Effect of Fatty Acids and Prostaglandin on Androgen Receptor Binding in Human Prostatic Cancer Cells

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SUMMARY: The effect of fatty acids and delta 12 prostaglandin J2 (PGJ) on androgen ([3H]R1881) binding to human prostatic cancer cytosol was investigated with glycerol density gradient analysis and dextran—charcoal assay. The binding of androgen to the prostatic cancer cytosol receptor proteins was not modulated by saturated fatty acids such as palmitic acid (C16:0) or stearic acid (C18:0). Unsaturated fatty acids (UFAs) such as palmitoleic acid (C16:1), linoleic acid (C18:2) and arachidonic acid (C20:4) showed concentration dependent inhibition on androgen binding to the receptor. The inhibitory potency of UFAs did not parallel to the number of cis double bonds and carbon; however, C20:4 had a marked inhibitory effect on the binding. PGJ at a low concentration (9.0 nM) stimulated androgen binding to the receptor up to 130% of the control value. At a higher PGJ concentration (30 nM), the androgen binding was markedly reduced. The inhibitory effect of PGJ on the androgen binding was more potent than that of UFAs. The results suggest that modulation of steroid hormone action by UFAs is a general characteristic of steroid hormone target cells regardless of species differences or malignant transformation of the cells.

INTRODUCTION
About 75% of all human prostatic cancers respond to endocrine therapy that includes orchiectomy, administration of estrogens, progestins, and antiandrogenic compounds. An explanation for the hormone sensitivity of the cancer is that nuclei and cytoplasm contain androgen receptor proteins (1, 2).
Accumulating evidence shows that fatty acids modulate the binding of the steroid hormone or thyroid hormone to its receptor in various organs of the rat (3–11). Moreover, the role of fatty acids has been emphasized in the field of oncology with the importance placed on cell multiplication through nutrition (12). Prostaglandin D2, an intermediate product in the metabolic chain designated arachidonate cascade, which originates from a UFA, arachidonic acid, shows potential antitumor activity in many malignant cell lines and tumors transplanted into nude mice (13–15).

The present study examined the effect of fatty acids or delta 12 prostaglandin J2 (PGJ), an active metabolite of prostaglandin D2 (16) on the binding of androgen ([3H]R1881) to the receptor protein in androgen–dependent serially transplantable human prostatic cancer (Honda tumor) (17) with glycerol density gradients analysis and dextran–charcoal assay.

MATERIALS AND METHODS

Tumor. The characteristics of the Honda tumor derived from metastatic carcinoma of the human prostate have been reported previously (17). The tumor has been serially transplanted in our laboratory, and it is now in the 75th passage. Serial transfer of the tumor was performed by transplanting fragments of tumor subcutaneously into the male nude mice (BALB/cJCL). All mice were maintained under pathogen–limited conditions.

Chemicals. [3H]R1881, R1881, and Aquasol–2 scintillation fluid were obtained from New England Nuclear (Boston, MA). Palmitic acid, stearic acid, palmitoleic acid, linoleic acid, arachidonic acid, triamsinolone acetate, and activated charcoal were all obtained from Sigma Chemical Co. (St. Louis, M.O.). Dextran was from Nakarai Chemical Ltd. (Kyoto, Japan). PGJ was a gift from Ono Poharmaceutical Co. (Tokyo, Japan).

Tissue Preparation. The tumor was pulverized after freezing in liquid nitrogen and homogenized 2 times in 30 sec with 30 sec cooling intervals. After filtration through nylon cloth, the homogenate was centrifuged at 800 X g for 15 min. The resultant supernatant was decanted and further homogenized with a Polytron PT homogenizer (Brinkman Westburg, NY). The homogenate was centrifuged at 10,000 rpm for 1 hr at 4°C with an ultracentrifuge (80P,
Hitachi, Hitachi, Japan). The resultant supernatant was used as a cytosol.

Glycerol density gradients. One ml of cytosol was incubated with 10 nM [3H]R1881 in TEDG buffer, containing 20 mM sodium molybdate with or without a 100-fold excess of unlabeled competing R1881. To measure androgen receptors, all assay tubes contained 5 μM of triamsinolone acetate. After overnight incubation at 0°C, 300 μl of the cytosol was layered on the linear 10 to 30% (v/v) glycerol density gradients and centrifuged at 105,000 x g for 16 hr at 4°C. Each gradient was collected from the bottom of the tubes in 3 drop fractions into 40 scintillation vials and counted for radioactivity in 4 ml of Aquasol-2 with a liquid scintillation counter (Ultrobeta; LKB, Sweden). [14C]methylated bovine serum albumin was run on a separate gradient to determine the approximate sedimentation coefficient (17). Protein was estimated by the method of Lowry (18).

Estimation of androgen binding capacity. The specific [3H]R1881 binding was estimated by dextran–charcoal (DCC) assay at 4°C. A sample of 500 μl of cytosol (3.7–4.2 mg of protein) was incubated in duplicate with 20 nM of [3H]R1881 in the presence and absence of 100–200 fold molar excess of unlabeled R1881, with 10 μl of 1 M sodium molybdate, and with 5 μl of 5 μM of TA for 18 hr at 4°C. Then the mixtures were layered on DCC pellets which were prepared from a mixture of 0.5% activated charcoal and 0.005% dextran in 1.5 ml of TEDG buffer with centrifugation for 10 min at 1,800 x g. The mixtures were stirred and incubated for 30 min at 4°C, followed by centrifugation for 10 min at 2,500 x g. The radioactivity of 100 μl of the supernatant was assayed with the same procedure described for glycerol density gradients.

RESULTS

The effect of saturated fatty acids (SFAs) or UFAs on the binding of [3H]R1881 to its receptor protein was studied by glycerol density gradient analysis. The density gradient profile of [3H]R1881 binding after coincubation of human prostatic cancer cytosol with arachidonic acid (C20:4), a UFA, showed remarkable dose–dependent inhibition at the peak of radioactivity (Fig.1). Another two UFAs, palmitoleic acid (C16:1) and linoleic acid (C18:2) also had an inhibitory effect on the androgen binding in a dose–dependent manner.
Although C18:2 weakly inhibited the binding, the rate of the inhibition was below 50% of the control value at a concentration of 150 μg/ml.

![Graph showing dpm vs fractions](image)

**Fig.1.** Glycerol density gradient analysis of the effect of arachidonic acid on androgen binding to human prostatic cancer cytosol. An aliquot of 1 ml of cytosol was incubated overnight at 0°C with 10 nM [3H]R1881 either alone (○) or in the presence of 50 μg (□), 100 μg (■), and 150 μg (○) of arachidonic acid. A sample of 300 μl of the mixture was layered on the linear 10 to 30% (V/V) glycerol density gradients. Ultracentrifugation was performed at 105,000 xg for 16 hr at 4°C. The radioactivity shown are net dpm after subtraction of nonspecific binding values.

(0.54 mM) (Fig.2). Among the UFAs examined, C20:4 was the most potent inhibitor of the androgen binding, and its effect was concentration-dependent (Fig.1, Fig.2). The inhibitory potency of the UFAs did not parallel the number of cis double bonds and carbon, since C18:2 was less active than C16:1 in its
inhibition of the binding (Fig.3).

![Bar chart showing inhibition of binding by fatty acids]  

**Fig.2.** Dose dependent inhibition by fatty acids on the peak of [3H]R1881 binding to human prostatic cancer cytosol receptor proteins. A sample of 500 µl of cytosol was incubated in duplicate with 10 nM of [3H]R1881 either alone or in the presence of increasing doses (50–150 µg) of palmitoleic acid (C16:1), linoleic acid (C18:2), and arachidonic acid (C20:4) respectively. The experimental procedures were the same as described for Fig.1. The values shown are the mean of two determinations.

The peak radioactivity shown in the glycerol density gradient profile was a slightly less than that of the control value after coincubation of the cancer cytosol with SFAs such as palmitic acid (C16:0) and stearic acid (C18:0). This was despite their high concentrations (0.59 mM and 0.53 mM respectively) in the incubation mixture with cytosol (Fig. 3, Fig.4).
PGJ had a two phase effect on [3H]R1881 binding to the receptor. A small amount of PGJ (9.0 nM) increased the androgen binding to the cytosolic receptor protein (up to 130%) followed by a decrease in the binding in proportion to the amount of PGJ added to the incubation mixtures (Fig.5).

![Bar chart showing percentage of control for different fatty acids](image)

**Fatty acid (150 µg)**

Fig.3. Comparison of inhibitory potency of fatty acids on the androgen binding to human prostatic cancer cytosol receptor proteins. All of the unsaturated fatty acids tested inhibited the binding. Inhibitory potency does not parallel the number of cis double bonds and carbon. The experimental conditions were the same as described for Fig.2.

**DISCUSSION**

Fatty acids modulate various cellular processes through the activity that induces dynamic changes in the binding of hormones to their receptors in cells and organs such as the liver, thyroid, mammary glands, uterus, and prostate of the rat (1–11). In the rat uterus and prostate, inhibition of the steroid binding to the receptor by UFAs has been shown to follow as a function of dose
increase, degree of saturation, and chain length. However, in human prostatic cancer, the inhibitory effect of UFAs did not appear to follow the order for rat tissue, since linoleic acid (C18:2) showed a less potent inhibitory effect.

Fig. 4. Glycerol density gradient analysis of the effect of stearic acid on androgen binding to human prostatic cancer cytosol receptor proteins. A sample of cytosol was incubated with 10 nM [3H]R1881 either alone (●) or in the presence of 150 µg (○) of stearic acid. The experimental conditions were the same as described for Fig.1.

on the binding than did palmitoleic acid (C16:1). When compared to the results obtained from normal rat tissue, malignant transformation of the characteristics in human prostatic cells could be related to the heterogeneous inhibitory effect of UFAs on the androgen binding. The inhibitory effect of UFAs on various steroid hormone receptors in rat tissues indicates that modulation of steroid hormone action by UFAs might be a general property of many steroid hormone target cells. The exact mechanism of this inhibition is not fully understood; however, it has been suggested that UFAs are interacting.
at a site on the receptor different from the hormone binding site, and changes in the conformation of the hormone binding site or receptor molecule could possibly reduce its affinity for the hormones (7,11).

Fig. 5. Effect of PGJ on the androgen binding in human prostatic cancer cytosol. A sample of 500 µl of cytosol was incubated with 20 nM of [3H]R1881 either alone or in the presence of increasing concentrations of PGJ (1–10 µg) for 18 hr at 4°C. The mixtures were stirred with 1.5 ml of dextran coated charcoal and incubated for 15 min at 2,800 rpm. The radioactivity of 100 µl of the supernatant was assayed by the same procedure as described for glycerol density gradients. The data shown are the mean of two determinations.

On the whole, a generality regarding these inhibitory effects of UFAs could be applied to androgen dependent human prostatic cancer. The results also indicate that steroid hormone dependency of the target cells is a fundamental property of the cells regardless of species difference and malignant transformation of the cells. However, some discrepancy in the effect of UFAs on the androgen binding in prostatic cancer cells compared to normal cells also suggest that hormone sensitivity of the cancer cells progressively changes, finally making the cells less hormone sensitive (19).

Prostaglandin D2, which is an intermediate metabolite in arachidonate cascade, is known to inhibit the growth of various cancer cell lines and tumors transplanted into nude mice (13–15). PGJ has been shown to be an active metabolite of PGD in many experimental systems (16). The androgen binding
capacity to the prostatic cancer cytosolic receptor protein was increased (up to 130%) in the presence of a small amount of PGJ (9.0 nM). The result is contradictory to the finding that a small amount of PGD is sufficient to inhibit the growth of tumor cells in cell culture systems (13). The mechanism of this increase remains to be elucidated. However, the inhibitory effect of PGJ at a concentration of over 30 nM seems more potent than that of UFAs, since approximately a tenth of the total amount of UFAs is necessary to cause nearly the same inhibitory effect on the binding. The mechanism of PGJ action could be different from that of the UFAs, since the inhibitory effect on cell growth by PGJ was observed at the cell division level (20). Conformational changes in side chain A and B of unsaturated ketonic configuration (21) has also been proposed as a factor related to the inhibition.

Fatty acids are particularly interesting because they are always subject to nutritional and metabolic variation, and the present results suggest that fatty acids may be useful nutritional agents for prevention of androgen-dependent cancer through their inhibitory effect on the androgen receptor binding.

REFERENCES