Imidazoline and $\alpha_2A$-Adrenoceptor Binding Sites in Postmenopausal Women before and after Estrogen Replacement Therapy

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**Background:** Platelet $\alpha_2A$-adrenoceptors ($\alpha_2A$AR) and imidazoline binding sites (subtype $I_1$) have been proposed as peripheral markers of brain stem receptors that mediate sympathetic outflow and are reported to be elevated in major depression.

**Methods:** In our study, $p[{^{125}}I]$-iodoclonidine was used to assess platelet $\alpha_2A$AR and $I_1$ binding sites in healthy postmenopausal women ($n = 34$) compared with healthy women of reproductive age ($n = 26$). Receptor determinations were repeated in 19 postmenopausal women following 59–60 days of estrogen replacement therapy (ERT; 0.1 mg estradiol transdermal patches).

**Results:** $I_1$ binding sites were twofold higher in platelets of postmenopausal women compared with women of reproductive age but were down-regulated (normalized) after 59–60 days of ERT. All other binding parameters, including platelet $\alpha_2A$AR density, were not different between groups nor were they changed after ERT. Platelet $I_1$ densities after 59–60 days of ERT were positively correlated with plasma luteinizing hormone concentrations.

**Conclusions:** It is suggested that increased imidazoline binding sites might be associated with mood and behavioral changes in postmenopausal women.

**Key Words:** Imidazoline receptors, $\alpha_2$ adrenoceptors, monoamine oxidase, estrogen, luteinizing hormone, menopause, depression

**Introduction**

Postmenopausal women are at high risk for osteoporosis (American Medical Association 1984) and cardiovascular disease (Stampfer et al 1991). Accordingly, estrogen replacement therapy (ERT) has been indicated for the prevention of osteoporosis and cardiovascular disease in postmenopausal women. Menopausal status may also influence cognitive problems (Yaffe et al 1998) and vulnerability to depression (Halbreich 1997; Halbreich and Lumley 1993). Some studies have suggested that ERT might improve cognition (Haskell et al 1997; Sherwin, 1988) and stabilize mood (Ditkoff et al 1991; Klaiber et al 1997) in postmenopausal women.

Clonidine is a centrally active, partial agonist for $\alpha_2$-adrenoceptors ($\alpha_2$AR) that elicits a number of cardiovascular (Parsons and Morledge 1970), hormonal (Siever and Uhde 1984), and cognitive effects (Ammassari-Teule et al 1991). Clonidine’s agonistic activities at $\alpha_2$AR can be discriminated from its nonadrenergic activities by induction of a G-protein “coupled” state of $\alpha_2$AR, yet not all of clonidine’s effects are mediated through adrenoceptors (Piletz et al 1994). Specifically, clonidine has nanomolar affinity for nonadrenergic $I_1$-imidazoline binding sites ($I_1$ sites: Ernsberger et al 1995a).

The molecular nature and function of $I_1$ sites has remained a subject of debate (Ernsberger and Haxhiu 1997) even while attempts to clone $I_1$ sites are being reported (Ivanov et al 1998b). In radioligand binding assays, $I_1$ sites can be distinguished from three subtypes of $\alpha_2$AR ($\alpha_2A$, $\alpha_2B$, and $\alpha_2C$-AR) by phenylephrine and guanidine compounds, which, in contrast to all $\alpha_2$AR subtypes, lack high affinities for $I_1$ sites (Ernsberger et al 1995a). Prolonged hypotension is induced by microinjection of imidazoline compounds, such as clonidine, into the rostroventral lateral medulla (RVLM) region of the brain stem (Bousquet et al 1989), the potency of which correlates with affinities of these agents at $I_1$ sites but not at any $\alpha_2$AR subtypes (Ernsberger et al 1995a). Based on pharmacologic studies, $I_1$ sites in the RVLM were proposed to reside on imidazoline receptors that regulate sympathetic outflow (Bousquet et al 1989; Ernsberger et al 1990). More recently, another subtype of I-sites ($I_2$ sites) has been identified as a modulatory domain within monoamine oxidase enzymes (Raddatz et al 1997; Tesson et al 1995). The latter finding has raised the possibility that imidazo-
lines might also mediate sympathetic outflow via the metabolism of catecholamines in the brainstem.

There is also evidence that platelet $I_1$ and $\alpha_{2A}$AR clonidine-binding sites may be co-dysregulated in depression. This is based on reports (Piletz et al. 1990, 1996a) that $I_1$ and $\alpha_{2A}$AR clonidine-binding sites are elevated in depressed patients compared with healthy control subjects, and that both sites are down-regulated following certain types of antidepressant treatments (Piletz et al. 1991, 1996b). Furthermore, platelet $\alpha_{2A}$AR and $I_1$ sites appear to possess identical binding properties as their counterparts in the brain (Piletz and Sletten 1993). Elevations in brain $I_1$ and $\alpha_{2A}$AR clonidine-binding sites have also been suggested (Callado et al. 1998; Garcia-Sevilla et al. 1996, 1999) in depressed suicide victims compared with sudden-death matched control subjects. Thus, high densities of $I_1$ and $\alpha_{2A}$AR sites may be associated with depressed mood (Garcia-Sevilla et al. 1999).

$\alpha_{2A}$AR in uteri and brain have been reported to be influenced by gonadal hormones and to regularly fluctuate along the menstrual cycle (Bottari et al. 1983; Orensanz et al. 1982; Roberts et al. 1979). On the other hand, we have found irregular fluctuations of platelet $\alpha_{2A}$AR and $I_1$ sites during the menstrual cycle in correlation with changes in plasma epinephrine and norepinephrine levels (Piletz et al. 1998). Women with dysphoric premenstrual symptoms also exhibited higher $[^{3}H]$-para-aminoclonidine binding to platelet $I_1$ and $\alpha_{2A}$AR sites than control subjects, which was especially pronounced during the symptomatic late luteal phase of the disorder (Halbreich et al. 1993).

To our knowledge, platelet $\alpha_{2A}$AR and $I_1$ sites have not previously been studied in postmenopausal women, and the effects of ERT on these sites have not been reported. Considering the possible role of these systems in the modulation of blood pressure and mood, their influence by menopause and ERT is of interest from both a clinical and a heuristic perspective.

**Methods and Materials**

**Subjects**

Thirty-four postmenopausal women (aged 52.6 ± 3.4 SD; range 45–59 years) and 26 women of reproductive age (aged 36.1 ± 6.5, range 22–45 years) participated in the baseline study. Of the postmenopausal women, 19 were retested for radioligand binding on days 59–60 of ERT (aged 52.2 ± 3.4 years). Six postmenopausal women completed ERT but were unable to be rescheduled for blood drawing until 2–3 days after estrogen (E$_2$) discontinuation (their posttreatment data will not be included here). Nine postmenopausal women discontinued ERT prematurely or were lost to follow-up.

All women of reproductive age as well as postmenopausal women were recruited, evaluated, and treated, and their samples were drawn and processed at the same site (Biobehavioral Program, State University of New York at Buffalo).

Eligibility of subjects was determined according to several criteria. Postmenopausal women had not experienced menstrual cycles for at least 2 years. They were at least 1 year after cessation of menopausal symptoms (including hot flashes, night sweats, and irritation by vaginal dryness) but were not older than 60 years of age. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations were within range for menopause, and no plasma progesterone was detected on at least two occasions before the study began. These postmenopausal women had no medical contraindications to ERT. The subjects weighed within 20% of ideal body weight, were physically healthy (including normal blood pressure and pulse), and met research diagnostic criteria (Spitzer et al. 1978) for “not currently mentally ill” for at least 2 years before the study. Ten of the enrolled women had lifetime histories of major depressive disorder (MDD) and were therefore treated as a subgroup. Women were excluded if they had any active physical illnesses or other disorders necessitating chemical or somatic treatments or had any Axis I diagnosis of the DSM-III-R (APA 1987) at the time of testing. Postmenopausal subjects were compared with women of reproductive status who had regular menstrual cycles and otherwise met the same inclusion and exclusion criteria. Women of reproductive age were also administered the Premenstrual Assessment Form (Halbreich et al. 1982) and daily rating forms (Endicott et al. 1986) to exclude subjects with dysphoric symptoms during their midfollicular phases or those with premenstrual syndrome. This study was approved by an institutional review committee. All subjects signed consent forms before the study.

Clinical procedures were as follows. Before enrollment, the women underwent a structured clinical interview with the Schedule for Affective Disorders and Schizophrenia (Endicott and Spitzer 1978), and research diagnostic criteria were derived (Spitzer et al. 1978). Postmenopausal women had their blood drawn before and after 59–60 days of Estraderm (estradiol transdermal system) treatment (0.1 mg). Control women of reproductive age were tested during their midfollicular cycle (days 7–10 of the menstrual cycle). Subjects monitored mood and behavior throughout the study with daily rating forms (Endicott et al. 1986) and were confirmed to be free of mood and behavioral abnormalities. Subjects refrained from chronic medications for at least 2 weeks before baseline blood drawings (candidates taking any chronic medications or recreational drugs were excluded during the screening). Alcohol, aspirin, or other over-the-counter pain medications were also excluded for at least 2 days before giving blood. Subjects had an overnight fast, and caffeine and cigarettes were not permitted until after the blood drawing. None of the patients or control subjects complained of increased anxiety or craving because of these restrictions. Symptoms were reviewed on the same day as the blood drawing according to the Schedule for Affective Disorders and Schizophrenia (Endicott et al. 1981) and the daily rating forms (Endicott et al. 1986) to ensure no changes in mental status. The women rested supine for at least 20 min before blood drawing, which was between 9:00 AM and 11:00 AM. The first 2–3 mL of blood was discarded, and then 90 mL of venous blood was collected.
slowly through an 18-gauge needle into a heparinized syringe (19 units/mL blood). An additional 5 mL of blood was collected to obtain plasma (stored at −70°C) for hormone determinations. The laboratory personnel were unaware of the subject’s age, diagnosis, or treatment (single blind).

Platelet Preparations

The preparation of intact platelets began according to a slight modification of the method of Corash (1980). Platelets were then washed four times in sterile Ca\(^{2+}\)/Mg\(^{2+}\)-free Hank’s balanced salt solution, pelleted, resuspended in the same solution with 0.1 mmol/L phenyl methylsulfonylfluoride, a protease inhibitor, and snap frozen at −80°C. After thawing, sonication, and centrifugation at 4°C (Piletz et al 1990), the platelet particulates were resuspended in ice-cold reaction buffer (designated washed lysates; formula of reaction buffer given below). Washed lysates were then layered over a discontinuous 14.5% and 34% sucrose gradient and centrifuged at 105,000 \(\times g\) for 90 min at 4°C using a Beckman (Allendale, NJ) SW-28 rotor in a Sorvall OTD60B ultracentrifuge (Dupont, Wilmington, DE). The interface layer, containing the plasma membranes, was diluted in 40 mL of ice-cold water, pelleted, and resuspended in reaction buffer (Piletz et al 1990). The concentration of protein was determined using a Lowry-Biuret reagent kit from Sigma Chemical Co. (St. Louis). Samples were stored at −80°C until use.

Binding Assays

Radioligand binding with \([\text{125I}]\)iodoclonidine (\([\text{125I}]\)PIC; New England Nuclear, Boston) was according to our previous procedure (Ernsberger et al 1995b; Piletz et al 1996b). Specific \(\alpha_{2A}\)AR binding was determined from “total binding” minus “nonadrenergic binding,” as displaced in the presence of 10 mmol/L norepinephrine. With another aliquot, \(I_1\) binding sites were measured under a mask of 10 mmol/L NE (Piletz et al 1996b). With another aliquot, \(I_1\) binding was determined from “total binding” minus “nonadrenergic binding,” as displaced in the presence of additional 100 mmol/L moxonidine (kindly contributed by Dr. Siegfried Schafer, Solvay Pharma, Hanover, Germany). In most cases, the binding assays entailed seven concentrations of radioligand per site (Piletz et al 1996b); however, in some cases, a low protein yield permitted only 4–6 concentrations of radioligand per site. All measurements were made in hexuplicate (i.e., three total and three nonspecific values for each concentration). Reactions were initiated by adding 0.015–0.03 mg plasma membrane proteins (up to 0.25 mL) to the radioligand and incubating at 21°C. The reaction buffer was 5 mmol/L HEPES, 0.5 mmol/L MgCl\(_2\), 0.5 mmol/L EGTA (adjusted to pH 7.4 with 0.1 mmol/L NaOH); with 0.1 mmol/L ascorbic acid added on the day of the binding assay. Trapped plasma membranes were washed three times with ice-cold wash buffer on thick glass fiber filters (Schleicher & Schuell #32, Keene, NH), and radioactivity was counted for 10 min per sample in a Cobra Gamma Counter (Packard Instruments, Meriden, CT) at 80% efficiency. Individual \(B_{\text{max}}\) and \(K_D\) values were quantified using LIGAND (McPherson 1985). Data were fit to both one-site and two-site models, but a one-site model was always preferred by an \(F\) test.

Hormonal concentrations were determined by radioimmunoassays (RIA) with commercially available kits. 17-Beta estradiol (E\(_2\)) was assayed (Diagnostic Products, Los Angeles) with 100\(\mu\)L aliquots of plasma that were incubated at room temperature for 3 hours in an antibody-coated tube with \([\text{125I}]\)-free estradiol. Bound estradiol was counted after decantation of the supernatant. The intra- and interassay coefficients of variation (CV) ranged from 4.0% to 8.1%; the sensitivity limit was 8 pg/mL. Plasma progesterone determinations (Diagnostic Products) followed procedures similar to those used for estradiol. The intra- and inter-assay CV ranged from 6% to 10%; the sensitivity limit was 0.05 ng/mL. Plasma testosterone was assayed by a solid phase RIA (Diagnostic Products) in an aliquot of 50 \(\mu\)L. Interassay CV ranged from 9%–13%, and intraassay CV ranged from 4% to 6%. The sensitivity limit for the testosterone assay was 0.04 ng/mL. Plasma FSH and LH were also determined with RIA reagents purchased from Diagnostic Products in 200-\(\mu\)L aliquots. The interassay CV ranged from 4% to 6% (FSH) and 2% to 7% (LH), and the intraassay CV ranged from 2% to 4% (FSH) and 1% to 2% (LH). The sensitivity limit for FSH was 0.06 mIU/mL and for LH was 0.1 mIU/mL.

Statistical Analyses

Subject groups were compared with each other using nonpaired Student’s \(t\) tests. Pre- to posttreatment values were compared with paired \(t\) tests. Pearson’s regression tests were performed for associations of radioligand binding values with hormone values. All \(p\) values are two tailed. The data are expressed as means ± standard deviations.

Results

As shown in Table 1 and Figure 1, platelet \(I_1\) density was significantly higher (pre-ERT) in postmenopausal women \((n = 34)\) compared with women of reproductive status \((n = 26); I_1\) \(B_{\text{max}}\) \(t = 5.4, p < .0001\). None of the other platelet radioligand binding parameters were significantly different between postmenopausal women and women of reproductive status \((\alpha_{2A}\)AR \(B_{\text{max}}\) \(t = 0.38 p = .7; \alpha_{2A}\)AR \(K_D\) \(t = 0.68, p = 0.5; I_1\) site \(K_D\) \(t = 1.17, p = .25\)). There was no correlation between the ages of postmenopausal women and any \(K_D\) or \(B_{\text{max}}\) parameters (pretreatment \(r_s = .04\) to .28, posttreatment \(r_s = -.02\) to -.24). Following treatment with E\(_2\) for 59–60 days, the \(I_1\) \(B_{\text{max}}\) values of postmenopausal women \((n = 19)\) decreased 35% \((t = 3.32, p = .004)\) to near normal levels. A comparison of the \(B_{\text{max}}\) data in postmenopausal women that completed treatment versus women of reproductive status (menstrual) is shown in Figure 1. The \(\alpha_{2A}\)AR \(B_{\text{max}}\) values of the women who completed 59–60 days of E\(_2\) treatment did not change \((t = 0.22, p = .829)\), as was the case with \(\alpha_{2A}\)AR \(K_D\) \((t = 0.55, p = .59)\). Also, \(I_1\) \(K_D\) values did not change in response to treatment with E\(_2\) \((t = 0.62, p = .55)\).
Before ERT, postmenopausal women had plasma E\textsubscript{2} concentrations of 7.4 ± 6.8 pg/mL, progesterone concentrations of 0.18 ± 0.13 ng/mL, LH concentrations of 81.4 ± 10.0 mIU/mL, FSH concentrations of 102.6 ± 52.1 mIU/mL, and testosterone concentrations of 0.24 ± 0.12 pg/mL. After 59–60 days of estrogen treatment, plasma E\textsubscript{2} concentrations were 63.6 ± 39.7 pg/mL (p < .0001 vs. baseline), plasma LH concentrations were 9.3 ± 5.7 mIU/mL (p < .0001 vs. baseline), plasma FSH concentrations were 39.2 ± 11.3 mIU/mL (p < .0001 vs. baseline), and plasma testosterone concentrations were 0.31 ± 0.40 pg/mL (ns). Progesterone concentrations after ERT were below the sensitivity of the RIA for most women. Baseline and posttreatment concentrations of E\textsubscript{2} or other hormones did not correlate with each other (r = .03, p = .86 for E\textsubscript{2}). Plasma E\textsubscript{2} concentrations after 30 days of ERT (61.6 ± 36.0 pg/mL) did not differ from E\textsubscript{2} concentrations after 59–60 days of ERT (t = 0.42, p = .63).

Correlations between Binding Sites and Hormones
At baseline (pre-ERT), none of the platelet receptor parameters were significantly correlated with any of the plasma hormone concentrations in postmenopausal women (r values ranged from .1 to .45, ns).

Because ERT induced a down-regulation of platelet I\textsubscript{1} sites (Table 1 and Figure 1), it was anticipated that E\textsubscript{2} concentrations at post-ERT might be negatively correlated with I\textsubscript{1} B\textsubscript{max} values. In fact, the opposite was observed. A positive correlation was found between posttreatment E\textsubscript{2} concentrations and I\textsubscript{1} B\textsubscript{max} values at the p < .04 significance level (r = .49, n = 18); however, pre-to-post treatment I\textsubscript{1} B\textsubscript{max} change scores (the magnitude of decrease) were positively correlated with posttreatment E\textsubscript{2} concentrations (r = .46, p = .04).

It was also anticipated that plasma LH concentrations, FSH concentrations, or both might be positively correlated with platelet I\textsubscript{1} B\textsubscript{max} values at posttreatment. This was because all three of these measures appeared to decrease following ERT. In accord with this prediction, plasma concentrations of LH after 59–60 days of ERT were highly positively correlated with I\textsubscript{1} B\textsubscript{max} values (r = .73, p = .001, n = 17) (Figure 2). Furthermore, the pre-to-post treatment I\textsubscript{1} B\textsubscript{max} change scores were negatively correlated with posttreatment LH concentrations (r = −.48, p = .04). Thus, higher E\textsubscript{2} and lower LH concentrations after ERT were associated with higher

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**Table 1. Platelets Imidazoline and α\textsubscript{2}AR Binding**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Baseline</th>
<th></th>
<th></th>
<th>Post-ERT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I\textsubscript{1} B\textsubscript{max}</td>
<td>I\textsubscript{1} K\textsubscript{D}</td>
<td>α\textsubscript{2} B\textsubscript{max}</td>
<td>α\textsubscript{2} K\textsubscript{D}</td>
<td>I\textsubscript{1} B\textsubscript{max}</td>
<td>I\textsubscript{1} K\textsubscript{D}</td>
</tr>
<tr>
<td>All postmenopausal women (n = 34)</td>
<td>177.6 ± 89.3a</td>
<td>4.2 ± 1.2</td>
<td>64.6 ± 59.8</td>
<td>0.66 ± 0.53</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Subjects who completed 60 days of E\textsubscript{2} (n = 19)</td>
<td>188.3 ± 63.3b</td>
<td>4.1 ± 1.2</td>
<td>76.6 ± 64.0</td>
<td>0.72 ± 0.59</td>
<td>122.7 ± 58.1</td>
<td>4.4 ± 1.7</td>
</tr>
<tr>
<td>Reproductive age women (n = 26)</td>
<td>84.6 ± 30.5</td>
<td>4.6 ± 1.6</td>
<td>60.0 ± 31.9</td>
<td>0.55 ± 0.61</td>
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</tr>
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Values represent mean ± SD. E\textsubscript{2}, 17-beta estradiol. a p < .0001 vs. women of reproductive age.
b p < .0001 vs. women of reproductive age and p = .004 vs. post–estrogen replacement therapy.

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**Figure 1.** Effect of menopause and estrogen therapy (ERT) on platelet α\textsubscript{2}-adrenergic and imidazoline-1 sites. [\textsuperscript{125}I]PIC, p[\textsuperscript{125}I]-iodoclonidine; prot, protein.

**Figure 2.** Platelet imidazoline binding site and plasma luteinizing hormone following estrogen replacement therapy in postmenopausal women. prot, protein.
change scores (i.e., more down-regulation) of $I_1 B_{max}$ values. No other significant correlation was observed between any of the hormone concentrations and either $I_1$ or $\alpha_{2A}AR$ parameters.

In general, there was no association between blood pressure or pulse rate with any of the platelet $I_1$ or $\alpha_{2A}AR$ parameters ($rs = .028-.184$). A negative correlation ($r = -.4040$, $p = .037$) was observed between platelet $\alpha_{2A}AR$ affinity ($K_D$) and pulse rate at baseline, however.

Subgroup with Lifetime History of MDD

Postmenopausal women with lifetime histories of MDD ($n = 10$) tended to have higher $I_1 B_{max}$ values than those who were never mentally ill ($n = 24$), but this did not reach statistical significance ($221 \pm 132$ vs. $159 \pm 69$ fmol/mg protein, $t = 1.62$, $p = .12$). Similarly, $I_1 K_D$ values did not differ ($4.1 \pm 1.0$ vs. $4.2 \pm 1.2$, $t = .3$, $p = .77$), nor did $\alpha_{2A}AR K_D$ values differ ($0.42 \pm 0.40$ versus $0.74 \pm 0.63$, $t = 1.39$, $p = .18$) in regard to lifetime histories of MDD. Postmenopausal women with lifetime histories of MDD differed in their $\alpha_{2A}AR B_{max}$ values at baseline, however, which were high ($73.8 \pm 61.2$ vs. $30.1 \pm 16.6$ fmol/mg protein, $t = 2.83$, $p = .01$). The two groups (with or without lifetime MDD) did not significantly differ in baseline hormonal concentrations.

Following 59–60 days of treatment with Estraderm, the women with past MDD ($n = 7$) did not differ from those without lifetime histories of MDD on any of the $I_1$ parameters; however, after $E_2$ treatment, the LH concentrations of women with histories of MDD decreased to lower concentrations than did the LH of women who were never mentally ill ($3.9 \pm 2.4$ vs. $11.1 \pm 5.6$ ug/L, $t = 3.23$, $p = .008$).

Discussion

We report two main findings. The first is a higher radioligand binding density of $I_1$ sites in platelets of postmenopausal women compared with women of reproductive age (Table 1 and Figure 1). The second is a down-regulation of platelet $I_1$ binding sites after 59–60 days of ERT (Table 1 and Figure 1). These findings are distinguished from $\alpha_{2A}AR$ sites on platelets, which were not different in postmenopausal women compared with women of reproductive age and were not affected by ERT.

We suggest that the high density of platelet $I_1$ binding sites observed in postmenopausal women might relate to altered hormonal status, rather than to age per se. This is for the following reasons: 1) In our study, there were no within-group correlations between ages and $I_1$ or $\alpha_{2A}AR$ binding parameters. 2) In our five previous studies (Halbreich et al 1993; Piletz et al 1990, 1996a, 1996b, 1998), with more than 90 subjects of varying diagnoses (male and female subjects, ages 20–65), no statistically significant associations have been found between ages and densities of platelet $I_1$ or $\alpha_{2A}AR$ sites. 3) Even considering only those reports (Piletz et al 1996a; 1996b, 1998) that used an identical binding assay and where a sizeable number of healthy female control subjects were available ($n = 44$), we could find no association between female ages (23–63 years) and densities of platelet $I_1$ or $\alpha_{2A}AR$ sites. To our knowledge, no other reports exist on aging effects on these platelet sites. Because no previous reports have even hinted at an effect of age per se on these sites, the best explanation for the present data seems to be the postmenopausal status of women and its related hormonal state.

$I_1$ and $\alpha_{2A}AR$ sites are known to be differentially distributed in the brain (DeVos et al 1994; Kamisaki et al 1990). $I_1$ sites are almost completely absent in the brain stem nucleus locus coeruleus, which contains a high density of $\alpha_{2A}AR$ (Ernsberger et al 1994). The frontal cortex is another region of high density of $\alpha_{2A}AR$, but where $I_1$ sites are of relatively low density (DeVos et al 1994; Kamisaki et al 1990). On the other hand, $I_1$ sites have been extensively studied in the RVLM brainstem nucleus (Bricca et al 1989; Ernsberger et al 1990), where they are associated with the hypotensive action of centrally applied imidazoline compounds. $I_1$ sites in the RVLM might serve a physiologic role in the tonic and reflex control of the sympathetic nervous system (Ernsberger and Haxhiu 1997). An endogenous clonidine displacement substance has also been characterized (Atlas 1991) and identified to contain agmatine, a decarboxylated arginine metabolite (Li et al 1994). Thus, agmatine and the imidazoline binding sites ($I_1$ and $I_2$) might compose a neurotransmitter-receptor system. Although agmatine is now realized to be only one of several molecules that constitute clonidine displacement substance (Piletz et al 1995; Sun et al 1995), agmatine fulfills several criteria for a neurotransmitter (Reis and Regunathan 1999).

We are unaware of any previous study on the influence of steroids on imidazoline binding sites (either $I_1$ or $I_2$); however, monoamine oxidase B (MAO-B) encodes an $I_2$ site (Raddatz and Lanier 1997; Tesson et al 1995) and platelet MAO-B activity levels have been studied in response to estrogen (Chakravorty and Halbreich 1997; Klaiber et al 1997). Thus, the down-regulation of platelet $I_1$ sites after ERT could be comparable to two previous reports (Holsboer et al 1983; Klaiber et al 1972) showing that estrogen therapy reduces plasma MAO activity in postmenopausal women with depressed mood. Furthermore, platelet MAO-B levels have been reported to be negatively correlated with serum estradiol levels during a hormone replacement therapy (Klaiber et al 1997). Age related changes (i.e., more down-regulation) of $I_1 B_{max}$ values. No other significant correlation was observed between any of the hormone concentrations and either $I_1$ or $\alpha_{2A}AR$ parameters.
though we did not measure platelet I₁ sites in our study, it is conceivable that the platelet I₁ site is physically related to I₂ sites.

Our finding of no effect of ERT on platelet α₂A AR sites in postmenopausal women is in accord with a previous study by Best and coworkers (Best et al. 1992) in which yohimbine binding to platelet α₂A AR sites was unchanged in postmenopausal women given an estradiol implant (100 mg) for 6 weeks. By contrast, another study reported increased platelet α₂A AR sites in human male volunteers treated with E₂ (U'Prichard and Snyder 1979), and three earlier studies of female rabbits reported that estrogen treatment decreases α₂A AR sites in platelets (Elliott et al. 1980; Mishra et al. 1985; Roberts et al. 1979). In our study, and in the study by Best and colleagues (Best et al. 1992), estradiol was administered for a much longer time (42–60 days) than in the human male study (4 days) or the rabbit studies (6–24 days). Therefore, the length of E₂ treatment and species and gender differences might be critical factors.

We have also reported (Halbreich et al. 1993) higher platelet α₂A AR and I₁ sites in women with dysphoric premenstrual syndrome compared with healthy control women. In that study, elevations in binding were most pronounced during the symptomatic late luteal phase of the disorder when plasma norepinephrine and adrenaline concentrations typically peak (Blum et al. 1992). The literature is uniformly negative for regular, cyclic fluctuations of platelet α₂A AR or I₁ sites along the human menstrual cycle (Jones et al. 1983; Piletz et al. 1998; Stowell et al. 1988; Sundaresan et al. 1985; Theodorou et al. 1987). In certain brain regions and in uteri, however, α₂A AR sites may be influenced by gonadal hormones and appear to fluctuate along the estrus cycle (Bottari et al. 1983; Orensanz et al. 1982).

One potential paradox was that post-ERT I₁ B_max values were positively correlated with plasma E₂ concentrations. This would not be expected if E₂ directly down-regulated I₁ sites; however, plasma E₂ concentrations were found to be positively associated with the magnitude of change in I₁ B_max values, which is in line with an E₂ down-regulation effect. We have previously reported (Piletz et al. 1998) irregular fluctuations of platelet α₂A AR and I₁ sites over two menstrual cycles in healthy women, which were not correlated with phase of the cycle but were instead correlated with plasma concentrations of adrenaline and norepinephrine. We have also reported (Ivanov et al. 1998a) that immunoreactive-I₁ sites on human megakaryoblastoma cells are up-regulated after 6 hours of exposure to norepinephrine in vitro. Similar treatments with estrogen in vitro were without effect (unpublished observations). Thus, the mechanisms of the down-regulation of platelet I₁ sites in women after ERT (Table 1) is still unclear. Future studies are needed to address whether indirect changes in plasma catecholamines might underlie platelet I₁ changes.

Another interesting observation was that post-ERT I₁ B_max values were positively correlated with plasma LH concentrations (Figure 2). Whether these are causally linked (i.e., LH or the I₁ site exerts a regulatory effect) or whether LH and I₁ sites are independently influenced by E₂ is open to speculation. The literature suggests no effect of clonidine on LH release (Kaufman and Vermeulen 1989). Any effect of LH on I₁ binding sites therefore is unknown and will require additional studies (in the same way that the possible effect of LH on mood and behavior has not been sufficiently investigated).

It is also interesting to note that the abnormal I₁ B_max values previously reported for depressed patients (Piletz et al. 1996b) are comparable in level to those of healthy postmenopausal women (Table 1). Of course, exact platelet I₁ B_max values are notoriously difficult to duplicate across studies for technical reasons (Ernsberger et al. 1995b). Even slight variations in platelet preparation may be critical (Corash 1980). In fact, all the platelets in our current study were prepared at a different site (Buffalo) than in our previous studies with depressed patients (Cleveland). Nevertheless, the B_max values in our postmenopausal women (177.6 ± 89.3 fmol/mg protein, n = 34) are surprisingly close to what we previously reported for a group of middle-aged male and female depressed patients (161 ± 66.3 fmol/mg, n = 26) (Piletz et al. 1996b). I₁ densities in postmenopausal women after ERT (122.7 ± 58.1 fmol/mg, n = 19) are also surprisingly close to what we previously reported for healthy middle-aged male and female subjects (126 ± 63.6 fmol/mg, n = 18) (Piletz et al. 1996b). Furthermore, the few postmenopausal women with lifetime histories of MDD had higher B_max values for α₂A AR (statistically significant) and I₁ sites (trend) compared with women without lifetime histories of MDD. If the platelet I₁ site becomes validated as a biological marker for depression (Piletz et al. 1994), the present findings in nondepressed postmenopausal women, as well as the down-regulation by estrogen, will have to be taken into account.

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