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Estradiol Attenuates α_2-Adrenoceptor–Mediated Inhibition of Hypothalamic Norepinephrine Release

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These studies tested the hypothesis that estradiol facilitates norepinephrine (NE) neurotransmission by modulating α_2-adrenoceptor–mediated inhibition of NE release. KCl-induced overflow of ^{3}H from superfused slices preloaded with ^{3}H-NE was Ca^{2+} dependent. Hypothalamic slices from estradiol-treated rats exposed to a single KCl pulse (S1) had modestly (20%) but significantly elevated NE release when compared to slices from ovariectomized (OVX) rats. Blockade of α_2-adrenoceptors by pretreatment with the imidazoline antagonists idazoxan (IDA) and RX821002 (RX) markedly facilitated NE release during S1 in hypothalamic slices from OVX rats; this facilitation was attenuated or absent in slices from estradiol-treated rats. In additional studies slices were stimulated twice, 24 min apart (S1 and S2), for 3 min with 20 mM KCl. In the absence of drug, the amount of ^{3}H-NE released during S2 was always less than the amount released during S1 (i.e., S2:S1 $\approx$ 0.6), regardless of whether slices were from OVX or estradiol-treated females. When 10 μM IDA was applied after S1 and 15 min prior to S2, the S2:S1 ratio increased to 1.8 $\pm$ 0.1 in hypothalamic slices from OVX animals. In contrast, the S2:S1 ratio rose only to 1.1 $\pm$ 0.2 in slices from estradiol-treated animals. RX applied before S2 markedly increased the S2:S1 ratio in both hypothalamic and preoptic area slices from OVX rats but failed to increase the S2:S1 ratio in slices from estradiol-treated rats. Interestingly, the modest effects of alkaloid α_2-antagonists such as yohimbine and rauwolscine on NE release in hypothalamic and preoptic area slices were not modified by estradiol. These results suggest that α_2-adrenergic inhibition of NE release is highly active in the hypothalamus of OVX female rats and that this inhibition is attenuated by estradiol. Furthermore, estradiol may specifically regulate the α_{2D}-adrenoceptor subtype.

[Key words: α_2-adrenoceptor, norepinephrine release, hypothalamus, estradiol, presynaptic inhibition, idazoxan, RX821002]

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The lordosis response is a mating posture that must be assumed by female rodents in order for fertilization to occur. This behavior is strictly regulated by the ovarian steroid hormones estradiol and progesterone (for review, see Pfaff, 1980; Etgen et al., 1992). The major neural target at which these hormones act to regulate lordosis is the ventromedial hypothalamus (Barfield et al., 1982). The dendritic fields lateral to the ventromedial hypothalamus are densely innervated by noradrenergic nerve terminals from the dorsal and ventral noradrenergic bundles (Moore and Bloom, 1979; Moore and Card, 1984). The importance of hypothalamic norepinephrine (NE) in the expression of lordosis has been demonstrated by a variety of studies. For example, lesions that eliminate NE input to the hypothalamus abolish the ability of estrogen and progesterone to facilitate lordosis behavior (Hansen et al., 1980, 1981). *In vivo* microdialysis experiments from our laboratory also demonstrate that hormonal activation of female reproductive behavior is accompanied by elevated hypothalamic NE release (Vathy and Etgen, 1989).

Furthermore, a common end point of many pharmacological agents that modulate lordosis responses may be an alteration of NE release in the hypothalamus. Cholinergic agents such as carbachol, which facilitate the lordosis response (Dohanich et al., 1984, 1990, 1991), can increase NE release in the hypothalamus (G. B. Karkanias and A. M. Etgen, unpublished observations) and other brain regions (Langer, 1981; Chesselet, 1984). Similarly, morphine applied directly to the ventromedial hypothalamus has been shown to inhibit lordosis in hormone-primed rats, and this behavioral inhibition is correlated with reduced NE release measured using microdialysis (Vathy et al., 1991). One major regulator of NE release is the α_2-adrenoceptor. Numerous studies have shown that α_2-adrenoceptor activation can diminish NE release from a variety of central and peripheral sites (for reviews, see Langer, 1974, 1981; Langer and Arbilla, 1981; Starke, 1977, 1981; Doxey and Roach, 1980; Dubocovich, 1984; Langer et al., 1985). Moreover, the demonstration that α_2-adrenoceptors are located at presynaptic noradrenergic nerve terminals provides the possibility that they mediate direct negative feedback inhibition of NE release (Farnebo and Hamberger, 1971; Kirpekar and Puig, 1971; Starke, 1971). The present studies were undertaken to test the hypothesis that estradiol facilitates hypothalamic NE transmission by attenuating α_2-adrenoceptor–mediated inhibition of NE release.

Materials and Methods

Animals and hormone treatments. Female Sprague–Dawley rats (150–175 gm) were obtained from Taconic Farms (Germantown, NY) and bilaterally ovariectomized (OVX), under Metofane anesthesia, 4–7 d prior to use. OVX rats were given subcutaneous injections of 2 μg

estradiol benzoate (EB) 24 and 48 hr prior to death. Some animals received EB plus 500 μg of progesterone (P) 3.5 hr prior to death. EB and P were dissolved in peanut oil and injected in a volume of 0.1 ml. OVX control animals received the appropriate number of injections of 0.1 ml peanut oil alone.

Tissue slice preparation. Animals were killed by decapitation and the brains rapidly removed and placed in ice-cold medium containing, in mM, 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$, and 10.5 glucose (Yamamoto, 1972) previously saturated with O_2:CO_2 (95:5). The entire hypothalamus and preoptic area (POA) were dissected over ice and removed as a block; 350-μm-thick slices were made using a McIlwain tissue chopper, beginning approximately 2 mm anterior to the mammillary bodies. Based on anatomical landmarks observed in comparable slices from fixed tissue, four slices of POA and three slices of middle hypothalamus (MH) were obtained as described by Etgen and Petitti (1986, 1987) and used in superfusion assays. The MH slices include the arcuate nucleus, the ventromedial nucleus, the dorsomedial nucleus, and much of the lateral hypothalamus.

Superfusion assay and drug treatments. Each slice was placed in an individual tissue culture well with 350 μl of medium containing 0.1 μM ^{3}H-NE and preincubated with shaking in an O_2:CO_2 (95:5)-saturated environment at 33°C for 45 min. Slices were then loaded into individual chambers of a Brandel SF2000 (Brandel, Inc., Gaithersburg, MD) automated superfusion apparatus and washed for 30 min at a flow rate of 1 ml/3 min with O_2:CO_2-saturated medium containing 1.0 μM desipramine (DMI), an NE reuptake blocker. Following the washout period, 20 consecutive, 3 min fractions were collected for each slice, and the ^{3}H content of the effluent was determined by liquid scintillation counting. After collection of the last fraction, the radioactivity remaining in the slice was determined by dissolving the slice in 1.0 M NaOH; this allowed calculation of the total tissue ^{3}H content at the start of each fraction.

Slices were stimulated once or twice (S1 at t = 15 min and S2 at t = 39 min) for 3 min with freshly oxygenated medium containing 1.0 μM DMI and either 20 or 50 mM KCl. In order to maintain solution osmolality, NaCl was removed on a mole-for-mole basis to compensate for the addition of KCl. The imidazoline α_2-antagonists idazoxan (IDA) and its 2-methoxy derivative RX821002 (RX) as well as the alkaloid α_2-antagonists yohimbine (YOH) and rauwolscine (RAU) were applied 15 min prior to S1 or S2 and remained present until the end of the experiment. The applied concentration of drug is rapidly reached and maintained (data not shown).

KCl-evoked release was expressed as percentage of total tissue ^{3}H, calculated by subtracting basal release from stimulus-evoked release and dividing by the total tissue ^{3}H content at the start of the fraction. Basal release was defined as the effluent from the four samples prior to the S1 and S2. Calculations of S1 and S2 included the four samples after application of KCl at t = 15 and 39 min, respectively. The S2:S1 ratios were calculated by dividing the percentage of total tissue ^{3}H released during S2 by that released during S1. Individual values from the four POA slices and the three MH slices were averaged to obtain a single value for the POA and MH, respectively, of each animal.

Statistics. When only two groups were being compared, t tests were used to evaluate significant differences between means. For more than two groups, analysis of variance was used to determine significant differences between means. Differences were considered significant if $p < 0.05$. Planned post hoc comparisons were made using the Newman–Keuls multiple-range test.

Materials. EB and P were purchased from Steraloids, Inc. (Wilton, NH). Metofane was obtained from Pitman-Moore, Inc. (Atlanta, GA). Radiolabeled ^{3}H-NE (specific activity, 56.9 Ci/mmol) was obtained from New England Nuclear (Boston, MA). IDA, RX, and RAU were purchased from Research Biochemicals, Inc. (Natick, MA). YOH and DMI were purchased from Sigma (St. Louis, MO).

Results

Characterization of basal and KCl-evoked efflux of ^{3}H-NE from MH and POA slices

KCl-evoked ^{3}H-NE overflow from MH and POA slices from OVX animals requires Ca^{2+} ions (Fig. 1), indicating that the evoked release utilizes vesicular exocytotic mechanisms (Del Castillo and Katz, 1954; Dodge and Rahamimoff, 1967; Baker et al., 1971; Llinás and Heuser, 1977; Israel and Lesbats, 1981;

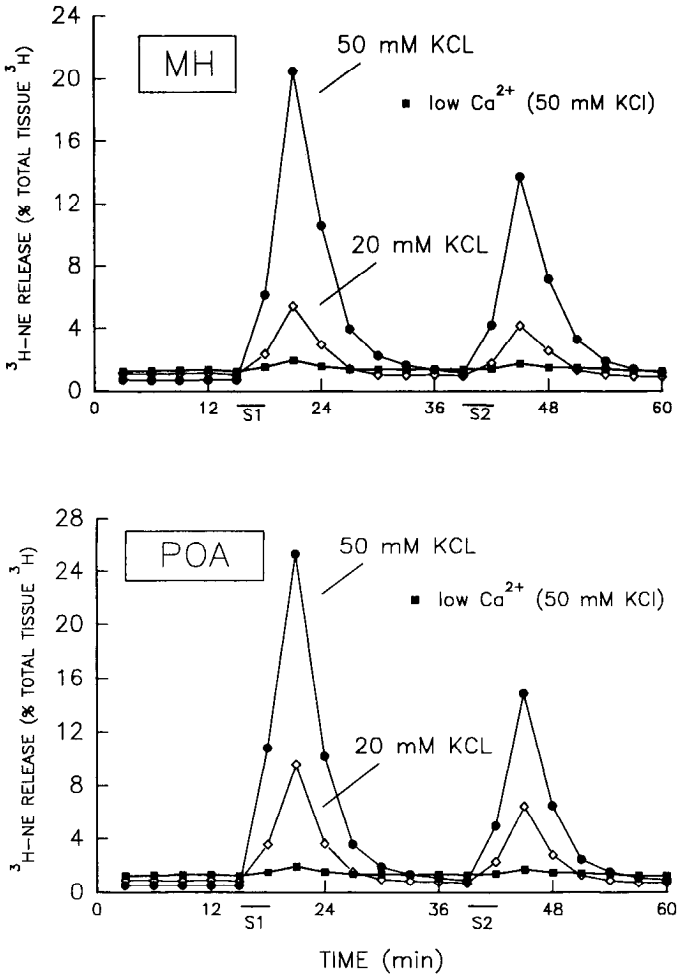

Figure 1. KCl-induced NE release is Ca^{2+} dependent. Release of ^{3}H-NE from MH and POA slices was evoked by two 3 min pulses (S1 and S2) of either 20 mM or 50 mM KCl separated by 24 min as indicated. Low-Ca^{2+} medium was made by excluding $CaCl_2$ from the buffer. The data are representative of three or four independent replications. The total ^{3}H uptake varied about fourfold among individual slices (range, 200,000–730,000 dpm/slice); despite this variability in total NE uptake, basal efflux when expressed as percentage of total tissue ^{3}H content varied less than 5% among slices.

Kretz et al., 1984). The low level of basal ^{3}H efflux was not affected by the removal of Ca^{2+} ions. Basal efflux of ^{3}H is approximately 40% NE and 60% deaminated metabolites, whereas stimulated release is greater than 65% authentic NE, as determined by high-pressure liquid chromatography with electrochemical detection. The magnitude of ^{3}H-NE release is also dependent on KCl concentration (Fig. 1). The likely dilution of the KCl pulse was determined with tracer compounds. Although the pulsed solution contained 20 or 50 mM KCl, it is likely that at the peak of the pulse, the concentration of KCl reaching the slice is diluted about 50% (data not shown). Based on these results, all subsequent experiments used 20 mM KCl to evoke NE efflux.

Estradiol effects on drug-free NE release

Table 1 shows that when MH slices are first stimulated with 20 mM KCl (S1), 20% more ^{3}H-NE is released from EB-exposed slices compared to OVX controls. In contrast, ^{3}H-NE release from POA slices is similar in both groups. Basal efflux is not affected by hormone treatment. Likewise, estradiol has no effect

Table 1. Estradiol increases KCl-evoked release during S1 in MH slices

	S1, % total tissue ^{3}H ($\bar{X}$ ± SEM)	
	OVX	Estradiol
MH	8.8 ± 0.3	10.8 ± 0.5*
POA	8.9 ± 0.4	7.8 ± 0.3

When slices from OVX control or estradiol-treated rats were first stimulated with 20 mM KCl, 20% more ^{3}H-NE was released by MH slices from estradiol-treated rats than from control females. ^{3}H-NE release from POA slices was similar in both groups. OVX, n = 42; estradiol, n = 37. The large n was attained because drug-free S1 values were analyzed from all experiments.

* $p < 0.05$ versus OVX MH, t test.

on the S2:S1 ratio, which is approximately 0.6 in all drug-free slices regardless of hormone treatment.

Effects of steroids on imidazoline α_2-antagonist augmentation of KCl-evoked ^{3}H-NE release

To determine whether α_2-adrenoceptor–mediated inhibition of NE release is an active mechanism in the hypothalamus and POA of female rats, we evaluated the effects of two imidazoline α_2-antagonists on ^{3}H-NE release in slices from OVX and OVX, EB-treated animals (Fig. 2). When 10 μm IDA is applied 15 min prior to S1, KCl-evoked ^{3}H-NE release is facilitated by 89% in slices from OVX rats compared to a 36% increase in MH slices from estradiol-treated animals ($p < 0.01$). Similarly, 10 μM RX applied 15 min prior to S1 in slices from OVX rats causes a 57% increase over control but elicits a significantly lower (20% increase) response in slices from estradiol-treated rats ($p < 0.01$). Although similar trends are obtained in POA slices, the differences between OVX and EB-exposed slices are not significant (Fig. 2).

Estradiol also attenuates α_2-antagonist facilitation of NE release from MH slices after S1 but during S2. Figure 3 shows representative ^{3}H-NE release data from individual MH slices from OVX control and EB-exposed rats that were stimulated twice with 20 mM KCl. The S2 was preceded by either 10 μM IDA or vehicle (control). Control MH slices from OVX and EB-treated rats have S2:S1 ratios of 0.67 ± 0.03 (n = 4) and 0.60 ± 0.04 (n = 4), respectively. When 10 μM IDA is applied 15 min prior to S2, the S2:S1 ratio rises to 1.8 ± 0.1 (n = 4) in

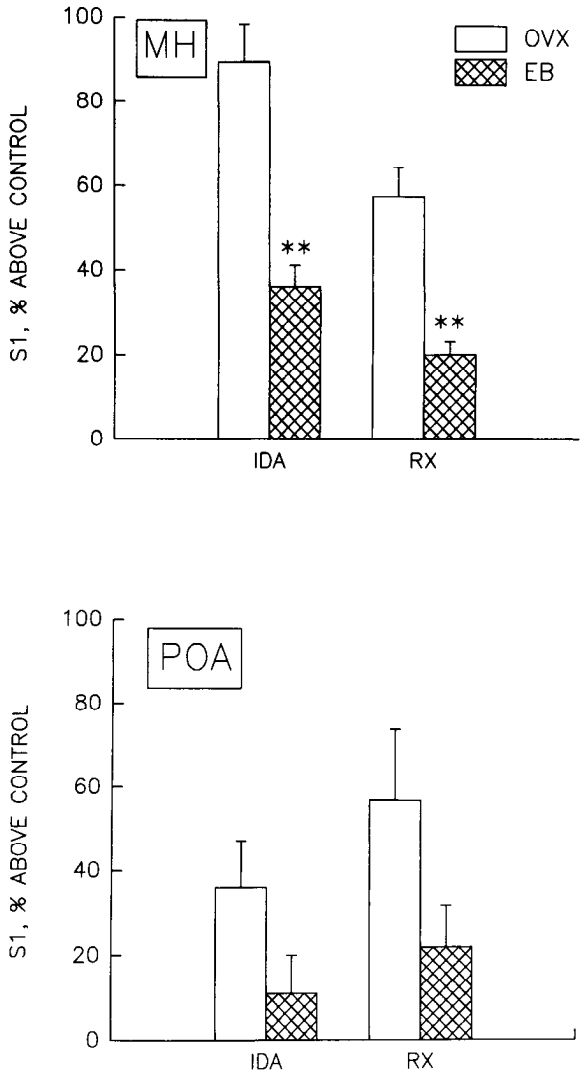

Figure 2. Estradiol attenuates α_2-antagonist facilitation of NE release from MH slices during S1. The α_2-antagonists IDA and RX (10 μM each) or vehicle (control) were applied to slices from OVX and OVX, EB-treated females 15 min prior to S1 and remained present until the end of the experiment. IDA, n = 3; RX, n = 6. Two-way ANOVA indicated a significant main effect of hormone in the MH (F = 15.5; df = 1,14; $p < 0.002$) but not POA (F = 2.6; df = 1,14; $p > 0.1$). **, $p < 0.01$ versus OVX, Newman-Keuls.

Table 2. IDA and RX effects on the S2/S1 ratio in POA slices from OVX and estradiol-treated rats

	S2:S1 ratio ($\bar{X}$ ± SEM)	
Treatment	OVX	Estradiol
Control	0.62 ± 0.02	0.56 ± 0.05
1 μM IDA	0.87 ± 0.08	0.79 ± 0.07
10 μM IDA	0.89 ± 0.11	0.74 ± 0.10
1 μM RX	0.50 ± 0.04	0.50 ± 0.002
10 μM RX	1.2 ± 0.08**	0.57 ± 0.03

IDA, RX, or vehicle (control) was applied 15 min prior to S2 and remained present until the end of the experiment (n = 4/group). Two-way ANOVA indicated no significant between-group differences in IDA-treated slices. For RX-treated slices, there was a significant main effect of hormone (F = 58.4; df = 2,23; $p < 0.0001$) and drug dose (F = 42.0; df = 2,23; $p < 0.0001$), and a significant hormone × drug dose interaction (F = 42.1; df = 2,23; $p < 0.0001$).

** $p < 0.01$ versus paired control and versus estradiol at same drug dose, Newman-Keuls.

control slices. In contrast, the ratio increases only to 1.1 ± 0.2 (n = 4) in EB slices (Fig. 3). The S2:S1 ratio of MH slices exposed to combined EB plus P resembles that of slices exposed to EB alone, increasing from a control level of 0.66 ± 0.02 (n = 4) to 1.0 ± 0.2 (n = 4) when 10 μM IDA is applied prior to S2. Moreover, IDA facilitation of KCl-evoked ^{3}H-NE release is dose dependent in hypothalamic slices from OVX control animals but not in slices from estradiol-exposed animals (Fig. 4). When 1 and 10 μM concentrations of IDA are applied to MH slices from control rats 15 min prior to S2, the S2:S1 ratio rises to 1.3 ± 0.1 (n = 4) and 1.8 ± 0.1 (n = 4), respectively. In contrast, the S2:S1 ratio of MH slices from EB-treated rats is ≈0.94 at both 1 and 10 μM IDA. In POA slices, neither 1 nor 10 μM IDA significantly increases the S2:S1 ratio, regardless of hormone treatment (Table 2). However, 10 μM RX applied 15 min prior to S2 increases the S2:S1 ratio to 1.52 ± 0.05 (n = 4) and 1.2 ± 0.1 (n = 4), respectively, in MH and POA slices from OVX

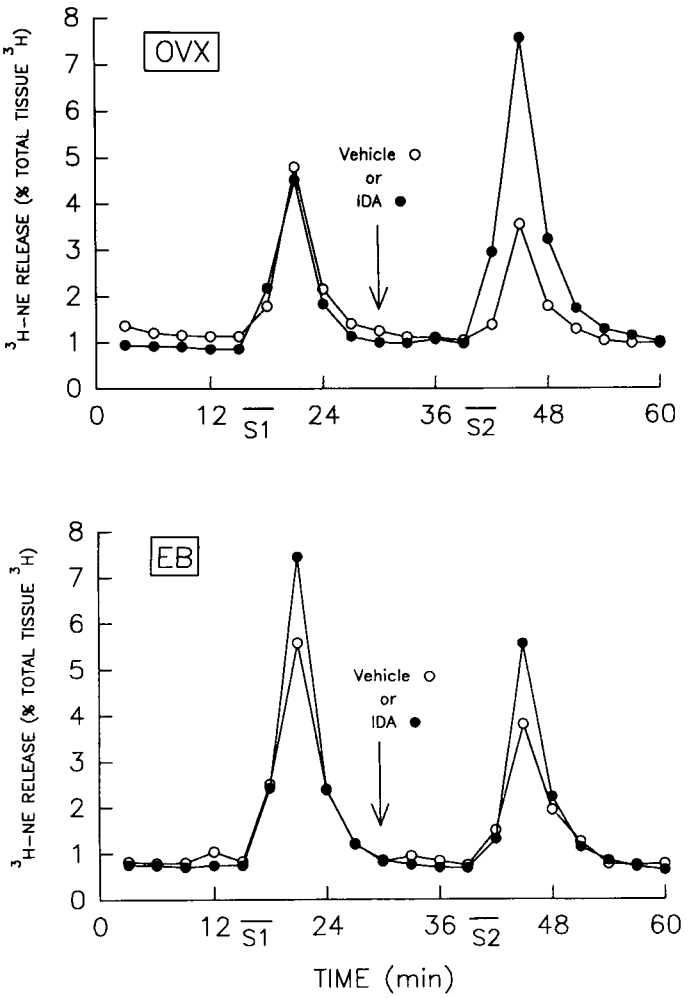

Figure 3. Estradiol attenuates α_2-antagonist facilitation of NE release from MH slices during S2. ^{3}H-NE release is shown from slices from OVX control and EB-exposed rats that were stimulated with 20 mM KCl in the absence (S1) or presence (S2) of vehicle (control) or 10 μM IDA. Control MH slices from OVX control and EB-treated rats had S2:S1 ratios of 0.67 ± 0.03 (n = 4) and 0.60 ± 0.04 (n = 4), respectively. When IDA was applied 15 min prior to S2, the S2:S1 ratio rose to 1.8 ± 0.1 (n = 4) in OVX slices and to 1.1 ± 0.2 (n = 4) in EB slices.

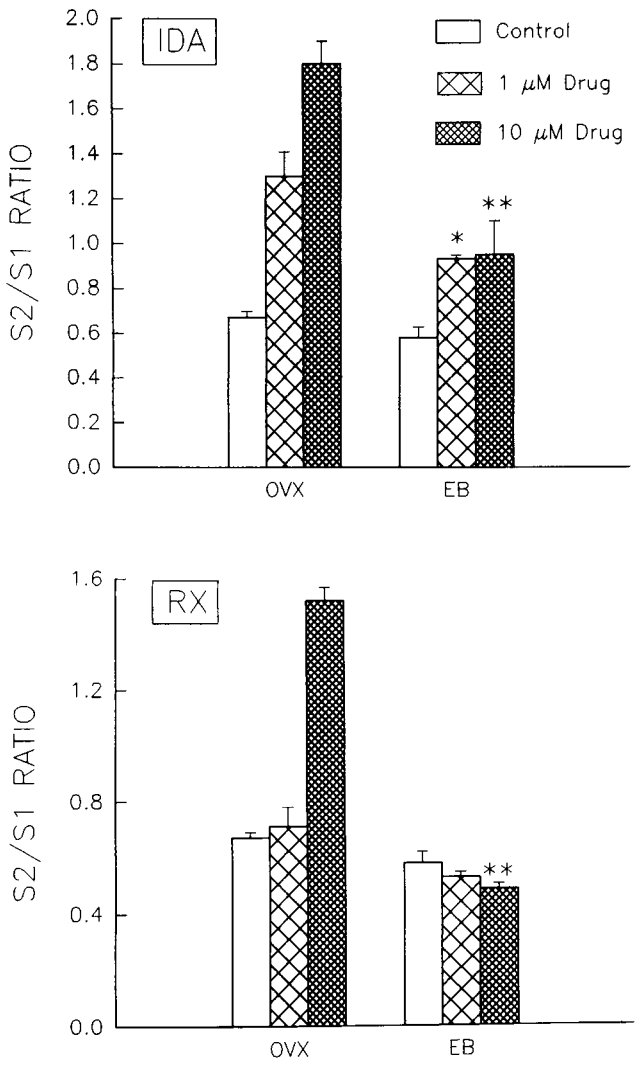

Figure 4. IDA and RX effects on the S2:S1 ratio in MH slices from OVX control and EB-treated rats. Drugs or vehicle (control) were applied to slices 15 min prior to S2 and remained present until the end of the experiment (n = 4/group). For IDA-treated slices, there were significant main effects of hormone (F = 23.4; df = 1,17; $p < 0.01$) drug dose (F = 54.2; df = 2,17; $p < 0.0001$), and a significant hormone × drug dose interaction (F = 5.7; df = 2,17; $p < 0.02$). For RX-treated slices, there were also significant main effects of hormone (F = 274; df = 1,21; $p < 0.0001$) and drug dose (F = 83.1; df = 2,21; $p < 0.0001$), and a significant hormone × drug dose interaction (F = 151; df = 2,21; $p < 0.0001$). *, $p < 0.05$ versus OVX at same drug dose, Newman-Keuls; **, $p < 0.01$ versus OVX at same drug dose, Newman-Keuls.

control females. Estradiol priming completely abolishes the ability of RX to augment NE release in slices from both brain regions (Fig. 4, Table 2).

Estradiol does not affect alkaloid α_2-antagonist effects

Additional studies were carried out with alkaloid α_2-antagonists to begin evaluating the receptor subtype specificity of α_2-antagonist augmentation of ^{3}H-NE release in hypothalamic and POA slices from female rats (Fig. 5). Interestingly, 10 μM concentrations of the alkaloid α_2-antagonists YOH and RAU fail to increase the S2:S1 ratio in MH and POA slices from either control or estradiol-treated animals. Only 1 μM YOH modestly increases the S2:S1 ratio in MH and POA slices from OVX animals, and this augmentation was not attenuated by EB (Fig. 5).

Discussion

The present study demonstrates that NE release in the hypothalamus of OVX female rats is under inhibitory control by α_2-adrenoceptors and that this inhibition is attenuated by estradiol, in a dose known to prime female reproductive behavior. The conclusion that α_2-adrenoceptors mediate a potent inhibition of NE release in the hypothalamus of OVX rats is supported by several findings. First, preincubation (15 min prior to S1) of slices from OVX animals with the imidazoline α_2-antagonists IDA and RX markedly potentiates KCl-evoked NE release. This demonstrates that in hypothalamic slices from OVX rats, KCl-evoked NE release is under a tonic, α_2-adrenoceptor–mediated inhibition. Hence, it appears that the low level of basal NE efflux is sufficient to inhibit depolarization-evoked NE release in slices from OVX animals. Similarly, in the experimental paradigm where both S1 and S2 were elicited, and the α_2-antagonists were applied 15 min prior to S2, a marked facilitation of NE release from OVX hypothalamic slices was elicited by α_2-antagonists. IDA and RX increased the S2:S1 ratio in MH slices from OVX

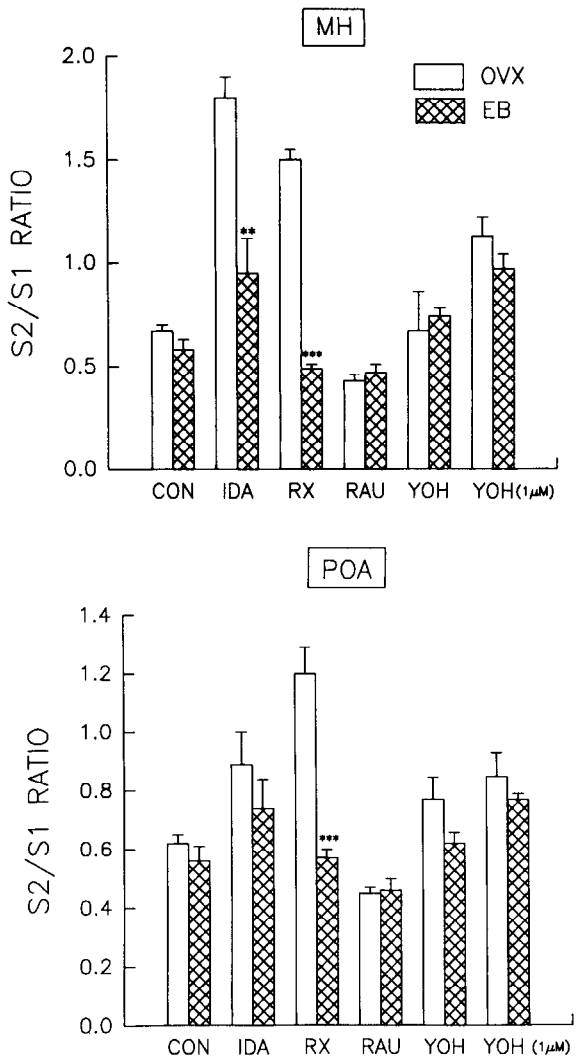

Figure 5. The effects of alkaloid α_2-antagonists on NE release are not modified by estradiol. Drugs (10 μM unless otherwise indicated) or vehicle (*CON*) were added 15 min prior to S2 and remained present until the end of the experiment. The alkaloid α_2-antagonist YOH at 1 μM produced a modest facilitation of ^{3}H-NE release that was not attenuated by EB treatment. Each value represents the mean (±SEM) of three to six independent replications. Data on IDA and RX are repeated from Figure 4 and Table 2 to facilitate comparisons. **, $p < 0.01$ versus paired OVX, t test; ***, $p < 0.001$ versus paired OVX, t test.

females approximately 300% relative to the S2:S1 ratio in the absence of drug. These findings demonstrate the existence of a potent α_2-adrenoceptor–mediated inhibition of NE release in slices from OVX animals that can be removed by preincubation with the α_2-antagonists IDA and RX.

It is interesting to note that the facilitatory effects of IDA and RX on NE release are greater when the drugs are applied between S1 and S2 than when applied prior to S1. This observation implicates NE released in response to the first KCl pulse as the agent responsible for producing α_2-adrenoceptor–mediated inhibition of NE release during S2. The negative feedback actions of NE released during S1 may also account in part for the approximately 40% reduction in the amount of ^{3}H-NE released during S2 (i.e., S2:S1 $\approx$ 0.6).

In marked contrast with MH slices from OVX control females, preincubation of MH slices from estradiol-primed rats with IDA and RX prior to S1 produced more moderate (20–36%) increases in NE release. Furthermore, IDA application prior to S2 in MH slices from estradiol-treated rats produced only a slight elevation in the S2:S1 ratio relative to the drug-free S2:S1, and RX failed to produce any facilitation of the S2:S1 ratio in either hypothalamic or POA slices from estradiol-treated rats. These observations are consistent with the notion that administration of estradiol *in vivo* significantly attenuates α_2-adrenoceptor–mediated inhibition of NE release in the hypothalamus and POA. This conclusion is further supported by the finding that NE release in response to a single drug-free KCl stimulation is 20% greater in MH slices from estradiol-treated rats than from OVX controls. The addition of P neither alleviates nor enhances the action of estradiol. In many respects, the POA and MH slices showed similar responses to drug and hormone administration, although the POA responses were generally diminished. For example, both MH and POA slices from OVX control animals showed RX facilitation of NE release that was attenuated by estradiol. Slices from both brain areas also displayed a modest facilitation of NE release by 1.0 μM YOH, a response that was not affected by estradiol. However, estradiol did not increase drug-free, KCl-evoked NE release in POA slices, and IDA did not significantly increase the S2:S1 ratio in POA slices regardless of hormone treatment. These findings might reflect regional differences in responsiveness to estradiol and/or the distribution of α_2-receptor subtypes.

The α_2-adrenoceptor agonist clonidine applied 15 min before S2 produced a slight reduction in the S2:S1 ratio in both control and estradiol-treated slices ($\approx$0.4; Karkanias and Etgen, unpublished observations) relative to the S2:S1 ratio in drug-free controls ($\approx$0.6). The small effects of the α_2-agonist clonidine were not unexpected since numerous studies have shown that experimental paradigms that favor antagonist effects (e.g., slow superfusion rates, high biophase concentrations of NE, high-frequency electrical stimulation) tend to show diminished agonist effects. This is attributed to the fact that when α_2-adrenoceptor occupancy is high, available receptor reserves for agonist occupancy and detection of agonist effects are diminished (for review, see Westfall, 1990). It should also be noted that clonidine is only a partial α_2-agonist, and there is limited information about its specificity for α_2-receptor subtypes. The presence of a small clonidine-mediated suppression of S2 in estradiol-treated slices indicates that the attenuation of hypothalamic α_2-adrenoceptor function may not be complete. However, the present experiments utilized a single dose of estradiol; higher doses may produce a further attenuation of α_2-adrenoceptor–mediated inhibition of NE release.

One possible mechanism by which estradiol could attenuate α_2-adrenoceptor–mediated inhibition of NE release is via an alteration of α_2-adrenoceptor binding properties. Previous reports have shown that estradiol can influence brain α_2-adrenoceptor number. Wilkinson and Herdon (1982) demonstrated that diethylstilbestrol, a potent synthetic estrogen, decreases α_2-adrenoceptor number in rat hypothalamus and amygdala. However, brain region differences in estradiol regulation of α_2-adrenoceptor number exist. For example, in studies measuring α_2-receptors with ^{3}H-*p*-aminoclonidine and receptor autoradiography, Johnson et al. (1985, 1988) reported that treatment of OVX guinea pigs with estradiol increases α_2-adrenoceptor binding in the POA but decreases binding in the ventromedial hypothalamus. Our work with ^{3}H-IDA binding in membrane fractions from the POA and MH detected no measurable estro-

gen-induced changes in α_2-adrenoceptor levels (Etgen and Karkanias, 1990). A possible explanation for the discrepancy between the present findings and those of Johnson et al. (1985, 1988) is that autoradiographic methods do not distinguish between pre- and postsynaptic α_2-receptors whereas the functional measures used herein are likely to reflect changes in presynaptic receptors only. Alternatively, our findings may be reconciled with the autoradiographic results if one considers that only a single concentration of ^{3}H-*p*-aminoclonidine was used in the receptor autoradiography study. It is possible that the decrease observed in α_2-adrenoceptor number in the hypothalamus is due to a decrease in α_2-adrenoceptor affinity. Antagonists are not sensitive to changes in agonist affinity states produced by interactions of the liganded receptor with G-proteins (Tsai and Lefkowitz, 1979; Lynch and Steer, 1981; Brodde et al., 1982; U'Prichard et al., 1983). Studies using the α_2-antagonist ^{3}H-IDA would therefore not detect a change in agonist affinity for the α_2-receptor, whereas the agonist ^{3}H-*p*-aminoclonidine might be sensitive to different receptor affinity states. We are presently conducting agonist competition studies in hypothalamic membranes to determine whether estradiol affects the agonist affinity states of α_2-adrenoceptors.

The pharmacology of the α_2-adrenoceptor mediating inhibition of NE release in hypothalamic/POA slices resembles the α_{2D}-adrenoceptor. This α_2-receptor subtype may be the rat homolog of the human α_{2A}-adrenoceptor found in human platelets (Chalberg et al., 1990; Bylund et al., 1991; Harrison et al., 1991; Lanier et al., 1991; Bylund, 1992). The cloning of the human platelet α_2-adrenoceptor was first reported by Lefkowitz and colleagues (Kobilka et al., 1987), and it is referred to as the $\alpha_{2\text{-}C10}$- or α_{2A}-adrenoceptor. A rat α_2-adrenoceptor was later cloned and found to have an amino acid sequence homology of 89% with the human α_{2A}-adrenoceptor (Chalberg et al., 1990; Lanier et al., 1991). Initial pharmacological characterization indicated that the rat α_2-receptor was most similar to the α_{2A}-adrenoceptor of the human platelet with one significant difference: the rat clone (RG20) had a markedly lower affinity for the alkaloid α_2-adrenoceptor antagonists YOH and RAU (10- and 20-fold, respectively) than the α_{2A}-adrenoceptor. Therefore, the rat receptor may be a fourth α_2-adrenoceptor subtype currently designated α_{2D} (see Bylund, 1992).

The present study found that the imidazoline α_2-antagonists IDA and RX were far more effective in facilitating NE release than the alkaloid α_2-antagonists YOH and RAU. In fact, only 1 μM YOH produced a modest facilitation of NE release, and this facilitation was not attenuated by estradiol. This pharmacological profile most closely resembles the α_{2D}-adrenoceptor subtype. Thus our findings provide the first evidence that α_2-adrenoceptor–mediated inhibition of NE release in the hypothalamus of female rats may be mediated by an α_{2D}-adrenoceptor subtype and that estradiol attenuates this putative α_{2D}-adrenoceptor–mediated inhibition.

To date, α_{2D}-adrenoceptors have been demonstrated in rat submaxillary gland (Michel et al., 1989), bovine pineal gland (Simonneaux et al., 1991), rat cerebral cortex and cerebellum (O'Rourke et al., 1992), possibly rat vas deferens (Smith and Docherty, 1992), and isolated perfused rat kidney (Schwartz and Malik, 1992). In addition, Zeng and Lynch (1991), using α_2-adrenoceptor subtype–specific hybridization clones, detected mRNA encoding the rat homolog of the α_{2A}-adrenoceptor in the midbrain, brainstem, spinal cord, pituitary, and diencephalon. Others have demonstrated the presence of the rat homolog of α_{2A}-adrenoceptors in rat brain cortex, medulla, and hypothalamus (MacKinnon et al., 1992; Rosin et al., 1992), as well as the locus coeruleus (Go et al., 1992; Scheinin et al., 1992). Furthermore, there is emerging evidence that α_{2D}-adrenoceptors are involved in inhibition of transmitter release in rat brain synaptosomes (Gobbi et al., 1990) and other tissues. Waterfall et al. (1985) showed that IDA was more effective than YOH or RAU against clonidine-induced inhibition of NE release in rat vas deferens. Smith and Docherty (1992) have also provided evidence that the α_2-adrenoceptor in rat vas deferens that inhibits NE release is similar to that of rat submandibular gland and may be the rat homolog of the human α_{2A}-adrenoceptor or the putative α_{2D}-adrenoceptor. In addition, Schwartz and Malik (1992) have characterized a presynaptic α_2-adrenoceptor that inhibits NE release from isolated, perfused rat kidney and closely resembles the α_{2D}-adrenoceptor.

It has also been reported that the imidazoline α_2-adrenoceptor antagonist IDA can bind to sites sometimes referred to as nonadrenergic IDA binding sites. Indeed, this pharmacological profile may have accounted for our earlier failure to detect hormone-dependent changes in hypothalamic α_2-adrenoceptor binding (Etgen and Karkanias, 1990). However, the imidazoline-α_2 antagonist RX does not bind to these nonadrenergic sites (Langin et al., 1990a,b; Senard et al., 1990). In our studies, estradiol was more effective in attenuating RX facilitation of NE release than the facilitation caused by IDA. Thus, estradiol is likely to be modifying NE release via attenuation of α_2-adrenoceptor–mediated action rather than via regulation of nonadrenergic IDA binding sites.

We previously demonstrated that female rats engaging in hormone-dependent reproductive behavior exhibit augmented release of NE from the ventromedial hypothalamus (Vathy and Etgen, 1989). The present results suggest that α_2-adrenoceptor–mediated inhibition of NE release is a highly active mechanism in the hypothalamus of OVX female rats and that estradiol, in doses known to prime female reproductive behavior, may facilitate hypothalamic NE release by reducing the ability of released NE to act as a negative feedback inhibitor of its own release. Furthermore, the receptor subtype involved may be the α_{2D}-adrenoceptor. An interesting direction for future research will be to determine the mechanism by which estradiol mediates the attenuation of α_2-adrenoceptor–mediated inhibition of NE release and whether estradiol affects other G_i-linked modulators of NE release.

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