activated by androgens in LNCaP prostate cancer cells. Activation of this receptor resulted in transient $[Ca^{2+}]i$ levels, which appear to depend on L-type Ca^{2+} channels in cell membranes. Furthermore, CPA, an antagonist of intracellular ARs, did not inhibit increases in androgen-induced Ca^{2+} influx. Moreover, testosterone bound to a large protein molecule (T-BSA) mimics these effects. These results suggest that the rapid responses triggered by DHT were not due to activation of the classic intracellular AR in LNCaP prostate cancer cells but rather through a G protein–linked membrane receptor.

Testosterone has been reported to induce $[Ca^{2+}]i$ increases in rat osteoblasts and myotubes, mice splenic T cells and macrophages, and human prostate cancer cells. Consistent with the previous study (Steinsapir et al, 1991), we found that DHT induced $[Ca^{2+}]i$ increases in LNCaP cells by using Fura-2 as a Ca^{2+} probe. Moreover, we have produced a series of fluorescence ratio images, in pseudocolor, from LNCaP cells preloaded with Fura-2/AM dye. The sequence showed a fast and transient fluorescence increase after testosterone addition. It has been reported that sustained elevation of $[Ca^{2+}]i$ with Ca^{2+} ionophores or inhibitors of Ca^{2+}-ATPase reduce AR expression (Gong et al, 1995) and promote apoptosis in prostate cancer cells (Tombal et al, 2000). The effect of physiologic exposure to androgen on Ca^{2+}-mediated functions remains to be investigated.

Another question is how DHT induces Ca^{2+} increases. The source of Ca^{2+} increase varies with cell type. In murine T cells, for example, mAR mediates ligand-induced Ca^{2+} influx through nonvoltage-gated, Ni^{2+}-blockable Ca^{2+} channels. In rat osteoblasts, testosterone induces both the influx of extracellular Ca^{2+} via voltage-gated Ca^{2+} channels and Ca^{2+} release from intracellular stores through GPCR, thereby activating phospholipase C via a PTX-sensitive G protein. Murine macrophages of the cell line IC-21 respond to testosterone with predominantly intracellular Ca^{2+} mobilization mediated through GPCR for testosterone. Our results suggest that the effects of androgen on LNCaP cells were abolished by extracellular Ca^{2+} removal or pretreatment with L-type Ca^{2+} channel inhibitors (nifedipine, verapamil, and diltiazem). These results are consistent with the finding of the previous study that androgens may directly cause Ca^{2+} entry through L-type calcium channels in LNCaP cells (Steinsapir et al, 1991). Furthermore, we found that pretreatment with endoplasmic reticulum ryanodine receptor blocker (procaine) or phospholipase C inhibitor (neomycin sulfate) did not alter the DHT-induced Ca^{2+} influx. These results support the possibility that androgens may cause Ca^{2+} increase in a manner dissociated from Ca^{2+} store depletion and intracellular Ca^{2+} stores activation.

It has not yet been determined whether the nongenomic effects of androgen are mediated through a novel mAR or through a c-Src kinase-AR complex. AR has been found to interact with the intracellular tyrosine kinase c-Src, triggering c-Src activation. The tyrosine kinase activity of c-Src is autoinhibited by the interaction between the tyrosine kinase domain and the Src homology 2 and Src homology 3 (SH3) domains. In response to DHT or the synthetic androgen R1881, AR interacts with the SH3 domain of c-Src. The association of AR with c-Src results in stimulation of c-Src kinase activity within 1 minute in the AR-positive LNCaP prostate cancer cell line in response to 10 nM R1881. The rapidity of c-Src kinase activation suggests that R1881 stimulates the c-Src pathway through a nongenomic mechanism (Heinlein and Chang, 2002). In the present study we found that a membrane-impermeant testosterone conjugate (T-BSA) induced similar effects on Ca^{2+} compared with the

free hormone, excluding the possibility that the rapid increase in Ca^{2+} due to androgen in LNCaP cells was mediated through the intracellular c-Src kinase-AR complex. Furthermore, we found that androgen-induced Ca^{2+} influx was not blunted by CPA, an antagonist of the intracellular AR, suggesting that these rapid effects are not mediated by the actions of classic intracellular AR but by a transmembrane AR.

The nongenomic action of androgen has been reported to be mediated by activation of tyrosine kinase receptors as well as GPCR. It was reported that an androgen analog, antiandrogen hydroxyflutamide, exerted a non-AR–mediated and nongenomic action in AR-negative prostate cancer cells through the epidermal growth factor receptor, a tyrosine kinase receptor (Lee et al, 2002). Yet in the present study, treatment of LNCaP cells with genistein, a tyrosine kinase inhibitor, did not modify the $[Ca^{2+}]i$ increase induced by testosterone. On the other hand, several lines of evidence demonstrate that androgens can activate PTX-sensitive G proteins. To determine if testosterone interacts with GPCR in LNCaP cells, we evaluated Ca^{2+} increases in LNCaP cells treated with GDPßS and PTX. The fact that G protein inhibitors blocked the fast effects of testosterone reinforced the idea of a membrane receptor for this hormone. Androgen binding by sex hormone–binding globulin (SHBG) could also activate the membrane SHBG receptor (SHBG-R), which has been suggested to be coupled to G proteins and to stimulate cyclic adenosine monophosphate and protein kinase A. However, SHBG-R is associated with G_S-containing G protein complexes (Rosner et al, 1999). We found that the $[Ca^{2+}]i$ increase in LNCaP cells was sensitive to PTX, which does not inhibit the G_S subfamily, suggesting that GPCR-mediating $[Ca^{2+}]i$ elevation in LNCaP cells is distinct from SHBG-R.

The existence of a novel membrane AR has been postulated by a number of authors based on the detection of specific androgen binding to plasma membranes in different cell types. Unfortunately, this putative membrane receptor has not yet been further purified or cloned, preventing a definitive characterization. A human membrane receptor for progesterone has been cloned, and a heteromeric membrane receptor for anabolic steroids has recently been isolated (Gerdes et al, 1998). The identification of distinct membrane receptors for other steroid hormones suggests that a novel membrane receptor for androgens may also exist. Moreover, a number of receptors are known to be coupled to more than one G subfamily (Gudermann et al, 1997). Therefore, it remains to be determined whether androgen action via GPCR occurs through a single receptor coupled to different G subfamilies, possibly in a tissue-specific manner, or through separate receptors each linked to specific G protein complexes.

The mechanism of the change in prostate cancer from being androgen responsive to androgen unresponsive is generally explained by clonal selection, adaptation, an alternative pathway of signal transduction, and AR involvement. Since androgen action is mediated by AR, abnormalities in AR are believed to play an important role in the progression of prostate cancer (Suzuki et al, 2003). Our experiment has put forward a new possibility that androgen may act on prostate cancer cells through a novel GPCR-dependent and AR-independent pathway. These results may provide a new explanation for the progression of prostate cancer.

To sum up, this study explored the effect of androgens on $[Ca^{2+}]i$ in hormone-sensitive human prostate cancer cells and examined the underlying mechanisms of action. The results suggest that androgens induced an L-type calcium channel–dependent $[Ca^{2+}]i$ increase in LNCaP prostate cancer cells. The rapid responses triggered by DHT did not appear to be mediated through classic intracellular AR, c-Src kinase-AR complex, or SHBG but through GPCR. The precise signal transduction pathways and effects of nongenomic action of androgens on prostate cancer cell proliferation, apoptosis, and migration remain to be further investigated.

Footnotes

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