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Effect of Progestins, Androgens, Estrogens and Antiestrogens on ^{3}H-thymidine Uptake by Human Endometrial and Endosalpinx Cells In Vitro

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Summary. The present study describes the effects of 11 steroid hormones and 3 non-steroidal antiestrogens on the ^{3}H-thymidine uptake by human endometrial and endosalpinx cells in long-term cultures. The compounds were added in various concentrations ranging from 10^{-9} to 10^{-4} M. Progesterone and 19-nortestosterone caused a dose-dependent reduction of the ^{3}H-thymidine incorporation resulting in a 94–98% inhibition at a concentration of 10^{-4} M, gestonoroncaproate and d,1-norgestrel were less effective causing a 49% and 40% decrease. Antiproliferative effects were also noted after the addition of androgens (testosterone, 5α-dihydrotestosterone, 5β-dihydrotestosterone and danazol). The inhibitory effect of testosterone was equivalent to progesterone at concentrations of 10^{-4} M. The addition of estrogens (estrone, estradiol-17β and estriol) and antiestrogens (tamoxifen, N-desmethyl- and 4-OH-tamoxifen) produced a dual respone in monolayer cultures as low concentrations (10^{-9}–10^{-6} M) were associated with a slightly increased ^{3}H-thymidine incorporation while pharmacological concentrations (10^{-5}–10^{-4} M) were followed by a significant decrease. Cells originating from the endosalpinx did not respond to either estradiol-17β or progesterone. These results suggest that in contrast to endometrium, the proliferation of endosalpinx cells is independent of sex steroids.

Key words: Progestins – Androgens – Estrogens – Antiestrogens – Human endometrial cells

Introduction

The antiproliferative effect of progesterone or progestogens on estrogen stimulated human endometrium is well established. A dose-dependent reduction of ^{3}H-thymidine incorporation into human endometrial cells by progesterone was demonstrated in culture systems by Norqvist (1970a). In short-term incubation studies it was also shown that the addition of progesterone resulted in a

significant reduction of DNA- and RNA-synthesis in specimens of endometrial carcinomas (Norqvist 1970b).

The present study describes the effects of 11 steroid hormones and 3 non-steroidal antiestrogens on the ^{3}H-thymidine uptake by human endometrial cells under long-term culture conditions (Kirk and Irwin 1980; Trent et al. 1980; Eckert and Katzenellenbogen 1981). The cells were grown as monolayer cultures.

Material and Methods

Specimens of endometrial tissue were obtained by curettage on day 7 or 8 of the menstrual cycle from healthy volunteers undergoing tubal ligation. The tissue was minced with scissors and subsequently digested in 20 ml phosphate buffered saline (PBS) containing 300 Mandl units collagenase for 3 h at 37° C under gentle shaking. The cell suspension was centrifuged at 800 × g and repetitively resuspended in PBS.

Finally, the cells were transferred to 20 ml Dulbecco's minimum essential medium (DMEM) containing 10% (v/v) fetal calf serum (FCS), 8 IU bovine insulin, 2.0 mg streptomycin, 200 IU penicillin and 100 µg ascorbic acid. After 4 days the cells were grown to monolayers consisting of fibroblasts and epithelium. The monolayers were treated with trypsin (0.5% w/v) in PBS at 37° C for 3 min to obtain single cell suspensions for the experimental procedure.

For control experiments cells originating from the endosalpinx of fallopian tubes were used. Fallopian tubes were obtained during hysterectomy. The inner layer of the tubes was exposed to 0.05% (w/v) trypsin for 3 h at 37° C and then rinsed with PBS. The cells obtained were incubated in DMEM as described for endometrial cells.

All hormonal compounds were dissolved in ethanol and diluted in DMEM to achieve various concentrations ranging from 1×10^{-4} M to 1×10^{-9} M. The highest ethanol concentration did not exceed 1% (v/v) per test tube.

Samples of 100 µl of each dilution medium were distributed on a microtiter plate. Approximately 5.000 cells were added to each sample. Finally, the medium was supplemented with 100.000 dpm ^{3}H-thymidine (SA: 20.0 Ci/mmol) and incubated for 15 h at 37° C. After termination of the incubation the cells were aspirated by a Dynatech Micromash and the DNA was fixed to nitrocellulose paper. After drying of the nitrocellulose platelets radioactivity was determined in a liquid scintillation counter (BF 3000, Bertold, Wildbad, FRG).

All chemicals used were obtained from Merck, Darmstadt, FRG, and were of analytical grade.

DMEM, PBS, FCS and collagenase were purchased from Serva Chemicals, Heidelberg, FRG.

Thymidine (Methyl-^{3}H) (SA: 20.0 Ci/mmol) was obtained from NEN, Dreieich, FRG. D,1-norgestrel was a gift from Schering AG, Berlin, FRG. Tamoxifen, 4-OH-tamoxifen, and N-desmethyl-tamoxifen were obtained from Imperial Chemical Industries. For statistical analysis Student's *t*-test for impaired data was applied.

Results

The hormonal compound used were classified according to their biological effect as

1. Gestogens	Progesterone, 19-nortestosterone, gestonoroncaproate, d,1-norgestrel
2. Androgens	Testosterone, 5α-dihydrotestosterone, 5β-dihydrotestosterone, danazol
3. Estrogens	Estrone, estradiol-17β, estriol
4. Antiestrogens	Tamoxifen, N-desmethyl-tamoxifen, 4-OH-tamoxifen

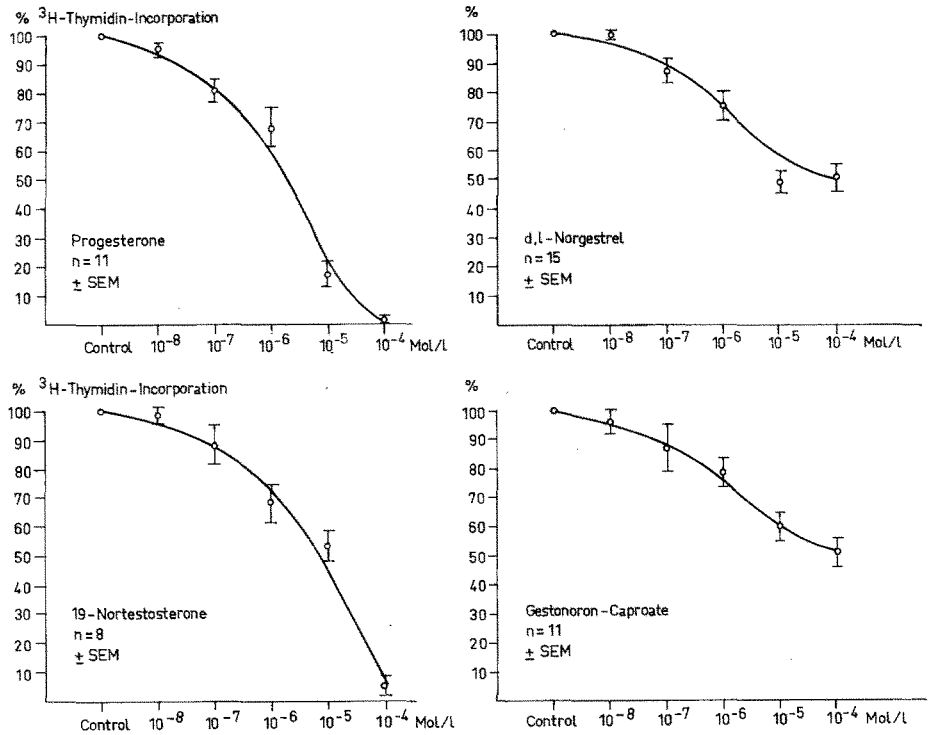

Fig. 1. Effect of various concentrations of progesterone 19-nortestosterone, d,1-norgestrel and gestonorone caproate on the uptake of ^{3}H-thymidine by human endometrial cells under long-term culture conditions

The average ^{3}H-thymidine incorporation in control experiments accounted for 2.500 dpm/5.000 cells. The addition of progesterone resulted in a dose dependent inhibition of the ^{3}H-thymidine uptake as shown in Fig. 1. A concentration of 10^{-4} M progesterone caused a 98% inhibition.

The inhibitory potency of 19-nortestosterone was almost equally effective resulting in a 94% inhibition at a concentration of 10^{-4} M. Concentrations of 10^{-4} M gestonoroncaproate and d,1-norgestrel reduced the ^{3}H-thymidine incorporation to 49% and 40% of the controls.

Significant reductions of ^{3}H-thymidine uptake were also noted after the addition of testosterone, 5α- and 5β-dihydrotestosterone at concentrations of 10^{-4} M ($P < 0.001$), as demonstrated in Fig. 2. 5β-dihydrotestosterone proved to be less effective than 5α-dihydrotestosterone at concentrations of 10^{-7} M and 10^{-6} M. At higher concentrations the inhibitory effects were identical. Addition of 10^{-4} M danazol caused a 66% reduction of the ^{3}H-thymidine incorporation being significantly less effective than testosterone ($P < 0.001$).

With the exception of 4-OH-tamoxifen estrogens and antiestrogens affected the ^{3}H-thymidine incorporation in a dual fashion as low concentrations between 10^{-9} M and 10^{-6} M were associated with an increase while higher concentrations

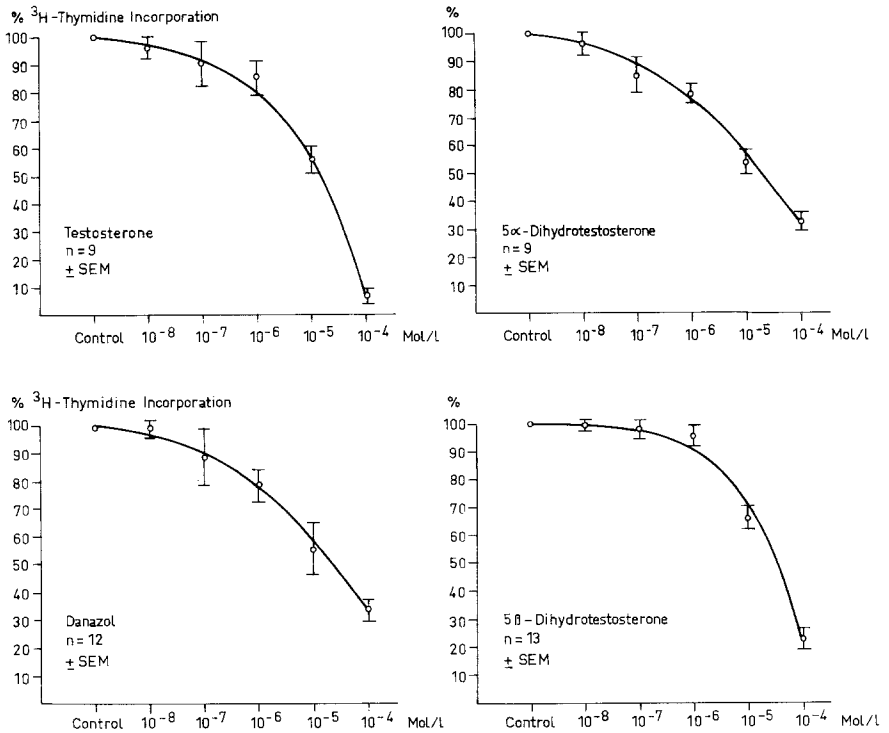

Fig. 2. Effect of various concentrations of androgens (testosterone, 5α-DHT, 5β-DHT and danazol) on the incorporation of ^{3}H-thymidine into human endometrial cells under long-term culture conditions

caused a decreased uptake of ^{3}H-thymidine, as shown in Figs. 3 and 4. The addition of 4-OH-tamoxifen resulted in a dose-dependent inhibition of the ^{3}H-thymidine incorporation exhibiting the highest potency of all antiestrogens tested. At a concentration of 10^{-4} M 4-OH-tamoxifen a 77% reduction of the ^{3}H-thymidine uptake was noted.

The average ^{3}H-thymidine uptake by endosalpinx cells accounted for 524 ± 170 dpm/5.000 cells being significantly lower than by endometrial cells ($P < 0.001$).

The uptake of radioactivity remained unaffected by either 10^{-5} M estradiol-17β (460 ± 134 dpm/5.000 cells) or by 10^{-4} M progesterone (420 ± 205 dpm/5.000 cells).

Medium without cells serving as background control contained 38 ± 7 dpm.

Discussion

The addition of 10^{-4} M progesterone or 10^{-4} M 19-nortestosterone to the culture medium is associated with an almost complete inhibition of ^{3}H-thymidine incorporation into the endometrial cells indicating a strong antiprolifera-

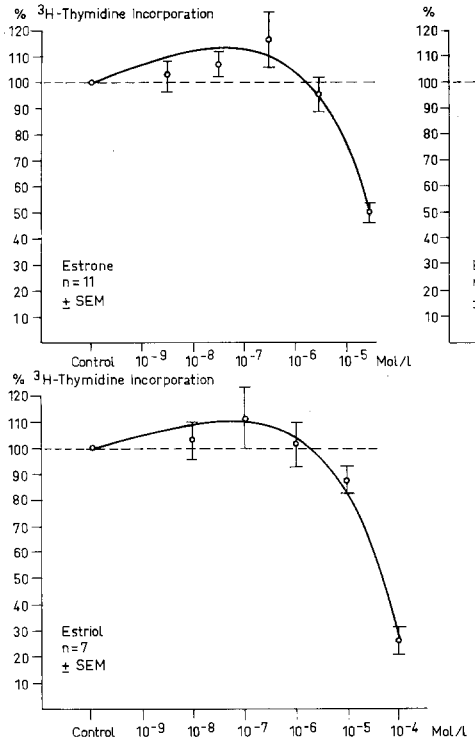

Fig. 3. Effect of estrogens in various concentrations (estrone, estradiol-17β, estriol) on the incorporation of ^{3}H-thymidine into human endometrial cells under long-term culture conditions

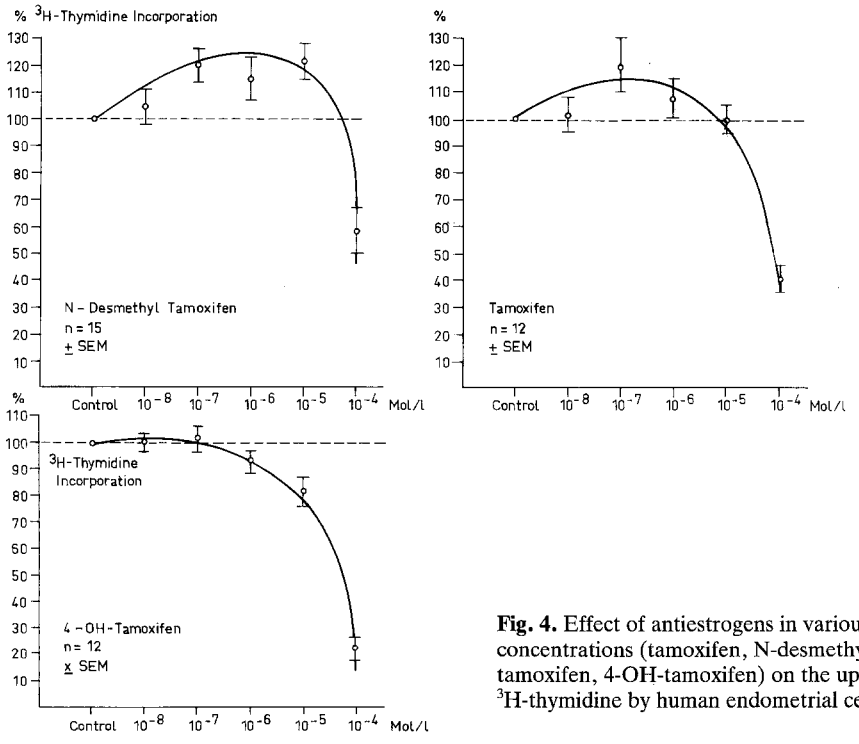

Fig. 4. Effect of antiestrogens in various concentrations (tamoxifen, N-desmethyl-tamoxifen, 4-OH-tamoxifen) on the uptake of ^{3}H-thymidine by human endometrial cells

tive effect. The antiproliferative effect of progestogens and androgens is clinically applied in the management of endometriosis and endometrial cancer (Norqvist 1970b; Darwich 1978).

D,1-norgestrel and gestonoron-caproate are known as highly active gestogenic agents as they bind with high affinity and specificity to the progesterone receptor (McGuire et al. 1974). However, the inhibitory effect on thymidine incorporation was less pronounced when compared to progesterone and 19-nortestosterone. Norgestrel is rapidly metabolized by human endometrium to biologically inactive compounds as shown in in-vitro-studies by Uniyal et al. (1977). Within 2 h 48–51% of the added norgestrel was converted to inactive metabolites. This rapid local metabolism may explain the reduced antiproliferative effect.

Androgens have been shown to bind to progesterone receptors (Chamnese et al. 1980; Kokko et al. 1982; Kokko 1983). The affinity of testosterone to the progesterone receptor was found to be in the order of 8% (Barile et al. 1979). Displacement of progesterone from its receptor has also been reported for danazol (Chamnese et al. 1980; Kokko et al. 1982; Kokko, 1983). The antiproliferative effect of danazol on human endometrium is well documented (Dmowski et al. 1971; Wentz et al. 1976; Barbieri et al. 1979; Dmowski 1979; Kokko et al. 1982). At a concentration of 10^{-4} M danazol reduced the ^{3}H-thymidine uptake to 35% of the controls. Similar effects were achieved by 5×10^{-6} M testosterone and 5×10^{-7} M progesterone. It is still an unsolved question whether androgens cause their antiproliferative effect via a specific androgen receptor or by competing with the progesterone receptor.

Friberg et al. (1978) provided evidence for the existence of DHT receptors in human endometrial cancer tissue. It has also been suggested that androgens compete with estradiol for binding sites in human endometrium (Kreitmann and Bayard 1979). Specific binding sites for DHT, progesterone and cortisol have been described in calf uterine tissue (Wagner et al. 1972).

Estrogens affect the ^{3}H-thymidine uptake in a biphasic fashion. Low concentrations cause a slight increase in ^{3}H-thymidine incorporation. Since the cell divisions in the culture systems are not synchronized (Paul 1980) only cells in the sensitive phase will respond to the hormonal stimulus. The desynchronization of the monolayer cultures may explain the moderate stimulating effect of physiological estrogen concentrations.

In physiological concentrations in the range of 10^{-7} M to 10^{-6} M estrone and estriol as well as estradiol-17β tend to increase the ^{3}H-thymidine uptake, in pharmacological concentrations, however, all 3 estrogens cause a significant reduction of the ^{3}H-thymidine incorporation. Similar inhibitory effects on the mitogenic activity in mouse uterine epithelium have been described by Lee (1980). Stimulatory and inhibitory effects of estrogens on uterine cell division as reported by Mukku et al. (1981) are well in accordance with the present data and correspond to the findings of Stormshak et al. (1976) and Kirkland et al. (1979). Mukku et al. (1982) described an initial increase of uterine DNA synthesis 24 h after a single E_2-administration, a decrease in DNA-synthesis was noted in response to a second E_2-injection when given 15–18 h after the first. Similar results were presented by Horwitz and McGuire (1978) indicating that a

nuclear processing of the estradiol receptor is required to obtain an estrogen related response.

Estrogenic potency can also be quantitated by determining the receptor affinity. According to data of Eckert and Katzenellenbogen (1981) the estrogenic potency of estrone was calculated 28%, of estriol 12% as compared to estradiol-17β being 100%. Pharmacological concentrations of several steroids and antiestrogens resulted in all experiments in a reduction of ^{3}H-thymidine uptake by endometrial cells suggesting an antiproliferative effect.

Tamoxifen and its active D-desmethyl- and 4-OH-derivatives cause in low concentrations an increase in ^{3}H-thymidine uptake. At concentrations of 10^{-4} M a significant inhibition of the ^{3}H-thymidine incorporation is noted. The strongest inhibitory effect is observed after the addition of 4-OH-tamoxifen.

Several reports have indicated that estrogenic and antiestrogenic properties of these compounds can be explained by a prolonged nuclear retention associated with a concommittant cytoplasmic receptor depletion which renders the tissue refractory to subsequent estrogen stimuli (Clark et al. 1974; Katzenellenbogen and Ferguson 1975).

The pharmacological effects of estrogens and antiestrogens are probably due to a receptor down regulation as proposed by Katzenellenbogen and Ferguson (1975) and Murai et al. (1979). In animal experiments it was clearly demonstrated that uterine growth response to estrogens was absolutely depending on the amount of cytoplasmatic estrogen receptors (Katzenellenbogen et al. 1977).

Cells originating from the endosalpinx were not affected by the addition of estradiol-17β or progesterone, despite the presence of estradiol and progesterone receptors (De Bower and Vanderkerck Klowe 1983; Devonto and Pino 1984). These findings indicate that the growth of endosalpinx cells is independent of sex steroids.

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