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Modulation of nuclear oestrogen receptor levels by oestrogen and antioestrogen

C.J. DIX & V.C. JORDAN

Department of Pharmacology, University of Leeds and Ludwig Institute for Cancer Research, Bern, Switzerland

In oestrogen target tissues oestradiol binds to cytoplasmic oestrogen receptors which translocate to the nucleus where they initiate RNA synthesis (Jensen & De Sombre, 1973). During the ensuing protein synthetic phase the cytoplasmic oestrogen receptor pool is replenished (Sarff & Gorski, 1971). Similarly non-steroidal antioestrogens translocate oestrogen receptors to the nucleus, but it has been postulated that antioestrogen-oestrogen receptor complexes inhibit the synthesis of oestrogen receptors therefore making the tissue refractory to oestrogens (Clark, Anderson & Peck, 1973). Some support for this concept has come from studies of antioestrogen action *in vitro* (Horwitz & McGuire, 1978).

If the nuclear levels of antioestrogen-oestrogen receptor complexes represent a labile pool which is being destroyed and then replaced by newly synthesized oestrogen receptors translocated from the cytoplasm, then inhibition of protein synthesis should rapidly reduce nuclear oestrogen receptor levels once this destruction or processing (Horwitz, Koseki & McGuire, 1978) is initiated.

In a preliminary experiment a dose of cycloheximide was determined that would inhibit oestradiol benzoate stimulated uterine cytoplasmic oestrogen receptor synthesis. Cycloheximide (1.25, 2.5, 5 or 10 μg in 0.1 ml saline) was administered s.c. to groups of immature rats (Alderley Park strain, 8 rats/group) every 2 h for 8 h before and 20 h after the s.c. administration of oestradiol benzoate (25 μg in 0.1 ml arachis oil). The 5 μg cycloheximide regimen was found to inhibit oestrogen receptor synthesis without causing severe toxic effects.

The antioestrogens tamoxifen (25 μg, *trans* 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenyl-but-1-ene) and monohydroxytamoxifen (25 μg 1-(4-β-dimethylaminoethoxyphenyl)-1-(4-hydroxyphenyl)-2-phenyl-but-1-ene) and the oestrogen oestradiol benzoate (25 μg) were administered s.c. to separate groups of immature rats (8 rats/group) every 12 hours. The antioestrogen experiment was repeated with the addition of the 5 μg cycloheximide regimen previously described. Groups of rats were sacrificed 2, 8, 16, 28 and 90 h after the first administration of oestrogen or antioestrogen and uterine nuclear oestrogen receptor concentrations determined using the method of Katzenellenbogen (1975). Nuclear oestrogen receptors increased reaching a maximum 8 h after monohydroxytamoxifen or oestradiol benzoate administration and 16 h after tamoxifen. For monohydroxytamoxifen and oestradiol benzoate treated groups the levels then decreased to control values by 90 h at which time the tamoxifen treated group still had elevated nuclear oestrogen receptor levels. In the presence of cycloheximide, the initial rise in nuclear oestrogen receptors after both antioestrogens was similar to that seen without cycloheximide treatment, however there was a more rapid decrease in nuclear oestrogen receptors in the cycloheximide treated groups, control levels being reached with both compounds.

The results show that antioestrogen-oestrogen receptor complexes are processed in the nucleus in a similar manner to oestrogen receptor complexes. Furthermore the rapid decrease in nuclear antioestrogen-oestrogen receptor complexes observed in the presence of cycloheximide indicates that oestrogen receptors are synthesized after antioestrogenic stimulation and that these receptors upon translocation to the nucleus contribute to the overall pattern of nuclear antioestrogen-oestrogen receptors observed after antioestrogen administration.

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Reduced antioestrogenic activity in derivatives of tamoxifen unable to undergo metabolic p-hydroxylation

K.E. ALLEN, E.R. CLARK & V.C. JORDAN

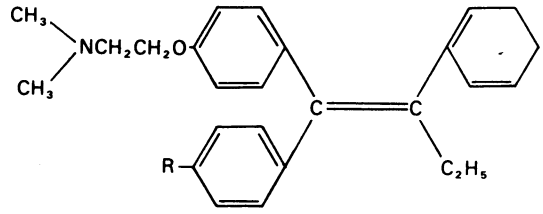
Department of Pharmacology, University of Leeds

Katzenellenbogen, Katzenellenbogen, Ferguson & Kranthammer (1978) have suggested that nonsteroidal antioestrogens are pro-drugs. In support of this concept the antioestrogen tamoxifen is metabolised in laboratory animals to the more potent monohydroxytamoxifen (Jordan, Collins, Rowsby & Prestwich, 1977). However, tamoxifen inhibits the growth of human breast cancer cells *in vitro* under conditions where metabolic transformation cannot be detected (Horwitz, Koseki & McGuire, 1978).

In the present study we have examined a series of tamoxifen derivatives (see Figure) for oestrogenic and antioestrogenic properties and also their ability to inhibit the binding of oestradiol-17β to the oestrogen receptor. These derivatives were designed with the aim of preventing the para-hydroxylation that occurs with tamoxifen.

In the 3 d uterine wet weight test (8 rats/group, 35–50 g, Alderley Park strain) all compounds investigated were partial oestrogen agonists. Similarly all compounds inhibited increases in uterine wet weight produced by oestradiol benzoate (0.16 µg in 0.1 ml arachis oil daily × 3 s.c.) in a dose related manner. The order of antioestrogenic potency was monohydroxytamoxifen > tamoxifen > *p* fluorotamoxifen ≡ *p* chlorotamoxifen ≡ *p* methyl tamoxifen.

In contrast tamoxifen was approximately equiactive with its *p* methyl, *p*-fluoro and *p*-chloro derivatives in their ability to inhibit [^{3}H]-oestradiol binding to rat uterine oestrogen receptors *in vitro*. Assays were undertaken with a 30 min incubation time at 30°C. The relative binding of tamoxifen and its derivatives



R	
H	tamoxifen
OH	monohydroxytamoxifen
CH_3	*p* methyl tamoxifen
F	*p* fluoro tamoxifen
Cl	*p* chloro tamoxifen

was approximately 0.1% of that observed with oestradiol-17β. Monohydroxytamoxifen was only slightly less active than oestradiol-17β.

In conclusion these results suggest that the biological activity of tamoxifen at low doses is the net result of its own activity and that of its more potent metabolite, monohydroxytamoxifen. However the results also demonstrate that the metabolic transformation of nonsteroidal antioestrogens is only an advantage and not a requirement for antioestrogenic activity.

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