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In vivo occupancy of female rat brain estrogen receptors by 17β-estradiol and tamoxifen

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Estrogens or antiestrogens are currently used by millions of women, but the interaction of these hormonal agents with brain estrogen receptors (ER) in vivo has not been characterized to date. Our goal was to assess, in vivo, the extent and regional distribution of brain ER occupancy in rats chronically exposed to 17β-estradiol (E_2) or tamoxifen (TAM). For that purpose, female ovariectomized Sprague–Dawley rats were implanted with subcutaneous pellets containing either placebo (OVX), E_2, or TAM for 3 weeks. ER occupancy in grossly dissected regions was quantified with 16α-[^{18}F]fluoroestradiol ([^{18}F]FES). Both E_2 and TAM produced significant decreases in radioligand uptake in the brain although the effect of E_2 was larger and more widespread than the effect of TAM. Detailed regional analysis of the interaction was then undertaken using a radioiodinated ligand, 11β-methoxy-16α-[^{125}I]iodo-estradiol ([^{125}I]MIE$_2$), and quantitative ex vivo autoradiography. E_2 treatment resulted in near-complete (86.6 ± 17.5%) inhibition of radioligand accumulation throughout the brain, while ER occupancy in the TAM group showed a marked regional distribution such that percentage inhibition ranged from 40.5 ± 15.6 in the ventrolateral part of the ventromedial hypothalamic nucleus to 84.6 ± 4.5 in the cortical amygdala. These results show that exposure to pharmacologically relevant levels of TAM produces a variable, region-specific pattern of brain ER occupancy, which may be influenced by the regional proportion of ER receptor subtypes. These findings may partially explain the highly variable and region-specific effects observed in neurochemical, metabolic, and functional studies of the effects of TAM in the brain of experimental animals as well as human subjects.

Keywords: SERMs; 16α-[^{18}F]fluoroestradiol; 11β-Methoxy-16α-[^{125}I]iodo-estradiol; Autoradiography

Introduction

Estrogens, like all steroids, easily diffuse through the blood–brain barrier to occupy estrogen receptors localized in specific brain regions in rodents and humans (Laflamme et al., 1998; Loy et al., 1998; Österlund et al., 1998; Pfaff and Keiner, 1973; Shughrue et al., 1997). There are at least three estrogen receptor (ER) subtypes represented in the brain, namely, ERα, ERβ, and membranal ER (ER-x or mER), exhibiting sub-type-specific regional distribution and presumably subserving different functions (Deecher et al., 2003; Kuiper et al., 1996; Pietras and Szego, 1977; Shughrue et al., 1997; Toran-Allerand et al., 2002; Tremblay et al., 1997). ER are believed to play distinct roles in high-order cognitive functions (Drake et al., 2000; Krug et al., 2003), mood (Carranza-Lira and Valentino-Figueroa, 1999; Österlund and Hurd, 2001), neuroprotection (Azcoitia et al., 1998; Behl, 2002; Dubal et al., 1998; Sawada et al., 2002; Weaver et al., 1997), and modulation of neurotransmitter receptors and transporters (Biegon and McEwen, 1982; Cyr et al., 2001; Kelly et al., 1992; Lagrange et al., 1997; Pecins-Thompson et al., 1998). Menopause is characterized by a drop in circulating estrogen levels and is associated with increased incidence of hot flushes, depression, and specific cognitive deficits. These effects of estrogen deprivation can be reversed with estrogen replacement therapy (Kimura, 1995; Sherwin, 1997).

Tamoxifen (TAM) is a useful adjuvant and prophylactic agent in the treatment and prevention of human breast cancer. It is the best known representative of the class of agents known as selective estrogen receptor modulators (SERMs), which have tissue-dependent agonistic or antagonistic activities at ER (Jordan, 2003; Jordan and Morrow, 1999; Turgeon et al., 2004). TAM was shown to cross the blood–brain barrier and accumulate in rat brain following a single injection (e.g., Biegon et al., 1996). Hot flushes are the most common side effect of TAM; observed in women as well as men and supporting an antagonist action of the drug at human brain ER (Anelli et al., 1994; Jordan, 2003; Jordan and Morrow, 1999). However, studies on the effect of TAM on brain metabolism and cognitive function report conflicting results: While some studies report impairment of cognitive functions and metabolism (Eberling

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et al., 2004; Shilling et al., 2003), other studies failed to detect brain metabolic deficits in TAM users and suggest that TAM actions in the brain resemble those of estrogen, supporting an agonist-like activity of the drug in the brain (e.g., Ernst et al., 2002). These observation and comparable animal data suggest that the interaction of TAM with ER in the brain is subtype- and region-selective (Cyr et al., 2001; Kimelberg et al., 2003; Mize et al., 2003; Watanabe et al., 1997; Zhou et al., 2002). The goal of the present study was to assess the in vivo extent and regional distribution of brain ER occupancy in ovariectomized rats exposed to 17β-estradiol (E_2) or TAM at physiologically or pharmacologically relevant doses and duration, using radioactively labeled E_2 derivatives with high specific activity and documented selectivity for ER (Brown et al., 1989; Moresco et al., 1995; Mortimer et al., 1996; Zielinski et al., 1986).

Materials and methods

Animals and treatments

Twenty-four ovariectomized Sprague–Dawley female rats (6–8 weeks old, 160–180 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA, USA) and housed in the Lawrence Berkeley National Laboratory (LBNL) animal facility under controlled temperature and light conditions, with food and water available ad libitum. One week after ovariectomy, animals (n = 8/group) were implanted subcutaneously under Nembutal (40 mg/kg ip) anesthesia with blank pellets (OVX) or pellets containing E_2 (0.72 mg) or TAM (68.0 mg) (Innovation Research of America, OH, USA) as previously described (Biegon et al., 1996). Pellets were designed to release controlled levels of the hormones over a 21- to 60-day period. The E_2 pellet provides plasma estradiol levels in the high physiological range and the TAM pellet provides levels compatible with those found to be effective in breast cancer (Biegon et al., 1996). All experimental protocols were approved by the LBNL animal use committee. Animals were checked daily for signs of infection and weighed on the day of pellet implantation and every 7 days thereafter until the end of the 21 days treatment period.

Radioligands

[^{18}F]fluoroestradiol ([^{18}F]FES) and 11β-methoxy-16α-[^{125}I]iodo-estradiol ([^{125}I]MIE$_2$) were synthesized following published procedures achieving specific activities of 1000 and 2000 Ci/mmol, respectively (Lim et al., 1996; Zielinski et al., 1986). The unlabeled precursor for MIE$_2$ was a gift from Prof. R.B. Hochberg of Yale University (New Haven, CT, USA).

In vivo ER labeling

Gross regional distribution with [^{18}F]FES

Twenty-one days after pellet implantation, rats (n = 4/group) were injected in the tail vein with approximately 0.5 mCi/kg [^{18}F]FES and killed 60 min postinjection (pi). This time point was chosen based on the published kinetics of this ligand in rat brain (Moresco et al., 1995) and our own pilot study (Biegon et al., 2003). Animals were killed by halothane overdose and exsanguination. The brain was quickly removed and rinsed in ice cold saline. Using free hand dissection, the hypothalamus, preoptic area, and amygdala were removed along with approximately 50 mg samples of cerebellum and prefrontal cortex. The uterus was removed as well, trimmed of fat, and blotted dry. The blood samples obtained from exsanguination and the tissue samples were placed in preweighed vials, counted in a gamma counter (Liquid Scintillator Analyzer 2500TR, Packard) with decay correction, and weighed.

Total counts in each sample were divided by the corresponding weight. Brain uptake of [^{18}F]FES was calculated as the ratio of counts/g in tissue over counts/g in blood. A ratio = 1 indicates no selective retention in tissue compared to blood levels. In order to assess ER occupancy in E_2- and TAM-treated rats relative to OVX, we calculated %occupancy = 100 × (1 − [tissue/blood − 1, treat] / [tissue/blood − 1, OVX]).

Statistical analysis

The effect of chronic exposure to E_2 and TAM on uterine weight and ER occupancy was tested, respectively, by one- and two-way analysis of variance (ANOVA), followed by post hoc comparisons by Fisher's PLSD test with $\alpha < 0.05$. Statistical analysis was carried out with StatView 4.1.

Ex vivo quantitative autoradiography with [^{125}I]MIE$_2$

A second set of animals treated with E_2, TAM, or blank pellets for 21 days were injected with approximately 0.1 mCi/kg 11β-methoxy-16α-[^{125}I]-iodo-estradiol ([^{125}I]MIE$_2$) in the tail vein (n = 4 for OVX, n = 3 for E_2 and TAM, since two animals had to be sacrificed prematurely due to an infection around the pellet). Animals were killed 120 min pi (Brown et al., 1989; Zielinski et al., 1986). Brains were quickly removed, rinsed, frozen in powdered dry ice, and kept at −80°C. Frozen brains were sectioned coronally in a cryostat from the prefrontal cortex to the midbrain. Brain sections (20 μm) were obtained at a cutting temperature of −15°C and thaw mounted onto microscope glass slides. Sections were collected at 200 μm intervals and exposed to radiation sensitive film (Kodak Biomax) for 14 days. Commercially available calibrated ^{125}I standards (Amerham microscales) were included in the exposure cassettes. Films were developed by hand in Kodak D-19, fixed and dried. The sections were then stained with cresyl violet for anatomical localization.

Quantitative analysis

Films were scanned (Umax PowerLook 2100 xL) and digitized using Adobe PhotoShop. Analysis was carried out with NIH Image software. In order to get absolute values (nCi/mg), the calibration curve was determined from the iodine standards. The mean density of radioactivity was measured in manually delineated regions of interest (ROIs). ROIs were drawn bilaterally over the images, guided by the corresponding histological sections and a rat brain atlas (Paxinos and Franklin, 2001). ROIs were placed in the medial, cortical, and dorsolateral nuclei of the amygdala, the arcuate, and ventrolateral ventromedial nuclei of the hypothalamus, the bed nucleus of the stria terminalis (anterior and posterior), central gray, frontal cortex, hippocampus (dorsal and ventral), medial preoptic area, anterior paraventricular thalamus, and striatum. Radioactivity in the striatum, a region devoid of ERs, was measured to assess the contribution of nonspecific binding of radioligand. The specific binding was defined as region minus

striatum. Test–retest examination of experimenter error revealed an error of less that 2% for larger regions (e.g., striatum) and less than 10% for smaller regions (e.g., amygdaloid nuclei).

ER occupancy in E_2- and TAM-treated animals, relative to OVX, was expressed as %occupancy = 100 × (1 − [specific binding, treated] / [specific binding, OVX]).

Statistical analysis

Treatment effect on ER occupancy was tested by a two-way ANOVA, followed by post hoc comparisons by Fisher's PLSD test with $\alpha < 0.05$. Statistical analysis was carried out with StatView 4.1.

Results

Pellet validation

Body and uterine weight gain were used to validate hormone release from the pellets during the treatment period and on the day of sacrifice, respectively, due to the known effects of ER agonists on these parameters. As expected, all ovariectomized females implanted with blank pellets gained weight steadily during the three week observation period (mean gain 7 ± 2.6%, 19 ± 8.7%, and 36 ± 5% over baseline 7, 14, and 21 days after implantation, respectively). Ovariectomized females treated with E_2 or TAM did not gain any weight during the same period (overall weight change

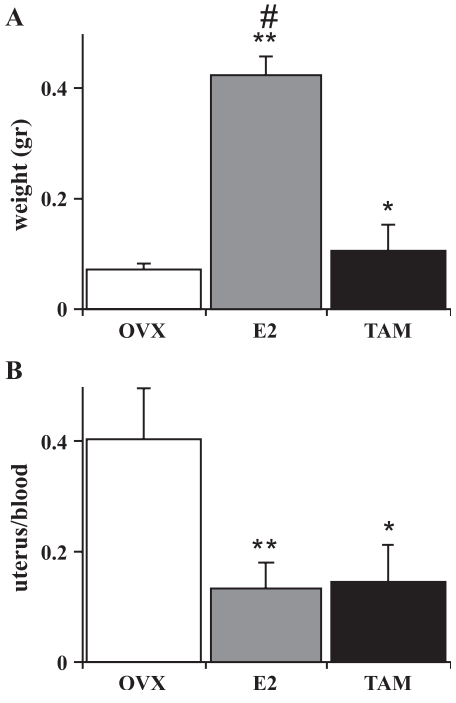

Fig. 1. Effects of E_2 and TAM on uterine weight (A) and ER occupation (B). (A) Uterine weight was significantly increased by treatment (ANOVA $P < 0.0001$). *$P < 0.005$, **$P < 0.0001$ E_2 and TAM vs. OVX; #$P < 0.0001$ E_2 vs. TAM (post hoc Fisher's PLSD). (B) ER binding, expressed as dpm/g uterus over dpm/g blood, was significantly reduced by treatment (ANOVA $P < 0.0001$). *$P = 0.0006$, **$P < 0.0004$ E_2 and TAM vs. OVX (post hoc Fisher's PLSD).

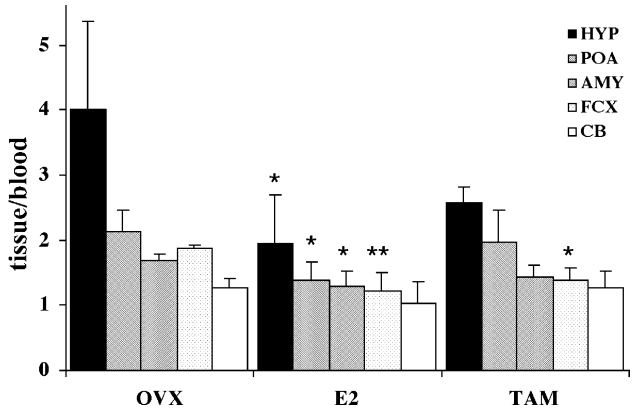

Fig. 2. Brain uptake ratios of [^{18}F]FES after E_2 and TAM treatment. Values are the mean (±SD) of four animals per group. Two-way ANOVA revealed a significant effect of treatment ($P < 0.0001$), region ($P < 0.0001$), as well as a significant interaction term ($P < 0.0001$). *$P < 0.05$, **$P < 0.005$, E_2 and TAM vs. OVX (post hoc Fisher's PLSD). Abbreviations: hypothalamus (HYP), preoptic area (POA), amygdala (AMY), frontal cortex (FCX), and cerebellum (CB).

−1.6 ± 4% to +0.5 ± 6% in E_2- and TAM-treated rats, respectively).

Mean uterus weight differed significantly between groups (ANOVA $P < 0.0001$; Fig. 1A.). E_2 produced a very large increase in uterine weight relative to OVX (502%, post hoc $P < 0.0001$), and TAM produced a relatively modest but statistically significant increase (49%, post hoc $P = 0.002$), as previously observed and expected from its mixed agonist–antagonist profile in this organ (Biegon et al., 1996). Uterine weight of E_2-treated rats was also significantly higher than for the TAM group (304%, post hoc $P < 0.0001$). Treatment with E_2 and TAM resulted in a highly significant decrease in uterine ligand uptake, indicating a significant >50% occupation of ER by both agents (ANOVA $P < 0.0001$; Fig. 1B.).

Effects of E_2 and TAM on ER in dissected brain regions

The regional distribution of the radioligand following in vivo administration in ovariectomized rats followed the known pattern of brain ER, with the highest tissue to blood ratio found in the ER-rich hypothalamus (Fig. 2). This ratio was strongly and significantly reduced in E_2-treated rats, while TAM produced smaller and region selective decreases (Fig. 2). In the E_2 group, ER occupation ranged from 60% to 84%; while in TAM rats, occupation varied from 14% to 55% (Table 1). The differences in inhibition patterns

Table 1
Reduction of brain FES uptake in estradiol- and tamoxifen-treated animals measured in grossly dissected regions

Region	E_2	TAM
Frontal cortex	75.7 ± 33.0	55.5 ± 19.8
Hypothalamus	68.8 ± 25.2	47.7 ± 8.4
Amygdala	59.2 ± 35.4	38.0 ± 28.7
Preoptic area	84.1 ± 10.6	14.3 ± 43.4

Results are expressed as mean% reduction of uptake compared to ovariectomized rats implanted with blank pellets and represent mean ± SD of four animals per treatment. Two-way ANOVA by treatment and region reveals a significant effect of treatment ($P = 0.01$).

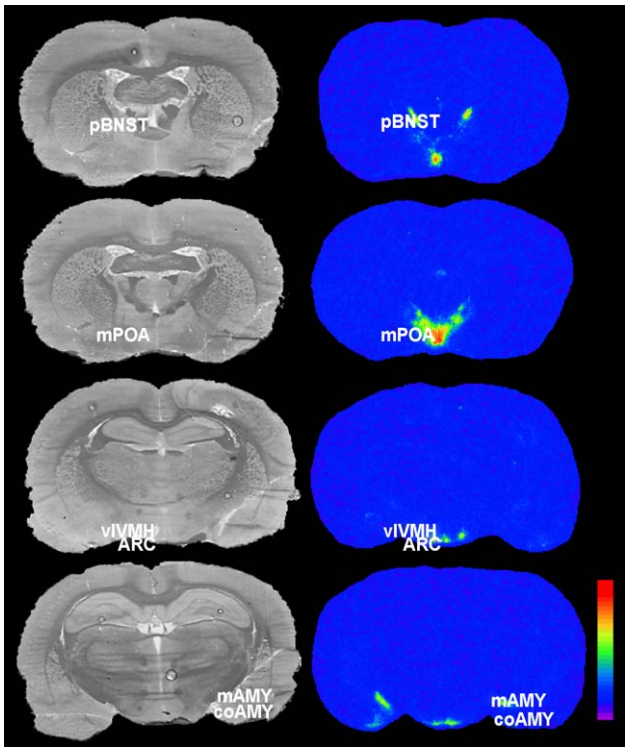

Fig. 3. ER distribution in ovariectomized (OVX) rats. Cresyl-violet-stained sections (left column) and [^{125}I]MIE$_2$ autoradiograms (right column) from an OVX animal showing posterior bed nucleus stria terminalis (pBNST) (top, bregma −0.92 mm), medial preoptic area (mPOA) (second row, bregma −1.30 mm), arcuate nucleus, hypothalamus (ARC), and ventromedial ventrolateral hypothalamic nucleus (vlVMH) (third row, bregma −3.14 mm), medial (mAMY), and cortical amygdala (coAMY) (bottom, bregma −3.60 mm). No binding was detectable in white matter regions. The rainbow scale used for the pseudo-color conversion is shown in the lower right corner (purple color for the lowest image values and red for the highest).

(and ER occupancy) between the two drugs were statistically significant by two-way ANOVA (E_2 vs. TAM occupancy; P = 0.011; treatment X region interaction P = 0.44, not significant).

The short half-life of [^{18}F], combined with the high uptake of the ligand in white matter (Biegon et al., 2003) and low density of ER in the brain, did not permit a more detailed, autoradiographic visualization of the regional effects of the hormonal agents, prompting the use of an iodinated agent.

In vivo occupancy of ER by E_2 and TAM studied with MIE$_2$ autoradiography

[^{125}I]MIE$_2$ autoradiograms of OVX animals demonstrated a highly specific and heterogenous distribution pattern closely matching the known distribution of ER in the rat brain (Fig. 3) (Pfaff and Keiner, 1973; Shughrue et al., 1997). ROIs were placed in the medial and cortical and dorsolateral amygdala, the arcuate nucleus of the hypothalamus, the bed nucleus of the stria terminalis (anterior and posterior), central gray, frontal cortex, hippocampus (dorsal and ventral), medial preoptic area, anterior paraventricular thalamus, ventrolateral ventromedial hypothalamus nucleus, and striatum. Two-way ANOVA by treatment and region revealed a highly significant effect of treatment ($P < 0.0001$) and region ($P < 0.0001$) as well as a highly significant treatment × region interaction ($P < 0.0001$) in the measured densities of radioactivity, supporting further post hoc analyses within and across groups and regions.

The uptake of [^{125}I]MIE$_2$ in OVX animals varied significantly among the regions considered (ANOVA by region $P < 0.0001$; Fig. 4). [^{125}I]MIE$_2$ levels were highest in the medial preoptic area followed by the posterior bed nucleus of the stria terminalis, medial amygdala, ventrolateral ventromedial hypothalamus, and the arcuate nucleus. Lower levels were detected in the anterior paraventricular thalamus, cortical amygdala, central gray, anterior bed nucleus of stria terminalis, and dorsolateral amygdala. The lowest values, indistinguishable from striatum under our experimental conditions were found in the dorsal hippocampus and frontal cortex. In addition, there was modest but noticeable uptake in the islands of Calleja, the periventricular nucleus, and the molecular layer of the cerebellar Purkinje cell layers. Due to the punctate distribution of ER in these small regions, they were not included in the quantitative analysis.

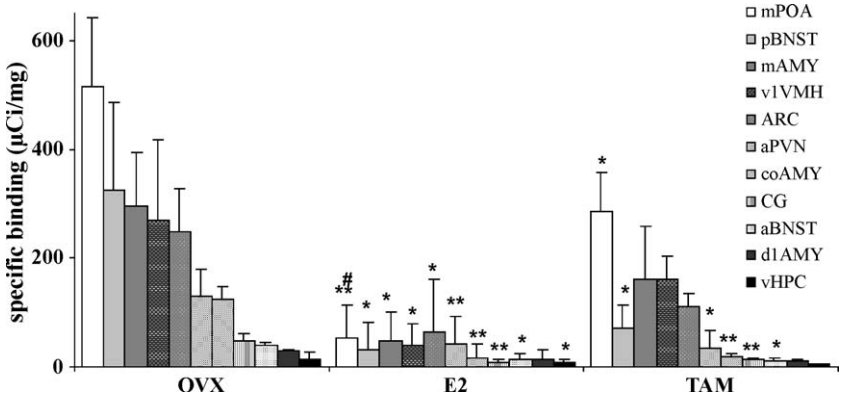

Fig. 4. Quantitative autoradiography of [^{125}I]MIE$_2$ uptake after E_2 and TAM treatment. Values are the mean (±SD) of three to four animals per group. Two-way ANOVA revealed a significant effect of treatment ($P < 0.0001$), region ($P < 0.0001$), as well as a significant interaction term ($P < 0.0001$). *$P < 0.05$, **$P < 0.005$ E_2 and TAM vs. OVX; $^{\#}P < 0.05$ E_2 vs. TAM (post hoc Fisher's PLSD). Abbreviations: medial preoptic area (mPOA), posterior bed nucleus stria terminalis (pBNST), medial amygdala (mAMY), ventrolateral ventromedial hypothalamic nucleus (vlVMH), arcuate nucleus hypothalamus (ARC), anterior paraventricular thalamus nucleus (aPVN), cortical amygdala (coAMY), central gray (CG), anterior bed nucleus stria terminalis (aBNST), dorsolateral amygdala (dlAMY), and ventral hippocampus (vHPC).

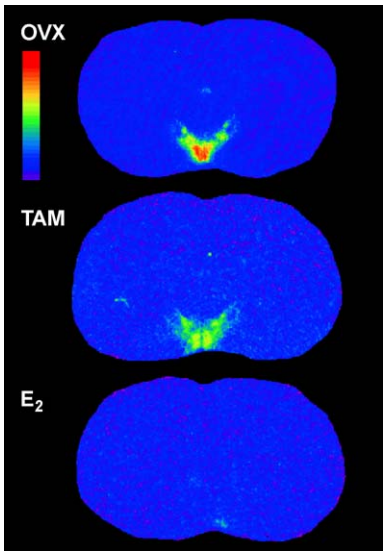

Fig. 5. Differential inhibition of MIE uptake by E_2 and TAM. Representative [^{125}I]MIE$_2$ binding after E_2 (bottom) and TAM (middle) treatment at the level of the medial preoptic area (bregma −1.30 mm). Inhibition of [^{125}I]MIE$_2$ binding was partial in TAM-treated rats while in E_2-treated animals no binding was visible. The rainbow scale used for the pseudocolor conversion is shown in the upper left part (purple color for the lowest image values and red for the highest).

The E_2-treated rats displayed very low and homogenous [^{125}I]MIE$_2$ uptake in the aforementioned ROIs (Fig. 5). The percentage of ER occupancy by E_2 was close to maximal across brain regions (mean 86.6 ± 17.5; Table 2).

By contrast, [^{125}I]MIE$_2$ uptake in TAM-treated animals was only partially inhibited (Figs. 4 and 5) and the degree of ER occupancy was region-dependent (ANOVA $P < 0.0001$), ranging from 40% to 85% (Table 2).

Discussion

Using two different techniques (brain dissection and autoradiography) and two radioligands ([^{18}F]FES and [^{125}I]MIE$_2$), we have demonstrated that the pattern of brain ER occupation resulting from 3 weeks of TAM treatment of ovariectomized female rats differs significantly from the occupancy by E_2. To our knowledge, this is the first study where ER occupancy has been assessed in vivo in rats chronically exposed to physiologically or pharmacologically relevant doses of E_2 and TAM. The hormonal agents were delivered through slow release pellets, implanted under the skin. The E_2 pellet is known to produce blood levels in the high physiological range, while the TAM dose provided blood levels similar to those found in women using TAM as a breast cancer adjuvant treatment (Biegon et al., 1996). The release of E_2 and TAM from the pellets in individual animals in the present experiment was ascertained by monitoring relevant physiological markers such as body weight gain and uterine hypertrophy. As expected, administration of E_2, a full agonist at all ER subtypes, produced a pronounced uterine hypertrophy in all of the animals. A smaller but significant effect was seen in all TAM-treated rats, as previously reported and expected from the profile of this drug in the uterus (e.g., Biegon et al., 1996). The degree of uterine ER occupation after E_2 and TAM treatment was very similar, with uterine [^{18}F]FES uptake reduced by more than 50% in both groups when compared to ovariectomized controls.

[^{18}F]FES has been used extensively to label ER in women in the context of breast cancer diagnosis and treatment (Dehdashti et al., 1995; Mankoff et al., 1997; Mortimer et al., 1996). In the brain, Moresco et al. (1995) have demonstrated specific in vivo labeling of ER in grossly dissected brain regions that could be blocked by acute injection of E_2 or DES. Using the same paradigm, we observed a near complete inhibition of ligand uptake in E_2-treated rats and a partial, region-dependent inhibition with TAM. The large and global reduction in ligand binding to ER caused by chronic E_2, which we report, probably reflects a combination of competitive occupancy of ER, as reported in female rat brain in the proestrous stage of the cycle (Yuan et al., 1995), or following acute E_2 administration (Moresco et al., 1995) and down-regulation of ER expression, as reported by Brown et al. (1996) following 8 days of exposure. TAM also reduced ligand uptake but the effect was region dependent, with smaller reduction in the amygdala and preoptic area compared to the hypothalamus.

Our attempt to study the regional distribution of the treatment effects at higher resolution with [^{18}F]FES ex vivo autoradiography has failed: Brain sections produced from animals killed 60 min after ligand injection and apposed to film produced blank autoradiograms, most probably due to a combination of the low levels of ER in the brain with the short half-life of [^{18}F] (109 min). Autoradiograms from animals killed at earlier time points were dominated by uptake in white matter regions (Biegon et al., 2003). This problem was resolved by using [^{125}I]MIE$_2$ (Brown et al., 1989; Zielinski et al., 1986), a specific ER radioligand labeled with an isotope with a half-life of 60 days.

The uptake of [^{125}I]MIE$_2$ in the brain of OVX animals followed the expected ER distribution: high density in preoptic and hypothalamic nuclei followed by amygdala, hippocampus, midbrain, and frontal cortex (Österlund et al., 1998; Pfaff and Keiner, 1973; Shughrue et al., 1997). The excellent match between

Table 2
Reduction of brain MIE uptake in estradiol- and tamoxifen-treated animals measured in discreet nuclei by quantitative autoradiography

Region	E_2	TAM
Cortical amygdala	87.7 ± 21.2	84.6 ± 4.5
Posterior bed nucleus stria terminalis	90.5 ± 15.7	77.7 ± 12.7
Anterior paraventricular thalamus	96.8 ± 4.0	74.2 ± 24.9
Central gray	84.1 ± 10.6	71.8 ± 8.1
Anterior bed nucleus stria terminalis	67.6 ± 32.4	69.7 ± 13.5
Arcuate nucleus hypothalamus	74.3 ± 39.6	55.1 ± 8.9*
Medial amygdala	83.9 ± 17.8	45.1 ± 32.5*
Medial preoptic area	89.9 ± 11.9	44.6 ± 14.1**
Ventrolateral ventromedial hypothalamus	85.5 ± 15.1	40.5 ± 15.6**

Results are expressed as mean% reduction of uptake compared to ovariectomized rats implanted with blank pellets and represent mean ± SD of four animals per treatment. ANOVA by region showed no regional variation in the estradiol-treated group ($P = 0.69$, mean reduction 86.6%) while in the TAM group ligand uptake was region dependent (ANOVA by region $P = 0.0001$, post hoc Fisher PLSD test $\alpha < 0.05$).

* $P < 0.05$, region vs. cortical amygdala.

** $P < 0.005$, region vs. cortical amygdala.

[^{125}I]MIE$_2$ uptake and the known distribution of brain ER confirm that this tracer provides specific in vivo imaging of brain ER.

After 3 weeks of E_2 treatment, rats displayed very low and homogenous [^{125}I]MIE$_2$ density in the brain. [^{125}I]MIE$_2$ uptake inhibition was close to maximal (mean 87%), indicative of high occupancy of all ER subtypes in all brain regions. By contrast, in TAM-treated animals, [^{125}I]MIE$_2$ uptake presented a heterogeneous regional distribution. [^{125}I]MIE$_2$ uptake inhibition by TAM was modest (< 50%) in the medial preoptic area and medial amygdala and similar to values obtained with E_2 in the bed nucleus of stria terminalis and cortical amygdala, reminiscent of the results obtained with FES and grossly dissected regions.

The pattern of ER occupation in TAM-treated rats appears to be correlated with the regional distribution of ER subtypes: [^{125}I]MIE$_2$ uptake inhibition was higher in ERα-dominated regions (cortical amygdala, central gray, and anterior paraventricular thalamus) and lower in regions where both subtypes are abundant (e.g., ventrolateral ventromedial hypothalamus, medial preoptic area, medial amygdala, and arcuate nucleus) (Laflamme et al., 1998; Österlund et al., 1998; Shughrue et al., 1997). The most likely explanation for this observation is that TAM is capable of down-regulating ER in "pure" ERα regions but not in regions with a mixed receptor subtype profile since recent literature suggests that the outcome of binding to ER does indeed depend on the subtype profile and drug/receptor confirmation (for a review, see Turgeon et al., 2004). However, the source of the regional in vivo and in vitro selectivity of TAM and other SERMs and ER antagonists (Cyr et al., 2001; Kimelberg et al., 2003; Mize et al., 2003; Watanabe et al., 1997; Zhou et al., 2002) certainly requires further study. It is becoming increasingly apparent that the effects of the SERMs TAM and raloxifene as well as the "pure" antagonist ICI 182 780 on brain ER are highly region dependent, although TAM has similar affinity to the alpha and beta estrogen receptor subtypes in vitro (Kuiper et al., 1997). A contribution of the membranal ER to the differential pattern is a distinct possibility, as it has been recently shown that ICI 182 780 can block fast, membranal effects of estrogen in the cortex but not the hippocampus, suggesting that membranal ER in different brain regions have distinct ligand binding and response patterns (Mize et al., 2003). It is also possible that TAM metabolites, such as the major active metabolite 4-OH TAM, contribute to the observed pattern. Interestingly, the affinity 4-OH TAM to the beta subtype is higher than its affinity to the alpha subtype in vitro (Kuiper et al., 1997).

Regardless of the mechanism, in vivo regional selectivity of TAM may explain several apparently conflicting observations regarding the actions of TAM, including the undisputed recurrence of hot flashes in women taking TAM as opposed to the controversy surrounding the type and frequency of cognitive side effects (Eberling et al., 2004; Ernst et al., 2002; Jordan, 2003; Jordan and Morrow, 1999). Similarly, low and variable occupancy of ER in specific regions after in vivo administration may explain conflicting results regarding the agonist vs. antagonist effects of TAM on various brain regions and markers (Cyr et al., 2001; Sumner et al., 1999; Zhou et al., 2002).

The relevance of our findings to humans should be tested by noninvasive (PET or SPECT) imaging of brain ER in women taking TAM or other ER modulators. Our results in rats, above, show that MIE$_2$ retains excellent specificity and high signal to noise for imaging brain ER in vivo. Labeled with appropriate isotopes (Iodine-123 for SPECT or Carbon-11 for PET), MIE$_2$ is a promising candidate for noninvasive studies of brain ER in humans. Such studies may help in the prediction and understanding of the clinical effects—both adverse and positive—of ER modulators in the human brain.

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