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Estrogenic control of monoamine oxidase A activity in human neuroblastoma cells expressing physiological concentrations of estrogen receptor

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Abstract

Several lines of evidence support the hypothesis of a role played by estrogens in the manifestation of affective disorders in women. The analysis of the mechanism of action of a number of antidepressant drugs clearly demonstrated the involvement of the catecholaminergic system in the etiology of these complex behavioral pathologies. The present in vitro study was therefore undertaken to investigate the presence of a functional link between estrogen and catecholamine metabolism in cells of neural origin. The model system utilized was a human neuroblastoma cell line which was obtained by stable transfection of the estrogen receptor cDNA (SK-ER3). The present study shows that in SK-ER3 activation of the estrogen receptor correlates with a marked decrease in monoamine oxidase A activity. This effect is observed following treatment with a physiological concentration of 17β-estradiol and can be blocked by the specific antagonist of the steroid receptor, ICI 182,780. Dibutyryl cyclic AMP acting, like estrogens, on the state of differentiation of SK-ER3 cells did not affect monoamine oxidase A activity. The present study provides strong evidence of a strict relationship between estrogen receptor and monoamine oxidase A activity in human cells of neural origin, thus favoring the hypothesis of an antidepressive effect of estrogens exerted via inhibition of the monoamine oxidative pathway.

Keywords: Monoamine oxidase A; Estrogen receptor; Neural cell; Catecholamine; Estrogen

1. Introduction

Mitochondrial monoamine oxidases A and B are the key enzymes for the metabolism of the cytoplasmic pool of monoamines. In the human brain, monoamine oxidase B is mainly localized in glial cells and in serotoninergic neurons, whereas monoamine oxidase A is mostly present in glia and in the nerve terminals of adrenergic neurons (Westlund et al., 1988; Saura Marti et al., 1992). It is generally believed that intraneural monoamine oxidase A has an important role in the catabolism of catecholamines and that the modulation of its activity has a crucial effect on the amount of catecholamines stored and released by synaptic vesicles (Cesura and Pletscher, 1992). This is the theoretical ground for the explanation of the effectiveness of monoamine oxidase A inhibitors in the treatment of affective disorders. In fact, these compounds are thought to cause hyperactivation of adrenergic terminals with subsequent down-regulation of specific receptors.

Since several reports are suggestive of a link between the manifestation of depressive symptoms and changes in the circulating levels of sex steroids (for a review see Keefe, 1994), the existence of a direct effect of sex hormones on the catecholamine system and in particular on the monoamine oxidase A activity could be postulated. An in vivo study (Youdim and Holzbauer, 1976), in fact, supports this view. However, the evidence of a direct relationship between estrogen receptor activation and modulation of catecholamine catabolism is still missing, partly due to the lack of appropriate cellular models in which the consequences

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of estrogen receptor activation can be conveniently examined.

Recently, a human neuroblastoma cell line expressing physiological levels of estrogen receptor was generated by stable transfection of the estrogen receptor cDNA into an estrogen receptor negative neuroblastoma cell line (SK-N-BE). In the generated cell line, named SK-ER3, the expressed hormone receptors seem to be fully functional (Ma et al., 1993a). Furthermore, exposure to 17β-estradiol causes morphological and biochemical differentiation of SK-ER3 cells towards a catecholaminergic phenotype (Ma et al., 1993b). This model therefore represents the best system currently available for the analysis of estrogen activity in cells of neural origin. The present study was undertaken with the specific aim to investigate whether the activation of estrogen receptors in SK-ER3 cells resulted in any change in monoamine oxidase A activity.

2. Materials and methods

2.1. Cell culture

The neuroblastoma cell line SK-N-BE and its subline SK-ER3, which is stably transfected with the human estrogen receptor cDNA, were grown as previously described (Ma et al., 1993a) in media deprived of steroids by charcoal stripping. It was previously demonstrated that this procedure leads to the removal of estradiol to undetectable levels (below 1 pM) (Vegeto et al., 1990). Therefore, the culture medium for these cells was composed of phenol red-free RPMI-1640 (Sigma) supplemented with 10% charcoal stripped fetal bovine serum.

All the experiments reported were done using cells between passages 36 and 40. Usually the cells were plated at a concentration of 1000 cells/cm^2, allowed to recover for 24 h and treated with the indicated compounds.

2.2. Determination of monoamine oxidase A activity

The pellets of approximately 10^7 cells were homogenized with 1 ml of phosphate buffer containing Triton X-100 (0.025%) at 4°C. Monoamine oxidase A activity was assayed by a radiometric method (Wurtman and Axelrod, 1963) upon incubation for 10 min at 37°C of homogenate aliquots corresponding to 50–100 μg protein, as previously described (Cesura et al., 1988). 200 μM [^{14}C]5-hydroxytryptamine (57.4 mCi/mmol from Amersham Int.) and 20 μM [^{14}C]phenylethylamine (50 mCi/mmol from New England Nuclear) were used as substrates for monoamine oxidase A and monoamine oxidase B, respectively. Protein content was determined by a spectrophotometric method (Bradford, 1976).

2.3. Immunoenzymatic quantification of estrogen receptor

Cytosolic extracts and immunoenzymatic quantification of the estrogen receptor were done as described previously (Maggi et al., 1989) with minor modifications. Briefly, at the end of the treatments, the culture medium was removed and the cells were trypsinized, resuspended in phosphate-buffered saline and centrifuged. The supernatants were removed and the cells were resuspended and centrifuged 2–3 times to eliminate any trace of trypsin or medium. Cells were finally resuspended in 1 ml of buffer (10 mM Tris, 1 mM EDTA, 10% glycerol and 1 mM dithiothreitol, pH 7.4) and homogenized. The homogenate was collected and centrifuged for 10 min at 10000 rpm in a Kontron A8.24 rotor; the supernatant was collected and centrifuged for 60 min at 100000 × g. The second supernatant was collected and assayed immunoenzymatically (Abbott Diagnostic Products) using a volume of cytosol of 100 μl. The protein content in cytosolic extracts was about 1–2 mg/ml. The protein content in the cytosol was assessed by the Bradford method (Bradford, 1976).

2.4. Statistical analysis

Values are expressed as means ± S.E.M. The statistical significance of the differences among various experimental groups was evaluated by two-way analysis of variance (ANOVA) with Tukey's multiple range test at 95% and 99%. The statistical significance of differences between two groups was evaluated with Student's t-test.

3. Results

In order to determine whether estrogens modulate monoamine oxidase activity in cells of neural origin, estrogen-responsive SK-ER3 cells were plated as described in the methodology section and treated with a physiological concentration (1 nM) of 17β-estradiol for 10 days. As shown in Table 1, in hormone treated cells, monoamine oxidase A activity was significantly reduced (−42%) with respect to control (untreated) cells. The same treatment did not cause any change in the SK-N-BE parental cell line not expressing the estrogen receptor. An attempt was also made to measure monoamine oxidase B activity which, if present, was below the limit of detection in both parental and estrogen receptor-transfected cells. Further experiments proved that the effect elicited in SK-ER3 by estradiol could be blocked by an estrogen receptor specific antagonist (ICI 182,780). As shown in Table 2, the presence of the estrogen receptor antagonist pre-

Table 1
Effect of 17β-estradiol on monoamine oxidase A activity in human neuroblastoma cells

	Monoamine oxidase A activity (pmol/mg protein/min)	
	Control	17β-Estradiol
SK-N-BE	633 ± 63	662 ± 44
SK-ER3	279 ± 37 [a]	154 ± 22 [b]

Cells were seeded as described in the methodological section. 24 h after plating the cells were treated with 1 nM 17β-estradiol or vehicle for 10 days. Results represent the average of four separate experiments where determinations were done in duplicate on three pools of cells from three Petri dishes each. [a] $P \leq 0.005$ with respect to SK-N-BE cells; [b] $P \leq 0.05$ with respect to SK-ER3 cells, by Student's t-test.

vented the 17β-estradiol-induced decrease in monoamine oxidase A activity.

This first set of experiments demonstrated that activation of estrogen receptors resulted in lowered monoamine oxidase A activity in SK-ER3 cells. Furthermore, the comparative analysis of monoamine oxidase A activity in untreated parental and transfected cells showed significant differences (Table 1). In SK-N-BE cells, in fact, monoamine oxidase A activity was over 2-fold higher than in the estrogen receptor-transfected SK-ER3 cells. We therefore speculated that the low levels of monoamine oxidase A activity detected in the SK-ER3 cells were the result of a (partial) state of activation of the estrogen receptor. This hypothesis was supported by a time-course experiment in which monoamine oxidase A activity was measured in SK-ER3 cells grown in the presence or absence of estrogen for 3, 6, 8 and 12 days. This study revealed an interesting pattern of monoamine oxidase A activity in control cells, which was found to increase with time in culture. Ten days after subculturing, monoamine oxidase A activity in untreated SK-ER3 cells was more than doubled with respect to that on day 3 in culture (Fig. 1). By contrast, when the cells were grown in the presence of estrogens, the levels of enzymatic activity were low for

Table 2
Monoamine oxidase A activity in SK-ER3 neuroblastoma cells following 17β-estradiol and ICI 182,780 treatment

Treatment	Monoamine oxidase A activity (pmol/mg protein/min)	% of controls
Control	236 ± 13	
17β-Estradiol (1 nM)	125 ± 6 [a]	52
ICI 182,780 (1 nM) + 17β-estradiol (1 nM)	196 ± 8	83

Results represent the average of three separate experiments where determinations were done in duplicate on 4–5 pools of cells from three separate Petri dishes. The treatments of the cells were given for 10 days. [a] $P < 0.01$ with respect to control cells and ICI 182,780 + 17β-estradiol by two-way ANOVA with Tukey's multiple range test.

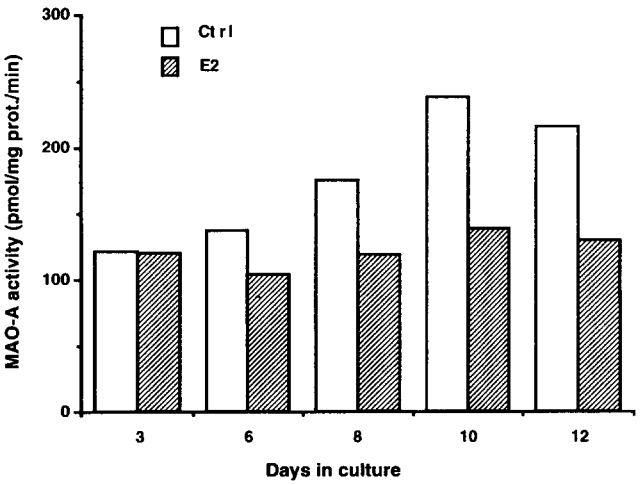

Fig. 1. Time course of the effect of 17β-estradiol on monoamine oxidase A activity in SK-ER3 cells. Cells were seeded in medium containing charcoal-stripped serum as described in the methodological section and treated with 1 nM 17β-estradiol or vehicle (0.01% ethanol) 24 h after plating. The cells were harvested at 3, 6, 8, 10 and 12 days after treatment respectively. Each bar represents the average of duplicate analysis of a pool of cells from four Petri dishes. The experiment was repeated twice with superimposable results. The experiment was also done by seeding the cells at time 0, treating them at different times and harvesting them on the same day. The results obtained were superimposable with those here reported. Empty bars: cells treated with vehicle; dashed bars: cells treated with 17β-estradiol.

the entire length of the study. A similar experiment done with SK-N-BE cells demonstrated that there was no change in monoamine oxidase A activity at any time tested (data not shown). It was therefore concluded that both estrogen-induced and time-induced changes in the activity of the catecholamine catabolic enzyme had to be attributed to the presence of the estrogen receptor.

To explain the low level of monoamine oxidase A activity found in control cells shortly after plating and its increase with time, we hypothesized the existence of a/some labile compound/s in fresh serum that was responsible for the estrogen receptor activation. To test this hypothesis, cells were plated, collected at various times following plating, and the cytosol was analyzed for the presence of estrogen receptor. It is known, in fact, that cellular fractionation studies can be utilized to analyze the state of activation of estrogen receptor: the unactive estrogen receptor is associated with the cytosolic fraction, while the activated estrogen receptor co-sediments with the nuclear pellet. The results shown in Fig. 2 revealed that, in SK-ER3 grown in the absence of estrogens, at a short time after plating (60 min) the levels of cytosolic estrogen receptor were decreased by about 70% with respect to time 0. These levels remained low for a few hours and started to increase at 12 h after subculturing. At 48 h the levels of cytosolic estrogen receptor were indistin-

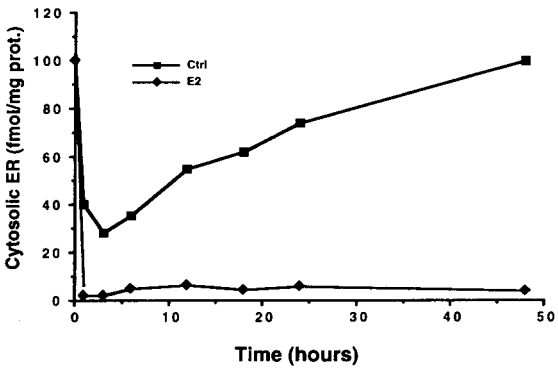

Fig. 2. Concentration of cytosolic estrogen receptor following addition of medium containing fresh serum. Cells were plated and allowed to grow to semi-confluence for about 4–5 days. The medium was then replaced and cells from separate sets of four plates each were harvested at the indicated times. The cytosolic fraction was prepared as indicated in the methodology section and the ER content was assessed by a commercial immuno-enzymatic test.

guishable from the levels at time 0. In cells treated with the hormone, the decreased concentration of cytosolic estrogen receptor was immediately manifest and remained so with time.

These results favor the hypothesis of the presence, in fresh medium, of one or more estrogen receptor activating compounds that are responsible for the disappearance of estrogen receptor from the cytosol. It is therefore conceivable that the reduced levels of monoamine oxidase A activity observed on the third day after plating of SK-ER3 cells are the consequence of the fresh medium-induced activation of the estrogen receptor. The increase in monoamine oxidase A activity observed later in the control cells is likely to reflect the decreased state of estrogen receptor activation. The persistence of the estrogen receptor in the activated state due to the addition of estradiol to the culture medium correlates with the low enzymatic activity found in the hormone-treated cells.

Previous studies done with SK-ER3 demonstrated that the morphology and the growth characteristics of this cell line are changed upon estrogen receptor activation (Ma et al., 1994). Therefore, it remained to be proven that the reduced estrogen-induced growth rate and increased state of differentiation of SK-ER3 were not the cause of the lowered monoamine oxidase A activity. In order to assess whether changes in the growth rate or in the state of differentiation could

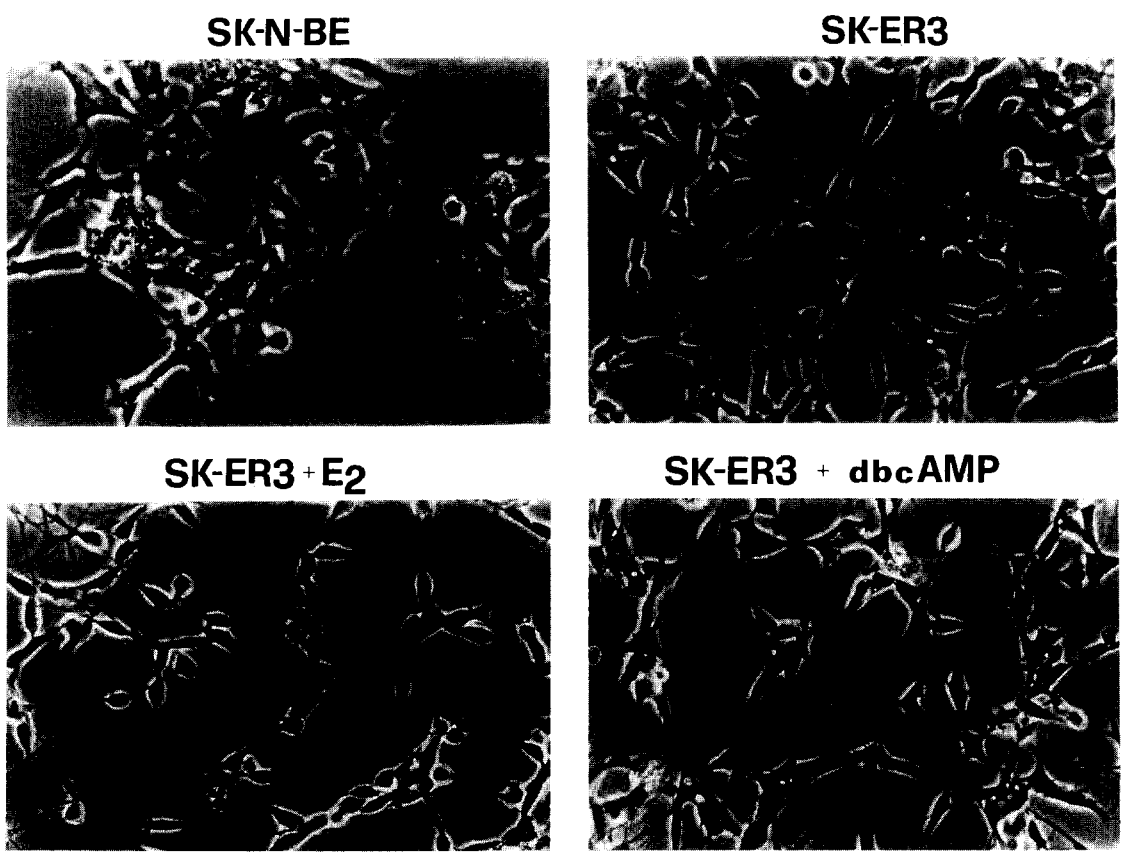

Fig. 3. Morphological differentiation of SK-ER3 cells following 17β-estradiol and dibutyryl cyclic AMP treatment. 24 h after plating, SK-ER3 cells were treated with vehicle, 1 nM 17β-estradiol (E_2) or 20 μM dibutyryl cAMP (dbcAMP). Cells were allowed to differentiate for 6 days and then photographed with phase-contrast optics. SK-N-BE parental cells were treated with vehicle (Nikon microscope 130 ×).

Table 3
Effect of 17β-estradiol and dibutyryl cyclic AMP on monoamine oxidase A activity in SK-ER3 neuroblastoma cells

Treatment	Monoamine oxidase A activity (pmol/mg protein/min)	% of controls
Control	242 ± 30	
17β-Estradiol (1 nM)	158 ± 22 [a]	65
Dibutyryl cyclic AMP (20 μM)	260 ± 28	107

Results represent the average of four separate experiments. The treatments were given for 10 days. In each experiment the determinations were done in duplicate on a minimum of three pools of cells grown in separate Petri dishes. [a] $P < 0.01$ vs. control and dibutyryl cyclic AMP by two-way ANOVA with Tukey's multiple range test.

affect monoamine oxidase A activity, SK-ER3 cells were treated with dibutyryl cyclic AMP, a compound known to induce growth arrest and differentiation of the parental cell line SK-N-BE. After dibutyryl cyclic AMP treatment, SK-ER3 cells indeed stopped growing and acquired a differentiated phenotype, similar to that observed with estradiol treatment (Fig. 3). However, this dibutyryl cyclic AMP-mediated differentiation of SK-ER3 cells did not change the activity of monoamine oxidase A (Table 3). These results, therefore, demonstrated that the state of differentiation of SK-ER3 cells is not correlated with changes in monoamine oxidase A activity.

4. Discussion

The present study shows that in the neuroblastoma cell line SK-ER3, activation of the estrogen receptor by 17β-estradiol results in a decrease in monoamine oxidase A activity. To our knowledge, this is the first demonstration of an effect of physiological concentrations of this steroid hormone on monoamine oxidase A activity in cells of neural origin. Previous reports of studies done in neuroblastoma PC12 cells indicated that 17β-estradiol can decrease monoamine oxidase A activity at concentrations as high as 1 μM (Youdim, 1991). It is known that PC12 cells synthesize estrogen receptors at very low concentrations (Sohrabi et al., 1993). This finding might explain the necessity of such a high concentration of the hormone to activate the receptor and to enable it to affect monoamine oxidase A activity.

The experiments reported underline the difficulties in studying the effects of estrogen on neuroblastoma cells due to the presence of serum factors that activate the estrogen receptor in the absence of the cognate hormone. This is in agreement with previous reports from other laboratories as well as our laboratory (Power et al., 1991; Aronica and Katzenellenbogen, 1993; Ma et al., 1994) which demonstrated that growth factors, also present in stripped serum, can trigger a series of intracellular events ultimately leading to the activation of ligand-free steroid receptors. In particular we demonstrated that, in SK-ER3 cells, insulin-like growth factor I and insulin-like growth factor II can activate the ligand-free estrogen receptor, most likely by activating a series of kinases which change the transcriptional activity of the estrogen receptor (Ma et al., 1994). The demonstration of the disappearance of the estrogen receptor from the cytosolic fraction of estrogen receptor-expressing cells shortly after plating further substantiates the above-mentioned studies.

What is the significance of the present set of experiments? Estrogens are known to have a profound influence on neural cells at different stages of mammalian life. During the embryonal/perinatal life of mammals, estrogens (resulting from the aromatase-mediated conversion of gonadal steroids) are instrumental for the sexual differentiation of the central nervous system (Phoenix et al., 1959). In adults, estrogens have been described to be involved in the regulation of a number of reproduction-related functions and behaviors (Metren et al., 1987). In adult humans, in particular in women, estrogens have been described as affecting mood in a positive manner, and the onset of selected types of depression (e.g. post-partum and post-menopausal depression, pre-menstrual tension) has been associated with a decrease in circulating estrogens (Maggi and Perez, 1985). The results presented here could lead us to hypothesize that the effect of estrogens on mood disorders in the adult brain is linked to a decreased catabolism of brain catecholamines. The effect of estrogens would therefore be similar to the effect of antidepressant monoamine oxidase A inhibitors.

It could be argued that the findings obtained with neuroblastoma cells are mostly representative of an effect of estrogens in immature cells and cannot be extrapolated to those occurring in the mature brain. At the present state of knowledge, it cannot be proven whether these observations for the cellular system used in the present study are more representative of events occurring in maturing than in fully differentiated neural cells, or vice versa. In this respect, it is worth mentioning that several reports suggest that monoamine oxidase A participates in the events leading to the sexual differentiation of the brain (Gonzales and Leret, 1992) and point mutations of monoamine oxidase A gene have been associated with the development of selected types of aggressive behaviors (Rastegar et al., 1993). Also in the adult rat brain monoamine oxidase A, in concert with sex steroids, has been reported to play an important role in the manifestation of sex-related behaviors (Brunner et al., 1993). These studies are therefore suggestive of the fact that the two systems are intertwined regardless the state of differentiation of the neural cells. It is conceivable that at

least some of the mechanisms exploited by estrogens for the regulation of neural cells (or neural circuitries) at an early time during ontogenesis are the same as those utilized by mature, fully differentiated cells.

The study here presented provides evidence of a close functional connection between 17β-estradiol and the metabolism of biogenic amines. The importance of this finding with regard to the clarification of the positive effect of this sex hormone on mood needs to be further analyzed.

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