Properties and Sex-Specific Differences of GABA_\alpha Receptors in Neurons Expressing \gamma 1 Subunit mRNA in the Preoptic Area of the Rat

SHOLEEN T. NETT, 1 JUAN CARLOS JORGE-RIVERA, 1 MARGARET MYERS, 1 ANN S. CLARK, 3 AND LESLIE P. HENDERSON 1,2

1Department of Physiology and 2Department of Biochemistry, Dartmouth Medical School; and 3Department of Psychology, Dartmouth College, Hanover, New Hampshire 03755

Neurons expressing GABA_\alpha receptors can be found in many brain regions, including those involved in sexual behaviors. In the preoptic area (POA), GABAergic neurons play a critical role in regulating hypothalamic function. Sex-specific differences have been detected in the levels of GABAergic transmission within the mPOA, with higher expression of the \gamma 1 subunit mRNA in male than female rats. This study aimed to characterize the biophysical and pharmacological properties of native GABA_A receptors in neurons of both sexes.

INTRODUCTION

Sexual dimorphism in neural structure has been described best in the medial preoptic region (mPOA) of the mammalian brain (for review, Döhler 1991; Gorski et al. 1978; Sakuma 1995). This region is critical for the regulation of neuroendocrine function and for the production of sexual behaviors (for review, Madeira and Lieberman 1995; Theodosius and Pouilain 1993). Within the mPOA, transmission mediated by \gamma-aminobutyric acid type A (GABA_A) receptors has been shown to regulate gonadotropin secretion (Adler and Crowley 1986; Moguilevsky et al. 1991) and to modulate the expression of both female sexual receptivity (for review; McCarthy 1995) and male copulatory behavior (Fernández-Guasti et al. 1986; for review, Meisel and Sachs 1994). Although GABAergic transmission within the mPOA plays a key role in these hypothalamic functions, no study to date has examined the physiological or pharmacological properties of GABA_A receptors in this region.

The GABA_A receptor is a heterooligomeric protein for which five families of mammalian subunit genes (\alpha 1–6, \beta 1–3, \gamma 1–3, \delta, and \epsilon) have been identified (Davies et al. 1997; for review, Macdonald and Olsen 1994; Whiting et al. 1997). It is believed that \alpha 2, \beta 3, and \gamma 1 subunits comprise the predominant class of endogenous receptors in neurons of the mPOA in the adult rat brain (Herbison and Fénelon 1995). Although \alpha 2 and \beta 3 subunit mRNAs are expressed throughout the rat brain, high levels of \gamma 1 subunit mRNA are limited to a small subset of interconnected forebrain regions that are known to be important in the regulation of sexual behaviors. Specifically, high levels of \gamma 1 expression are detected in the mPOA, the medial amygdala, the lateral septal nuclei, and the bed nucleus of the stria terminalis (Araki et al. 1992, 1993; Herbison and Fénelon 1995; Wisden et al. 1992; Ymer et al. 1990). The highly restricted distribution in expression of this subunit mRNA has led to the suggestion that \gamma 1-containing GABA_A receptors may play a critical role in the regulation of hypothalamic function (Herbison and Fénelon 1995; Ymer et al. 1990).

Chloride flux through the GABA_A receptor is modulated by a number of compounds acting at the benzodiazepine (BZ) recognition site (for review, Macdonald and Olsen 1994; Sieghart 1995). The effects of specific modulators depend on both \alpha and \gamma subunit composition (Macdonald and Olsen 1994; Sieghart 1995). \gamma 1 subunits primarily coassemble with \alpha 2 subunits in vivo (Wisden and Seeburg 1992) to create a receptor with a BZ type II pharmacological profile (for review, Sieghart 1995). Two classes of compounds that act at the BZ site, the imidazopyridines and the \beta-carbolines, exhibit significantly different effects at recombinant receptors containing \gamma 1 versus \gamma 2 subunits. Specifically, the imidazopyridine, zolpidem, is a positive modulator of recombinant receptors containing \alpha 2 and \gamma 2 subunits, but is either...
Electrophysiological recording

For slice recordings, BZ modulators were applied via the bath in the present study, we have assessed the properties of GABA A added to aCSF at a final concentration of 10 μM and 1 μM to contain GABA-containing neurons that are believed to contain a subunit (Bormann and Kettenmann 1988). In contrast to Sigma Chemical (St. Louis, MO), Recordings were made at 20 ± 2°C, at a holding potential (Vh) of −80 mV; and for slice recordings, preferentially from the superficial surface. The imidazopyridine, zolpidem hemi-tartrate (N,N-6-trimethyl-2-(4-methylphenyl)-imidazo[1,2-a]-pyridine-3-acetamide; a gift from Synthelabo Recherche, Bagneux Cedex, France) was dissolved in water (pH 4.8) and added to aCSF at final concentrations of 1 mM to 20 μM. β-CCM (9H-pyrido [3,4-b] indole-3-carboxylic acid methyl ester) and diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) were purchased from RBI (Natick, MA), dissolved in dimethyl sulfoxide (DMSO), and added to aCSF at a final concentration of 10 and 1 μM, respectively (0.01% DMSO). To determine a baseline for establishing drug effects, averaged peak current amplitudes of responses in the absence of any drug were measured at 3 min intervals for a period of 12 min. The standard deviation of response amplitudes over this time period was ±8% for all cells (n = 5). This measurement reflected inclusion of a few outlying individual data points. Exclusion of these few points provided an estimate of ±7% deviation of peak current amplitude over time in the absence of drug. BZ modulators were categorized as “without effect” if drug-induced changes were <7% of the initial value.

For slice recordings, BZ modulators were applied via the bath using gravity flow and a Hamilton multiport valve to switch between aCSF with and without BZ modulators. For all recordings, 3−5 min of predrug data were acquired. The bath was then changed to aCSF containing diazepam, β-CCM or zolpidem, and 3 min later, data were again acquired for 3−5 min. The bath was then switched back to aCSF alone and 3 min were allowed to pass before postdrug data were collected. For application of GABA plus BZ modulators to acutely isolated cells, solution exchange of the double barreled theta glass was accomplished by use of solenoid valves attached to a vacuum line (Zhu and Vicini 1997), thus neurons were pre-equilibrated with modulators and saline before being exposed to BZ modulators plus GABA. Concentration-response data for modulation of peak current amplitude by different concentrations of zolpidem was fitted according to Kapur and MacDonald (1996).

Data from slices and isolated cells were acquired and analyzed as described previously (Smith et al. 1996) with the use of a List EPC-7 amplifier (AL A Scientific Instruments, Westbury, NY) and either an Atari Mega 4 computer system or a PowerMac 8600. For analysis of sIPSCs, the acquisition program Acquire (Instrutech, Elmont, NY) was adapted to acquire randomly occurring, spontaneous synaptic currents. To avoid distortion due to cable properties, only events with 10−90% rise times ≥2 ms were included in data averages (Smith et al. 1996). For sIPSCs, >50 individual responses from each cell were averaged, and the software program, Review, was used to estimate rise times and to provide multiple iterative, exponential fits to current decays. Responses elicited with ultrafast perfusion were acquired with either the program Acquire or Pulse and analyzed by either Review or PulseFit (Instrutech). Data from both acutely isolated cells and slice recordings were digitized at 23.6 kHz and filtered at 4 kHz for analysis. Values are means ± SE. Statistical significance was determined using a two-tailed Student’s t-test.
tailed Student’s t-test for comparison of means and a χ² test of association for comparison of percentages.

In situ hybridization

In situ hybridizations were carried out according to Wisden et al. (1992), with minor modifications, for animals of both sexes at PNI4 (the mean age of animals used for physiological experiments). Oligonucleotides corresponding to subunit residues 341–354 of the γ1 cDNA (Ymer et al. 1990) and corresponding to subunit residues α1: 342–356, α2: 340–344, and α3: 355–369 (Wisden et al. 1992) were synthesized by Operon Technologies (Alameda, CA) and 3’ end-labeled using a 10:1 molar ratio of α-[35S]-dATP (Du Pont/New England Nuclear, Boston, MA; 1,000–1,270 Ci/mmole) to oligonucleotide using terminal deoxynucleotidyl transferase (Promega, Madison, WI). Unincorporated, free nucleotides were removed using Centri-Sep columns (Princeton Separations, Adelphia, NJ). Labeled probe (specific activity 0.5–1 × 10⁶ cpm/μg) was resuspended in a hybridization mixture consisting of 50% deionized formamide, 4× SSC, 1× Denhardt’s solution, 100 μg/ml salmon sperm DNA, 150 μg/ml yeast tRNA, 50 mM dithiothreitol, and 10% dextran sulfate at a concentration of 1.1 × 10⁷ cpm/100 μl. Animals were decapitated, brains quickly removed, frozen on dry ice, and stored at −80°C for 1–4 wk. Sections (20 μm) were cut using a Hacker-Bright model OTF cryostat, mounted on poly-L-lysine-coated slides, fixed with 4% paraformaldehyde (Fisher Scientific, Springfield, NJ) for 20 min, washed once with 3× and then twice in 1× phosphate-buffered saline (PBS), and dehydrated in serial ethanol (EtOH) (30, 60, 80, 95, and 100%).

Before hybridization, slides were brought to room temperature, washed once with 0.1 M glycine in 1× PBS for 3 min, twice for 10 min each in 1× PBS, incubated for 10 min in 0.1 M triethanolamine/0.25% acetic anhydride, washed twice for 15 min each in 2× SSC, dehydrated in serial EtOH, and air dried. Hybridization was carried out in a moistened chamber at 42°C overnight. After hybridization, sections were washed for 5 min at room temperature in 1× SSC, followed by three 30 min washes at 55°C in 1× SSC in a shaking water bath, and a final 5 min wash in 1× SSC at room temperature. Sections were dehydrated in 30% EtOH/0.6 M NaCl, 60% EtOH/0.6 M NaCl, 80% EtOH, 95% EtOH, and 100% EtOH, air dried for ~1 h, and apposed to Biomax MR film (Eastman Kodak, New Haven, CT) for 2–3 wk. Included in each film cassette was a slide mounted with 14C microscales (Amersham, Arlington Heights, IL), which was used to quantify densitometric signals (Huntsman et al. 1994) and slides incubated with labeled probe and a 50-fold excess of unlabeled oligonucleotide to assess nonspecific hybridization. Films were developed using Kodak GBX developer and replenisher. Subsequent to exposure to film, slides were stained with cresyl violet. Quantitative analysis of the autoradiograms was performed using the MCID system (Imaging Research, St. Catherine, Ontario, CA). Densitometric measurements were taken from the central portion of the mPOA, a region containing the SDN-POA. The region analyzed by densitometry was comparable in extent to the region sampled for slice recording. The density values are expressed as means ± SE from 5–10 sections of the mPOA for each animal examined. Sex-specific comparisons of levels of signal corresponding to each subunit-specific probe were always made within a single experiment with probe labeled on the same day and all sections hybridized on the same day. Data were analyzed using a one-way analysis of variance (ANOVA) followed by posthoc comparisons using the Scheffé test.

RESULTS

Functional analysis of GABA_A-mediated sIPSCs in neurons from the mPOA

Although putative γ1-containing receptors in astroglia have been characterized (Bormann and Kettenmann 1988), no study to date has defined the properties of native, neuronal γ1-containing receptors. Because steroid hormones can act both during early postnatal development (Smith et al. 1996) and during adulthood to regulate GABA_A-mediated responses (Brusgaard et al. 1997; Herbsis and Fénelon 1995), experiments were performed on neurons from rats between postnatal day 10 (PN10) and PN20, ages corresponding to a developmental window subsequent to the critical period for most of the organizational actions of gonadal steroids, but preceding activation changes associated with the onset of puberty (for review, Döhler 1991; Rhee et al. 1990a,b). Properties of synaptic GABA_A receptors were assessed from analysis of sIPSCs recorded in intact brain slices. Recordings were made from the central portion of the mPOA in the presence of pharmacological blockers of glutamatergic transmission (see METHODS). Under these conditions, sIPSCs were observed in >90% of mPOA neurons recorded from both sexes (Fig. 1A). Spontaneous IPSCs were completely and reversibly blocked by 10 μM bicuculline (n = 5; data not shown). Analysis of the frequency of sIPSCs indicated that values were similar for recordings from mPOA neurons of both male and female rats (1.44 ± 0.23 s⁻¹ for females, n = 21 and 1.57 ± 0.23 s⁻¹ for males, n = 20). Current decays were best fitted by the sum of two exponential components with time constants, τ₁ and τ₂, of 11.43 ± 0.37 ms and 44.08 ± 2.01 ms for females (n = 68) and 12.51 ± 0.51 ms and 46.06 ± 1.85 ms (n = 57) for males (Fig. 1, B and C). No significant differences were evident for recordings from females versus males in the values of τ₁ or τ₂ or the percentage of the peak current amplitude contributed by each kinetic component. Mean peak amplitudes were 112.9 ± 4.7 pA for females and 116.0 ± 6.7 pA for males (Fig. 1D). Further assessment of the distribution of current amplitudes indicated peaks corresponding to 43, 85, and 131 pA for males (Fig. 1E), and a similar distribution with peaks corresponding to 44, 76, and 134 pA for females (data not shown). Average peak current amplitudes of miniature IPSCs (mIPSCs) recorded in the presence of 1 μM tetrodotoxin were 48.0 ± 2.0 pA (n = 3), suggesting that the observed peak in the distribution of sIPSCs at 44 pA corresponds to single mIPSCs and subsequent peaks to multiples of these events. No statistical differences were evident in the distribution of current amplitudes between the sexes, and the coefficients of variation for the amplitude distributions were 0.60 for males and 0.57 for females.

Modulation of sIPSCs by compounds acting at the BZ recognition site of the GABA_A receptor

The presence of a γ subunit is required for modulation of GABA_A receptor function by compounds acting at the BZ site. The imidazopyridine, zolpidem, and the benzodiazepine, diazepam, are considered classic agonists for BZI and BZII type GABA_A receptors, respectively. Conversely, β-carbolines are characterized as classical inverse agonists that negatively modulate GABA_A receptor function (for review, Sieghart 1995). Previous studies have demonstrated that specific subtypes of recombinant receptors expressed in heterologous cells and native receptors in glial cells show a paradoxical pattern of modulation by zolpidem and the β-
FIG. 1. γ-Aminobutyric acid type A (GABA_A) receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) in neurons from the medial preoptic area (mPOA). A: representative superimposed sIPSCs recorded in the whole cell configuration from in an intact slice preparation. $V_h = -80$ mV. Scale bar: 50 pA; 20 ms. B: representative averaged sIPSC from this neuron. Current decay was fitted by 2 exponential components with time constants, $\tau_1$ and $\tau_2$. Heavier line: exponential fit to the data. Lighter lines: individual components of the fit that correspond to $\tau_1$ and $\tau_2$. Numbers in parentheses: contribution of each kinetic component to the peak current. Scale bar: 50 pA; 20 ms. C: average time constants ($\tau_1$ and $\tau_2$) were calculated for each cell and averaged for recordings made from mPOA neurons from male and female rats. Error bars: SE. $n$: number of cells. D: average peak current amplitudes were calculated for each cell and averaged for recordings made from mPOA neurons from male and female rats. Error bars: SE. $n$: number of cells. E: distribution of individual event amplitudes calculated for 10 neurons from the mPOA of male rats. Events (>100) were analyzed for each cell. Peaks were evident at 43, 85, and 131 pA. Distribution of events for mPOA neurons from females was similar (data not shown). Bin width: 5 pA.

Carbolines that has been attributed to the presence of a γ1 versus a γ2 subunit (Bormann and Kettenmann 1988; Puia et al. 1991; Wafford et al. 1993). Specifically, while diazepam potentiates either γ1- or γ2-containing receptors, zolpidem acts as an inverse agonist (or is without effect), and the β-carboline, β-CCM, acts as a positive modulator (or is without effect) at receptors containing a γ1 subunit. To determine if the high levels of γ1 subunit mRNA in the mPOA result in the expression of neuronal receptors with functional properties consistent with those reported for recombinant receptors, we analyzed modulation of sIPSCs in mPOA neurons by the benzodiazepine, diazepam, the β-carboline, β-CCM, and the imidazopyridine, zolpidem. Drugs were applied to acutely isolated slices at 1–20 μM, concentrations previously shown to modulate the moderate affinity $\alpha 2/\beta x/\gamma(1$ or 2) recombinant receptors (Puia et al. 1991), as well as receptors in glial cells (Bormann and Kettenmann 1988).

Analysis of modulation of sIPSCs in neurons from the mPOA indicated that BZ-like compounds elicited a paradoxical pattern of modulation of sIPSCs similar to that reported for GABA-elicited responses from recombinant $\alpha 2/\beta x/\gamma$ receptors and receptors in glial cells. The overall effect of diazepam was to potentiate sIPSCs, while β-CCM induced no significant effect and zolpidem induced a negative modulation of current amplitudes (Figs. 2 and 3). Specifically, diazepam elicited potentiation of sIPSCs in 77% of the mPOA neurons tested ($n = 22$), increasing either peak amplitude (68% of cells) and/or prolonging current decay (59% of cells). In contrast to the profile of pharmacological sensitivity typically seen with γ2-containing receptors, zolpidem induced positive modulation in only 22% of the neurons tested ($n = 37$), and β-CCM induced negative modulation in only 20% of the cells examined ($n = 37$). All three pharmacological profiles are consistent with those reported for recombinant γ1-containing receptors or receptors in glial cells. Both zolpidem and β-CCM were able to elicit classical responses predicted for γ2-containing receptors in other
Sex-specific differences in modulation of sIPSCs by zolpidem

Further analysis of the effects of modulators acting at the BZ site of the GABA<sub>A</sub> receptor revealed significant sex-specific differences in the efficacy of zolpidem to modulate sIPSC amplitudes in neurons of the mPOA (Fig. 3). Zolpidem significantly decreased the average peak current amplitude to 77.3 ± 4.2% (n = 19) of the control amplitude for neurons from male (P < 0.05), but had no significant effect on peak amplitudes in neurons from female (104.1 ± 3.9%; n = 18) rats. The average ratio of peak amplitude in the presence of the modulator to peak amplitude in control saline from mPOA neurons from males was significantly lower than that for females (P < 0.001) (Fig. 3B). Moreover, zolpidem was found to induce negative modulation in 85% of mPOA neurons tested from males (n = 19), but in only 11% of neurons recorded in the mPOA from females (n = 18; P < 0.005). No significant effects on the values of τ<sub>1</sub>, τ<sub>2</sub>, or the relative contribution of each component to the total peak current amplitude were evident on analysis of sIPSCs exposed to zolpidem. With regard to other modulators that act at the BZ binding site, no significant sex-specific differences were detected in the ability of β-CCM to modulate sIPSC amplitude or kinetics in mPOA neurons (Fig. 3). Diazepam induced slightly greater modulation of peak current amplitude in mPOA neurons from females than from males; an effect that approached, but did not attain significance (Fig. 3). Diazepam was found to prolong current decays in 77% of neurons from females and in 60% of neurons from males. Diazepam did not induce changes in current decay kinetics in neurons that did not also show enhancement of amplitudes. For those neurons in which diazepam did increase the peak current amplitude, current decay, as estimated by a monexponential fit, was increased 1.3-fold in neurons from females and 1.2-fold in neurons from males. Neither increase in current decay was significant.

Zolpidem modulation of currents elicited by ultrafast perfusion of GABA to isolated mPOA neurons

Modulation of sIPSCs by compounds acting at the BZ site was assessed at concentrations shown previously to modulate currents for recombinant receptors (Piuia et al. 1991) and receptors in glial cells (Bormann and Kettenmann 1988). It has been suggested, however, that drug diffusion may be limited in slice recordings and that the actual concentrations of modulators present at synapses is far less than those applied (Brussaard et al. 1997; Rovira and Ben-Ari 1993). To examine the ability of zolpidem to modulate GABA<sub>A</sub> receptor-mediated currents under conditions where drug concentrations delivered are equal to those reaching the cell, access is not restricted, and washout of drugs is not problematic, we also assessed the effects of zolpidem on currents elicited by ultrafast perfusion of 1 mM GABA onto neurons acutely isolated from the mPOA. Application of 1 mM GABA for 3 ms was chosen to mimic GABA concentrations present at the synaptic cleft (Jones and Westbrook 1995). Greater than 90% mPOA neurons from either sex for which GΩ seals were obtained were responsive to GABA (Fig. 4A). Current decays were best fitted by the sum of three exponential components (Fig. 4A) with average values of τ<sub>1</sub> = 8.39 ± 0.60 ms, τ<sub>2</sub> = 57.11 ± 2.49 ms, and τ<sub>3</sub> = 145.90 ± 16.71 ms for females (n = 32) and τ<sub>1</sub> = 8.55 ± 0.37 ms, τ<sub>2</sub> = 54.22 ± 1.77 ms, and τ<sub>3</sub> = 220.74 ± 48.37 ms for males (n = 103) (Fig. 4B).

Previous studies have shown that BZs modulate currents elicited by millimolar concentrations of GABA (Lavoie and Twyman 1996; Mellor and Randall 1997). To assess the effects of zolpidem on currents elicited by ultrafast perfusion of GABA, solutions were switched from saline alone in one port of the theta glass and saline plus 1 mM GABA in the second port to saline plus zolpidem and saline plus 1 mM GABA plus zolpidem by changing the reservoirs of solution for each side of the theta tubing. Control experiments in which solution switching was made from one reservoir containing 1 mM GABA to a separate reservoir containing 1 mM GABA (but no zolpidem) showed no change in current amplitude (99.5 ± 9.3% of control, n = 11). When zolpidem was co-applied with GABA, negative modulation of current amplitudes was induced.
FIG. 3. Sex-specific differences in modulation of GABA_A-mediated sIPSCs by compounds acting at the BZ binding site. A: representative, superimposed, averaged responses in mPOA neurons in intact slices from female rats (top) illustrating potentiation of currents by diazepam (left), no change in response to β-CCM (middle), and no change in response to zolpidem (right). Representative averaged responses in mPOA neurons in intact slices from male rats (bottom) illustrating potentiation of currents by diazepam (left), no change in response to β-CCM (middle), and diminution of the response by zolpidem (right). Scale bar: 50 pA; 20 ms. B: scatter plot indicating potentiation of depression of peak current amplitudes observed in individual neurons of the mPOA from male or female animals exposed to diazepam, β-CCM, or zolpidem. Each data point represents the percentage change in peak current amplitude for an individual neuron for females (■) or males (●). C: cumulative data indicating the percentage enhancement or percentage diminution of control peak sIPSC amplitude elicited by diazepam (left), β-CCM (middle), and zolpidem (right) for neurons from females and males (as indicated). Modulation of average peak current amplitudes was assessed by plotting the ratio of the average peak current estimated during exposure to modulator to the average peak current prior to its addition. No significant sex-specific differences were evident in the ability of diazepam or β-CCM to modulate the responses, however zolpidem decreased the peak current amplitude in mPOA neurons of male rats to 77 ± 4% of the initial amplitude (P < 0.05), but had no effect on amplitudes of sIPSCs in mPOA neurons from female rats (104 ± 4%). *The average ratio (Ipeak mod/Ipeak con) for responses from mPOA neurons from males was significantly lower than that for females (P < 0.001). Error bars: SE. n: number of cells.

in 100% of mPOA neurons from male rats tested with concentrations of zolpidem ranging from 100 nM to 20 μM (n = 29) (Fig. 5A and B). The maximum inhibition induced was ~50% of the peak control amplitude and was observed with 1, 10, and 20 μM zolpidem. The negative modulation in current amplitude induced by zolpidem was reversible (Fig. 5A), and the concentration response relationship indicated an IC_{50} = 58 nM zolpidem (Fig. 5B). No significant differences in the time constants (τ_1, τ_2, and τ_3) of current decay were induced by 10–20 μM zolpidem. However, the percentage of the peak current
amplitude attributed to $\tau_1$ was increased significantly ($P < 0.01$) from $25.14 \pm 3.90\%$ of the peak response in control solutions to $41.86 \pm 3.37\%$ during zolpidem application ($n = 21$). This effect was completely reversible on return to control saline plus GABA alone. In marked contrast to the maximal decrease elicited by co-application of $\mu$M concentrations of zolpidem and 1 mM GABA to mPOA neurons isolated from male rats, no modulation of currents was produced by 10 $\mu$M zolpidem in neurons isolated from females ($100.0 \pm 7.9\%$ of peak control amplitude; $n = 8$) (Fig. 5, A and B). These data are consistent with the negative modulation of sIPSCs induced by zolpidem in intact slices from male rats and the lack of effect of this modulator on sIPSCs recorded from mPOA neurons in intact slices from females.

**In situ hybridization of subunit-specific mRNAs in the mPOA**

Previous studies using both in situ hybridization and immunocytochemical analyses have demonstrated that $\gamma_1$ subunit mRNA and protein are both highly expressed in and highly restricted to the mPOA and a limited number of other forebrain nuclei (Araki et al. 1992, 1993; Herbison and Fénélon 1995; Wisden et al. 1992; Ymer et al. 1990), while levels of $\gamma_2$ and $\gamma_3$ mRNA are low to negligible (Wisden et al. 1992). However, these previous studies did not compare levels of GABAA receptor subunit mRNAs in male versus female rats. To determine if the ability of zolpidem to modulate currents in the mPOA is correlated with sex-specific differences in the levels of $\alpha$ or $\gamma$ subunit mRNAs, in situ hybridization experiments were performed. As has been reported previously (Wisden et al. 1992), signals corresponding to either the $\gamma_2$ or $\gamma_3$ subunit mRNAs were weak to negligible in the mPOA (data not shown). Consistent with the pharmacological modulation of GABAergic currents by zolpidem, densitometric analysis indicated that high levels of $\gamma_1$ mRNA were detected in the mPOA for both sexes with signal intensities strongest in the central region of the mPOA (Fig. 6). Although expression in the mPOA was clearly evident in both sexes, signal corresponding to the $\gamma_1$ subunit-specific probe was significantly higher ($P < 0.05$) for male than female animals (Fig. 6). The efficacy and potency of zolpidem is dependent on $\alpha$, as well as $\gamma$, subunit composition (for review, Sieghart 1995). Neither $\alpha_4$ nor $\alpha_6$ subunit mRNA is expressed to an appreciable extent in the mPOA (Wisden et al. 1992); however, levels of $\alpha_1$, $\alpha_2/3$, and $\alpha_5$ are predicted to correlate with high affinity BZI type receptors, moderate affinity BZII receptors, and very low affinity BZIII receptors, respectively (for review, Sieghart 1995). Densitometric analysis of the signal corresponding to $^{35}$S-labeled oligonucleotides specific for $\alpha_1$, $\alpha_2$, and $\alpha_5$ indicated that all three transcripts are expressed within the mPOA of both male and female rats; however, no significant sex-specific differences in the levels of any of these $\alpha$ subunit mRNAs were detected (Fig. 7). Previous studies have shown that neither the $\beta_1$ nor the $\beta_2$ mRNA is strongly expressed in the mPOA at any developmental age and that levels of $\beta_3$ mRNA are comparable from birth to adulthood (Zhang et al. 1991). Moreover, because $\beta$ subunits are not believed to play a major role in conferring sensitivity to BZ-like modulators (for review, Sieghart 1995), sex-specific differences in the levels of $\beta$ transcripts were not examined.

**DISCUSSION**

Region-specific regulation of GABAA receptor subunit genes has provided for a vast degree of diversity in receptor function (for review, Macdonald and Olsen 1994; Sieghart 1995). A number of previous reports have described the unique distribution of the $\gamma_1$ subunit mRNA within the rat brain and hypothesized that this distribution may contribute critical aspects to hypotalamic function (Herbison and Fénélon 1995; Ymer et al. 1990). The properties of native, neuronal $\gamma_1$-containing receptors have not been described previously. Biophysical characterization of GABAA-mediated currents from neurons of the mPOA indicated that sIPSCs had unitary peak current amplitudes of ~40 pA and decayed biexponentially (Fig. 1). No significant differences were evident in the amplitudes, frequencies, or decay kinetics of sIPSCs from mPOA neurons of female versus male rats.

Analysis of macroscopic currents elicited by ultrafast perfusion of GABA to acutely isolated cells indicated that current
FIG. 5. Dose-response relationship for zolpidem modulation of GABA-elicited responses in acutely isolated neurons of the mPOA. A: representative whole cell responses elicited by a 3 ms/1 mM application of GABA to mPOA neurons from a female (top) and male (bottom) rat (V_h = -80 mV). Solutions were then exchanged to preequilibrate the cell by exposing it to aCSF + 10 μM zolpidem and subsequent responses elicited by a 3 ms application of 1 mM GABA + 10 μM zolpidem. In the presence of μM zolpidem, peak current amplitudes were decreased in mPOA neurons isolated from male rats. Returning solutions to aCSF and applying a 3 ms pulse of 1 mM GABA alone (wash), indicated that the decrease in peak amplitude elicited by zolpidem in males was reversible. In contrast, this concentration of zolpidem elicited no effect on GABA-induced currents in neurons from female rats. Scale bar: 200 pA; 100 ms. B: concentration-response relationship for mPOA neurons acutely isolated from male rats (l) indicating the modulation of control responses by the indicated concentrations of zolpidem. Error bars: SE. n: number of cells. Maximal inhibition of ~50% was observed with 1, 10, and 20 μM zolpidem, and the decrease was reversible. IC_{50} = 58 nM. No effect of 10 μM zolpidem was observed in mPOA neurons from female rats (■).

decays were best fitted by three exponential functions. The first two time constants of current decay agree well with those assessed from analysis of sIPSCs (Figs. 1 and 4). The third, slower time constant, evident in some cells exposed to GABA with ultrafast perfusion, was only rarely observed in sIPSCs. Differences in kinetics of currents elicited by ultrafast perfusion versus IPSCs have been observed for other GABA_A receptor-mediated responses in other brain regions (Mellor and Randall 1997). These differences may reflect activation of a population of receptors with distinct kinetic properties from those present in the subsynaptic membrane or may arise if some parts of the whole cell are exposed to GABA with a slower time course than would be expected for an excised patch (Rossi and Hamann 1998). The parameters describing current amplitudes and decay for both sIPSCs and fast perfusion responses fall within a range of values reported for GABA_A-receptor mediated responses.

FIG. 6. Sex-specific differences in γ1 mRNA levels in the mPOA. Representative dark field autoradiograms of coronal sections from a male (top) and a female (bottom) rat brain hybridized with an 35S-labeled oligonucleotide specific for the γ1 subunit mRNA (left). High levels of γ1 mRNA are evident in the mPOA of both sexes; however, densitometric analysis of film autoradiograms (right) indicated that signal intensity for the γ1 subunit mRNA was significantly greater in the mPOA of male than female rats (*P < 0.05). Error bars: SE. Numbers above bars indicate animals.
that steroid modulators also act as inverse agonists in the mPOA, while inducing positive modulation in regions with high levels of γ2 mRNA (Jorge-Rivera and Henderson 1998). Taken together, these data raise the possibility that preferential expression of the γ1 subunit may confer a unique profile of pharmacological properties to neurons in the mPOA that extends to a broad range of allosteric modulators of the GABA$_A$ receptor.

Positive allosteric modulators are believed to enhance the rate of GABA association to the first agonist binding site (for review, Sieghart 1995), and experiments using ultrafast perfusion of GABA and diazepam support this hypothesis (Lavoie and Tywman 1996). Under saturating concentrations of GABA, positive BZ modulators are predicted to prolong current decay, but to have little effect on peak current amplitude. Numerous previous studies indicate that positive BZ modulators do not enhance mIPSC amplitudes, suggesting that at many central synapses release results in saturating concentrations of GABA in the synaptic cleft (for discussion, Mellor and Randall 1997). In the present study, diazepam was observed to potentiate sIPSC amplitudes. Similar potentiation of GABA$_A$ receptor-mediated current amplitudes by positive modulators has been reported for other central synapses (Freking et al. 1995; Mellor and Randall 1997; Nusser et al. 1997) and for currents elicited by ultrafast perfusion of 1 mM GABA to recombinant receptors in heterologous cells (Lavoie and Tywman 1996). Spontaneous IPSCs recorded in the mPOA appear to reflect responses arising from single and multiple quanta (Fig. 1), and enhancement of sIPSC amplitudes in the mPOA may reflect the integration of multiple mIPSCs whose durations are prolonged by the action of positive BZ modulators (Mody et al. 1994). Alternatively, it has been suggested that the ability of BZ compounds to potentiate current amplitudes arises because GABA concentrations at some central synapses are not saturating (Freking et al. 1995; Nusser et al. 1997) or that BZ compounds may act to recruit receptors (Lavoie and Tywman 1996).

In contrast to diazepam, zolpidem induced no modulation of sIPSCs recorded in mPOA neurons from female rats and negative modulation of peak sIPSC amplitude and peak amplitude of currents elicited by ultrafast perfusion of 1 mM GABA recorded in mPOA neurons from male rats. Zolpidem-induced decreases in the amplitudes of either sIPSCs or responses elicited by ultrafast perfusion of GABA to mPOA neurons from male rats were not accompanied by significant changes in the values of individual time constants of current decay. However, co-application of 1 mM GABA with 10–20 μM zolpidem, concentrations that induced a maximal decrease of peak current amplitude of ~50%, did induce a significant increase in the contribution of $\tau_1$ to the peak response. Similar decreases in peak current amplitude accompanied by speeding of deactivation have been reported for the partial inverse agonist Ro15–4513 (Mellor and Randall 1997).

In addition to the unique profile of BZ sensitivity of GABA$_A$ receptor-mediated currents in mPOA neurons versus other brain regions, our data also demonstrate that within a given region the efficacy of BZ modulators may vary between the sexes. Specifically, we have demonstrated that zolpidem acts as an inverse agonist of both sIPSCs and...
responses elicited by fast perfusion of GABA in neurons of the mPOA from male rats, but does not modulate either sIPSCs or responses elicited by fast perfusion in neurons from female rats. Results from in situ hybridization experiments indicate that levels of γ1 subunit mRNA in the mPOA are higher in male than female rats. It is clear that BZ sensitivity will be dependent on α as well as γ subunit composition (for review, Sieghart 1995). In situ hybridization and immunocytochemical analyses indicate that α2 and β2/3 subunits are the most abundantly expressed transcripts and subunits in the mPOA of juvenile female rats (Jung et al. 1992) and adult rats for which sex was not determined (Wiesen et al. 1992). Results from in situ hybridization experiments presented here indicate that α1, α2, and α5 mRNAs are all detectable within the mPOA of both sexes and that α2 is the most prevalent transcript. Moreover, our results show that there are no significant differences in the expression of any of the three α transcripts between male and female rats. That α1, α2, and α5 transcripts in the mPOA of female and male rats are equivalent suggests that the difference in γ1 mRNA levels cannot be attributed simply to an overall lower level of GABA_{α_1} receptor subunit gene expression or to simply fewer cells in the female versus in the male rat mPOA. Although the sex-specific difference in zolpidem sensitivity appears to be best correlated with specific differences in levels of γ1 subunit mRNA between the two sexes, it is clear that the relationship between mRNA levels of the nucleus as a whole and pharmacological profiles of individual cells is at best a broad correlation. Emulsion autoradiography of γ1 mRNA expression in the mPOA of adult rats indicates that within the SDN-POA, there is heterogeneity in both the numbers of neurons expressing detectable levels of this transcript and in the relative levels expressed (Clark et al. 1998). It is therefore likely that some of the heterogeneity in the ability of BZ-like compounds to modulate sIPSCs in the mPOA arises from cell to cell differences in the levels of receptors containing not just γ1 subunits, but heterogeneous complements of α subunits as well. Analysis of mRNA levels by single cell polymerase chain reaction techniques in individual neurons assayed for zolpidem sensitivity will provide the best assessment as to the role of differential expression of the γ1 subunit mRNA in the observed sex-specific difference in the ability of this modulator to affect GABA_{α_1} receptor-mediated responses.

There is a noted paucity of published data on the effects of BZs in the hypothalamus or other forebrain structures important for the production of sexual and reproductive behaviors (for review, Wilson 1996). The different profile of sensitivity to BZ modulation for neurons in the mPOA compared with other brain regions may have implications for regulation of hormonally-controlled functions. Specifically, endogenous BZ compounds have been reported to be synthesized by ependymal cells in the hypothalamus (Malagon et al. 1993; Tong et al. 1991), and are believed to exert β-carboline-like effects at the GABA_{α_1} receptor (Bormann et al. 1985), regulate aggressive behaviors (Kavaliers and Hirst 1986), and modulate GnRH expression in the hypothalamus (Li and Pelletier 1995). Our results would predict that, in contrast to other hypothalamic regions, GABAergic transmission in neurons in the mPOA would not be negatively modulated by these putative, endogenous β-carboline-like compounds. In addition, gonadal steroid-dependent regulation of γ1 mRNA levels may produce differences in the sensitivity of mPOA neurons to BZ modulators within a given sex. In particular, levels of γ1 subunit mRNA have been shown to be increased in the mPOA of adult, gonadectomized female rats given exogenous 17β-estradiol (Herbison and Fénelon 1995) and to be regulated across the estrous cycle in gonadally-intact female rats (Clark et al. 1998). Recent reports have demonstrated that fluctuations in gonadal steroids in female rats alter GABA_{α_1} receptor subunit composition and result in significant changes in the sensitivity of neurons to both neurosteroid (Brussaard et al. 1998) and BZ (Smith et al. 1998) modulators. These steroid-induced changes in receptor subunit composition, in turn, are believed to contribute to the execution of appropriate reproductive behaviors (Brussaard et al. 1998) and to underlie the insensitivity to BZ anxiolytic drugs that is associated with PMS and postpartum syndrome (Smith et al. 1998). Although the number of studies is limited, BZ compounds are known to act both in a sex-specific manner and to affect sexual behaviors. Sensitivity to diazepam has been shown to vary both in a sex-specific and hormone-dependent fashion in rats (Fernández-Guasti and Picazo 1990; Fernández-Guasti and Picazo 1997; Pesce et al. 1994) and in humans (Sundström et al. 1997). In addition, intracranial infusion of diazepam has been shown to facilitate sexual receptivity (McCarthy et al. 1995). Finally BZs have been shown clinically to result in sexual dysfunction (Fava and Borofsky 1991; Ghadirian et al. 1992). These studies suggest that BZ modulation of GABA_{α_1} receptor function may play an important role in sex-specific behaviors. Our results suggest that the high level of the γ1 subunit mRNA within the mPOA results in expression of GABA_{α_1} receptors with a distinct pharmacological sensitivity. Moreover, the ability of gonadal steroids to regulate γ1 expression in the mPOA (Clark et al. 1998; Herbison and Fénelon 1995), and thus sensitivity to allosteric modulators, suggests that GABA_{α_1} receptors within the hypothalamus may show a degree of plasticity in function that is dependent on hormonal state.

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Address for reprint requests: L.P. Henderson, Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755.

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