Functional Characterization of GABA_A Receptors in Neonatal Hypothalamic Brain Slice

REN-QI HUANG AND GLENN H. DILLON
Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, Texas 76107
Received 10 October 2001; accepted in final form 19 June 2002

Huang, Ren-Qi, and Glenn H. Dillon. Functional characterization of GABA_A receptors in neonatal hypothalamic brain slice. J Neurophysiol 88: 1655–1663, 2002; 10.1152/jn.00822.2001. The hypothalamus influences a number of autonomic functions. The activity of hypothalamic neurons is modulated in part by release of the inhibitory neurotransmitter GABA onto these neurons. GABA_A receptors are formed from a number of distinct subunits, designated α, β, γ, δ, ε, and θ, many of which have multiple isoforms. Little data exist, however, on the functional characteristics of the GABA_A receptors present on hypothalamic neurons. To gain insight into which GABA_A receptor subunits are functionally expressed in the hypothalamus, we used an array of pharmacologic assessments. Whole cell recordings were made from thin hypothalamic slices obtained from 1- to 14-day-old rats. GABA_A receptor-mediated currents were detected in all neurons tested and had an average EC_50 of 20 ± 1.6 μM. Hypothalamic GABA_A receptors were modulated by diazepam (EC_50 = 0.060 μM), zolpidem (EC_50 = 0.19 μM), lorzepene (EC_50 = 4.4 μM), methyl-6,7-dimethoxy-4-ethyl-β-carboline (EC_50 = 7.7 μM), and 5α-pregnan-3α-hydroxy-20-one (3α-OH-DHP). Conversely, these receptors were inhibited by Zn_2+ (IC_50 = 70.5 μM), dehydroepiandrosterone sulfate (IC_50 = 16.7 μM), and picrotoxin (IC_50 = 2.6 μM). The α4/6-selective antagonist furosemide (10–1,000 μM) was ineffective in all hypothalamic neurons tested. The results of our pharmacological analysis suggest that hypothalamic neurons express functional GABA_A receptor subtypes that incorporate α1 and/or α2 subunits, β2 and/or β3 subunits, and γ2 subunit. Our results suggest receptors expressing α3–α6, β1, γ1, and δ, if present, represent a minor component of functional hypothalamic GABA_A receptors.

INTRODUCTION

GABA_A receptors consist of a pseudosymmetrical, pentameric array of transmembrane subunits that form a receptor/Cl^- ion channel complex. GABA changes from an excitatory to an inhibitory neurotransmitter within 2 wk of birth, due to reversal of the Cl^- gradient (Obrietan and van den Pol 1995; Rivera et al. 1999). GABA_A receptor function is allosterically modulated by a variety of endogenous factors such as phosphorylation, pH, neurosteroids and Zn_2+ (Hevers and Lüddens 1998; Huang and Dillon 1999; Moss and Smart 1996). In addition, a number of pharmacologic agents modulate the receptor, including benzodiazepines, barbiturates, general anesthetics, and convulsants (see Hevers and Lüddens 1998). Based on a repertoire of ≥20 subunits (α1–6, β1–3, γ1–3, δ, π, ε, ρ1–3, π, and θ), the molecular architecture of native GABA_A receptors is extremely heterogeneous (Bonnert et al. 1999; Hevers and Lüddens 1998). Pharmacological studies of recombinant receptors have shown that individual subunits and their subtypes confer different sensitivities to GABA_A receptor modulators (Hevers and Lüddens 1998).

The hypothalamus plays an important role in regulation of a number of autonomic functions, including food intake, body temperature, cardiorespiratory activity, nociception/analgesia, circadian rhythms, and the endocrine system (for review, see Meister 1993). GABA suppresses the activity of hypothalamic neurons and has been suggested to be the dominant inhibitory neurotransmitter in the hypothalamus (Decavel and van den Pol 1990). GABA_A receptors as measured by [3H]muscimol binding are found throughout the hypothalamus (Xia and Haddad 1992). In situ hybridization studies of the hypothalamus have demonstrated that mRNA for α2, β3, γ2, and ε subunits is highly expressed, whereas mRNA for α1, α3, α5, β1, and γ1 subunits is moderately expressed (Whiting et al. 1997; Wisden et al. 1992). In addition, message for β2 and γ3 subunits is minimally expressed, whereas that for α4, α6, and δ subunits is negligible or absent (Wisden et al. 1992). Immunocytochemical studies suggest the existence of α1, α2, β2, β3, and γ2 subunits in hypothalamic magnocellular neurons (Fenelon and Herbsion 1995) and α1, α2, α5, β2/β3, and γ2 in other hypothalamic regions (Davis et al. 2000; Fritschy and Mohler 1995; Pirker et al. 2000). Although the aforementioned techniques are clearly informative, it is accepted that conclusions derived using them may be limited. For instance, mRNA levels do not necessarily correlate with expression of functional receptors (Saha et al. 2001). Moreover, results from immunohistochemical studies may be impacted by tissue preparation and integrity, antibody selectivity, and the possible labeling of incompletely assembled and/or subcellular receptors. Thus the aim of the present study was to conduct a pharmacological analysis of a physiologically intact system to obtain functional evidence of expression of GABA_A receptor subunits that are purported to exist in the hypothalamus.

METHODS

Hypothalamic brain slices

Sprague Dawley rats (Indianapolis, IN), postnatal day (P) 1–14 (either sex) were rapidly decapitated. Chemical anesthesia was not
used because of its well-known influence on GABA_A receptors (Franks and Lieb 1994). All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All stages of brain dissection and tissue slicing were conducted in ice-cold (~4°C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 124 NaCl, 5.0 KCl, 1.3 MgSO_4_, 26 NaHCO_3_, 1.24 KH_2PO_4_, 2.4 CaCl_2_, and 10 glucose; 300 mosM and pH ~7.4 after equilibration with a 95%–5% CO_2 gas mixture (carbogen). Thin hypothalamic slices (~200 μm) were cut with a vibratome (VSL, World Precision Instruments); slices were submerged in ACSF (22–25°C) aerated with the carbogen gas mixture. Slices were transferred to a recording chamber (~2 ml) and superfused continuously (7–10 ml/min, 22–25°C) with saline. To minimize synaptic influences on neurons under investigation, experiments were conducted in a synaptic blockade medium consisting of the following (in mM): 128 NaCl, 3.0 KCl, 11.4 MgCl_2_, 10 HEPES, 2.0 CaCl_2_, and 10 glucose, 300 mosM and pH 7.3. This superfusion medium has been shown to block both evoked and spontaneous chemical synaptic potentials in medullary neurons (Dean et al. 1997).

Individual hypothalamic neurons within the slice were visualized using an upright, fixed stage microscope (Nikon Optiphot-2UD) equipped with standard Hoffman modulation contrast (HMC) optics and a video camera system (Sony model XC-75 CCD video camera module, Tandy video monitor). Pipette tip location was acquired at low magnification (×4 via the CCD camera). The anatomical location of each recorded neuron was determined by comparison to plates in a stereotaxic atlas (Paxinos and Watros 1986).

**Whole cell patch-clamp recording**

Whole cell patch recordings were made at room temperature (22–25°C). Except during acquisition of current-voltage relationships, cells were voltage-clamped at ~60 mV. Patch pipettes of borosilicate glass (M1B150F, World Precision Instruments) were pulled (Flaming/Brown, P-87/PC, Sutter Instrument, Novato, CA) to a tip resistance of 1–2 MΩ. The recorded currents were monitored at ~60 mV. The recorded currents were filtered at 5 kHz, monitored on an oscilloscope and a chart recorder (Gould TA240), and stored on a computer (pClamp 6.0, Axon Instruments) for subsequent analysis. Sixty to 80% series resistance compensation was applied at the amplifier. To monitor the possibility that access resistance changed over time or during different experimental conditions, at the initiation of each recording, we measured and stored on our digital oscilloscope the current response to a 5-mV voltage pulse. This stored trace was continually referenced throughout the recording. If a change in access resistance was observed throughout the recording period, the patch was aborted and the data were not included in the analysis.

**Data analysis**

Concentration-response profiles were generated for GABA and a number of modulatory compounds. Agonist concentration-response profiles were fitted to the following equation: $I/I_{max} = \frac{[\text{agonist}]/(IC_{50} + [\text{agonist}])}{1 + \frac{[\text{agonist}]}{IC_{50}}}$, where $I$ and $I_{max}$ represent the normalized GABA-induced current at a given concentration and the maximum current induced by a saturating concentration of agonist, respectively. $IC_{50}$ is the half-maximal effective agonist concentration, and $n$ is the Hill coefficient. Ligand applications were separated by 3-min intervals to allow for recovery from desensitization when present.

**Results**

In all hypothalamic neurons tested, application of GABA activated an inward current when the membrane potential was voltage clamped at ~60 mV. The recorded currents were outwardly rectifying, reversed at the Cl~ equilibrium potential, and completely blocked by the GABA_A receptor antagonist bicuculline (Fig. 1, A and B). These characteristics demonstrate that the evoked currents were due to activation of GABA_A receptors.

**GABA sensitivity**

The sensitivity to GABA provides some information about receptor subunit composition. The concentration-dependent response of hypothalamic neurons to GABA (1–3,000 μM) is illustrated in Fig. 1C. The maximal amplitude of GABA-gated currents, typically achieved at a concentration of 300 μM, was 2.735 ± 189 pA ($n = 27$). EC_{50} values ranged from 11.4 to 44 μM (median = 20 μM, $n = 11$) and Hill coefficient values ranged from 1.2 to 2.25 (median = 1.6, $n = 11$). The mean GABA EC_{50} (20 ± 1.6 μM) and Hill coefficients (1.7 ± 0.2) of the entire group were similar to the median values, suggesting a single population of cells.

**Diazepam, zolpidem and Zn^{2+} sensitivity**

The benzodiazepine-site ligand diazepam is known to be dependent on the presence of the γ subunit in the receptor (Knofflach et al. 1996; McKernan et al. 1995). We assessed diazepam’s ability to potentiate hypothalamic GABA-activated current in the present investigation. Coapplication of diazepam (0.01–3 μM) with 10 μM GABA significantly enhanced GABA-gated current; threshold for the stimulatory effect was 30 nM, and maximal potentiation (to 206 ± 12% of control) was achieved at a concentration of 3 μM (Fig. 2, A and D). The EC_{50} and Hill...
Zolpidem has high affinity for GABA$_A$ receptors containing an $\alpha_1$ subunit and is nearly insensitive to GABA$_A$ receptors containing $\alpha_5$ subunits (Hevers and Lüddens 1998; Wingrove et al. 1994). Zolpidem (0.01–10 $\mu$M) markedly potentiated the current induced by 10 $\mu$M GABA in all cells ($n = 14$) recorded from the hypothalamus (Fig. 2B). The average EC$_{50}$ was 0.19 ± 0.07 $\mu$M and 3 $\mu$M zolpidem maximally potentiated the GABA current to 210 ± 18% of the control GABA response (Fig. 2D).

Inhibition of GABA$_A$ receptors by Zn$^{2+}$ is influenced by the subunit composition, in particular the presence of the $\gamma$ subunit (Gingrich and Burkat 1998; Smart et al. 1991). As shown in Fig. 2C, GABA-activated currents in hypothalamic neurons were sensitive to varying concentrations of Zn$^{2+}$ (1–1,000 $\mu$M). In the presence of 10 $\mu$M GABA, 1 $\mu$M Zn$^{2+}$ caused a slight but significant potentiation of steady-state currents in 5/5 cells tested (to 114 ± 4.9% of control, $P < 0.05$). Concentrations of Zn$^{2+}$ beyond 1 $\mu$M inhibited GABA-gated currents with an IC$_{50}$ of 70.5 ± 17 $\mu$M and a Hill coefficient value of 0.9 ± 0.14 ($n = 5$, Fig. 2D). Currents were decreased to ~10% of control in the presence of 1 mM Zn$^{2+}$. The responses to both diazepam and Zn$^{2+}$ indicate hypothalamic GABA$_A$ receptors express $\alpha$, $\beta$, and $\gamma$ subunits.

Loreclezole and DMCM sensitivity

Loreclezole is often used to distinguish $\beta_2$–3-containing receptors from $\beta_1$-containing receptors because it is more potent in the former receptors than the latter (Wingrove et al. 1994). As Fig. 3A shows, coapplication of loreclezole (0.3–100 $\mu$M) with 10 $\mu$M GABA enhanced the GABA current in all the cells tested ($n = 18$) in a concentration-dependent manner. Loreclezole was insoluble at concentrations >30 $\mu$M. However, the potentiating effect appeared nearly saturated at that concentration, so an EC$_{50}$ value could be estimated (mean EC$_{50}$ = 4.4 ± 0.7 $\mu$M, range = 1.8–10.0 $\mu$M). The maximal enhancement of peak current was 194 ± 12% of control (range: 119–272%) with 30 $\mu$M loreclezole. At higher concentrations (>10 $\mu$M), loreclezole produced an increase in the current decay rate (Fig. 3A).

Positive modulation of GABA$_A$ receptors by the $\beta$-carboline DMCM is also dependent on the $\beta$ subunit isoform. Figure 3B shows that concentrations of DMCM at ≥10 $\mu$M caused a modest potentiation of the GABA response. The maximal effect (to 141 ± 12.8% of control) was observed at 30 $\mu$M DMCM. The average EC$_{50}$ from 11 cells was 7.7 ± 3.2 $\mu$M.

3α-OH-DHP and furosemide sensitivity

3α-OH-DHP, an endogenous progesterone metabolite, has been shown to differentially modulate GABA$_A$ receptor function in a subunit-selective manner (Brussaard et al. 1997; Maitra and Reynolds 1999; Wohlforth et al. 2002; Zhu et al. 1996). As shown in Fig. 4A, 3α-OH-DHP potentiated GABA-activated currents in a concentration-dependent manner. Because the effect of 3α-OH-DHP could not be readily washed out, it was necessary to use a new slice for determination of effects of different concentrations of 3α-OH-DHP. Thus full concentration-response profiles were not collected. In addition, because 3α-OH-DHP can directly open GABA$_A$ receptor-Cl$^-$ channels at concentrations >1 $\mu$M (Ueno et al. 1997), we did not evaluate concentrations >1 $\mu$M in the present investigation. Nevertheless, it is apparent that hypothalamic GABA$_A$ receptors are markedly sensitive to 3α-OH-DHP, as GABA currents increased to 187 ± 20 and to 423 ± 48% of the control in response to 0.3 $\mu$M ($n = 7$) or 1 $\mu$M 3α-OH-DHP ($n = 8$), respectively (Fig. 4B).

Furosemide is a loop diuretic that acts as a selective, non-competitive antagonist of $\alpha_4$- or $\alpha_6$-containing GABA$_A$ receptors, with IC$_{50}$ values in the micromolar range (Knolflach et al. 1996; Korpi and Lüddens 1997). In 26 hypothalamic neurons...
tested, 300 \( \mu \text{M} \) furosemide, a concentration that inhibited the response to GABA by 39 ± 2% of control in rat recombinant \( \alpha_6\beta_2\gamma_2 \) receptors (\( n = 7 \), data not shown), had no effect on GABA-activated current. Increasing the furosemide concentration ≤1 \( \text{mM} \) was also without effect in all hypothalamic neurons tested (\( n = 4 \)).

**Picrotoxin and DHEAS sensitivity**

The CNS convulsant picrotoxin exerts its effects via blockade of GABA\( _A \) receptors. Although picrotoxin appears to be an effective blocker in most preparations (Bell-Horner et al. 2000; Krishek et al. 1996; Newland and Cull-Candy 1992), subunit-specific differences in affinity of picrotoxin for GABA\( _A \) receptor blockade do exist (Bell-Horner et al. 2000). Examples of typical responses of a hypothalamic neuron to picrotoxin-induced inhibition of the GABA response are illustrated in Fig. 5A. When coapplied with 10 \( \mu \text{M} \) GABA, picrotoxin (0.1–30 \( \mu \text{M} \)) induced a modest inhibition of peak GABA current and subsequently markedly enhanced the rate of current decay. The \( IC_{50} \) value for picrotoxin inhibition of steady-state current was 2.6 ± 0.4 \( \mu \text{M} \), and the Hill coefficient was 1.1 ± 0.1 (Fig. 5C).

DHEAS is a noncompetitive antagonist of GABA\( _A \) receptors in a number of preparations (Majewka et al. 1990). Figure 5B illustrates the DHEAS-mediated inhibition of the response to 10 \( \mu \text{M} \) GABA in a hypothalamic neuron. DHEAS reversibly inhibited the current amplitude and accelerated current decay in a concentration-dependent fashion. Coapplication of 300 \( \mu \text{M} \) DHEAS with 10 \( \mu \text{M} \) GABA reduced the GABA steady-state current to 7.7 ± 3.5% of control. The \( IC_{50} \) and Hill
coefficients were $16.7 \pm 2.3$ μM and $0.9 \pm 0.1$, respectively, for DHEAS inhibition of GABA-activated current in hypothalamic neurons ($n = 6$, Fig. 5C).

The efficacies and potencies of the different ligands modulating GABA<sub>A</sub> receptors in hypothalamic neurons are summarized in Table 1. Locations of all hypothalamic neurons ($n = 69$) studied in this investigation are shown in Fig. 6. All neurons tested were in the periventricular hypothalamus, and were located in the posterior, dorsomedial, lateral, ventromedial, and arcuate hypothalamic nuclei.

**DISCUSSION**

The involvement of GABA in regulating activity of hypothalamic neurons is well documented (Decavel and van den Pol 1990; Shonis and Waldrop 1995). Whereas several studies have assessed the potential expression of GABA<sub>A</sub> receptor subunits in the hypothalamus, a functional characterization of these receptors has not been conducted. Thus we have performed a pharmacologic and physiologic analysis of hypothalamic GABA<sub>A</sub> receptors. We chose to use the thin brain slice preparation in this analysis. This system more closely resembles the physiologic condition of the intact brain than isolated cultured cells and at the same time permits the rapid and consistent exposure of neurons to various exogenous ligands.

**Analysis of α subunit isoform**

The response to GABA itself varies significantly between receptors, depending on which α subunit is present. The sensitivity to GABA of α6- and α5-containing receptors (Hevers and Lüddens 1998; Knoflach et al. 1993) is 5- to 20-fold greater than those expressing α1, α2, or α3 subunits (Bell-Horner et al. 2000; Huang and Dillon 1998). The GABA EC<sub>50</sub> for these latter receptors is 15–30 μM (Bell-Horner et al. 2000), which is comparable to the value we obtained in hypothalamic neurons (20 μM). The fact that the individual EC<sub>50</sub> values were relatively evenly distributed around the mean does not support the existence of subsets of GABA<sub>A</sub> receptors with widely disparate GABA affinities.

Effects of diazepam are also influenced by α subunit isoform. The EC<sub>50</sub> of diazepam for stimulation of hypothalamic GABA-gated current was 60 nM in the present investigation. This is nearly identical (65 nM) to that reported by Amin et al.
GABAA receptor those incorporating the neurons tested appeared to express as their predominant cacy of diazepam is signifi
enant receptors expressing hypothalamic neurons responded to diazepam, and recombi
ary substances for diazepam stimulation of recombinant GABA \(_2\) receptors, which is approxi-
maximal response to diazepam was 311% of control in rat hypothalamic neurons.

\begin{table}
\centering
\caption{Relative efficacy and affinity of drugs tested for their modulatory effect on GABA\(_k\) receptor current in rat hypothalamic neurons.}
\begin{tabular}{llll}
\hline
Drug & \(n\) & Maximal Response & EC\(_{50}\)/IC\(_{50}\) (\(\mu M\))
\hline
Diazepam (0.03–1 \(\mu M\)) & 3–7 & 206 ± 12 & 0.060 ± 0.017
Zolpidem (0.01–10 \(\mu M\)) & 9–14 & 210 ± 18 & 0.19 ± 0.07
*ZtCl\(_2\) (1–1000 \(\mu M\)) & 5 & 7.6 ± 2.7 & 70.5 ± 17.3
Loreclezole (0.3–30 \(\mu M\)) & 18 & 194 ± 12 & 4.5 ± 0.86
DMCM (0.03–30 \(\mu M\)) & 11 & 141 ± 12.8 & 7.7 ± 3.2
3α-OH-DHP (0.3, 1 \(\mu M\)) & 8 & 423 ± 48 at 1 \(\mu M\) & ND
Furosemide (up to 1 mM) & 26 & No effect
*Picrotoxin (0.1–30 \(\mu M\)) & 5 & 7.3 ± 1.3 & 2.6 ± 0.43
*DHEAS (1–300 \(\mu M\)) & 6 & 7.7 ± 2.8 & 16.7 ± 2.3
\hline
\end{tabular}
\end{table}

Maximal response is expressed as a percentage of the control response to GABA (100%). Data are the means ± SE for the indicated number (\(n\)) of cells studied. Multiple values for \(n\) indicate varying numbers of cells tested at different concentrations of those concentration-response profiles. The number in brackets indicates the concentration range tested. EC\(_{50}\) or IC\(_{50}\) values were calculated from the concentration-response relationships fitted in Figs. 1–6 as described in Methods. ND, not determined. Values for antagonists (*) represent effect on steady-state GABA currents; values for agonists represent effect on peak GABA current amplitude. DMCM, methyl-6,7-dimethoxy-4-ethyl-carboline; 3α-OH-DHP, 3α-pregnan-3α-hydroxy-20-one; DHEAS, dehydroepiandrosterone sulfate.

diazepam (Knoflach et al. 1996). Sensitivity to diazepam does not of course eliminate the possibility that receptors expressing \(\alpha 4\) or \(\alpha 6\) subunits may also be present in hypothalamic neu-
rons. However, all hypothalamic neurons were also insensitive

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Inhibition of GABA-activated currents by picrotoxin (PTX) and dehydroepiandrosterone sulfate (DHEAS). A and B: representative traces recorded from individual neurons. Both antagonists inhibited peak current and accelerated current decay in a concentration-dependent manner. The duration of all drug application was 10 s. C: mean concentration-response profiles for inhibition of GABA current by picrotoxin or DHEAS. Each point is a mean of all drug application was 10 s.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{Schematic representation of majority of recording sites studied in the experiment. Slices in A and D represent the rostral and caudal extremes, respectively, encompassing the hypothalamic region that was investigated. ARC, arcuate hypothalamic nucleus; DM, dorsomedial hypothalamic nucleus; DMD, dorsomedial hypothalamic nucleus, diffuse; LH, lateral hypothalamic area; PH, posterior hypothalamic area; VMH, ventromedial hypothalamic nucleus. The drawing sections were adapted from a stereotaxic atlas for adult rats (Paxinos and Watson 1986).}
\end{figure}
to the diuretic furosemide. Furosemide blocks \(\alpha_4\) and \(\alpha_6\)-expressing receptors with \(\mu\)M affinity but is ineffective in other receptor configurations (Knoffel et al., 1996; Korpi and Lüddens 1997). Thus the results together suggest few hypothalamic neurons utilize receptors incorporating \(\alpha_4\) or \(\alpha_6\) subunits as the predominant \(\alpha\) subunit isoform.

Zolpidem has essentially no effect in \(\alpha_5\)-containing receptors and displays high affinity for \(\alpha_1\)- and \(\alpha_2\)-containing receptors (Hadingham et al. 1993; Lüddens et al. 1995). All neurons tested in the present investigation were stimulated by zolpidem, suggesting a lack of neurons that express predominantly \(\alpha_5\)-containing receptors. Taken together, results with GABA, diazepam, and zolpidem are consistent with the existence of functional hypothalamic GABA\(_A\) receptors that express predominantly \(\alpha_1\) and/or \(\alpha_2\) isoforms of the \(\alpha\) subunit. Receptors incorporating \(\alpha_3\), \(\alpha_4\), \(\alpha_5\), and \(\alpha_6\) subunits, if present, represent minority populations. Our findings expand significantly on experiments that have used visualization techniques to evaluate subunit expression in the hypothalamus. Wisden et al. (1992) found minimal and undetectable levels of \(\alpha_4\) and \(\alpha_6\) mRNA, respectively. mRNA for all other \(\alpha\) subunits was present to varying degrees, with mRNA for the \(\alpha_2\) isoform being most consistently and strongly evident. Immunohistochemical studies indicated the existence of both \(\alpha_1\) and \(\alpha_2\) subunits and a lack of expression of \(\alpha_4\) or \(\alpha_6\) subunits in the most parts of hypothalamus (Davis et al. 2000; Fritschy et al. 1994; Pirker et al. 2000). Davis et al. (2000) has shown that the density of immunoreactivity for \(\alpha_1\) and \(\alpha_2\) subunits varies with specific hypothalamic nuclei and that the relative density of these subunits in some hypothalamic nuclei switches by P20. It should be noted that because our recordings were obtained only in animals up to P14 in age, conclusions regarding functional subunit expression must be restricted to this age range.

**Analysis of \(\beta\) subunit isoform**

The potentiating action of loreclezole depends on the presence of either \(\beta_2\)- or \(\beta_3\)-containing GABA\(_A\) receptors and is absent in \(\beta_1\)-containing receptors (Hevers and Lüddens 1998; Wingrove et al. 1994). In the present study, loreclezole potentiated GABA-activated currents in all hypothalamic neurons tested. Loreclezole at high concentrations can also directly gate the GABA\(_A\) receptor, and this effect is also influenced by the \(\beta\) subunit isoform (Sanna et al. 1996). We have confirmed this direct activation effect of loreclezole in recombinant \(\alpha_1\beta_2\gamma_2\) receptors (unpublished observations). Thus the present report the effect on GABA\(_A\) receptors of high loreclezole likely comprise both modulatory and direct gating effects. However, the subunit-selective effects for both allosteric modulation and direct activation are the same (Sanna et al. 1996). Thus our conclusions are unchanged.

Positive modulation of GABA\(_A\) receptors by the \(\beta\)-carbol ine DMCM has been suggested to be due to interaction at the same site (Asn290) responsible for loreclezole stimulation of receptors expressing \(\beta_2/\beta_3\) subunits (Stevenson et al. 1995). DMCM (\(\geq 10\) \(\mu\)M) caused a modest potentiation of hypothalamic GABA\(_A\) receptors, suggesting the functional expression of receptors incorporating \(\beta_2\) and/or \(\beta_3\) subunits. It should be noted that we cannot rule out the possible coexistence of \(\beta_1\) with \(\beta_2/\beta_3\) subunits in the same neuron because the sensitivity to loreclezole or DMCM does not necessarily mean the absence of \(\beta_1\) subtype. In general, the \(\beta_2\) subunit is recognized as the most ubiquitous in the CNS. With regard to expression of mRNA, the \(\beta_3\) isoforms appears to be the most highly expressed in the hypothalamus (Wisden et al. 1992), whereas protein detection using immunocytochemical studies indicate the presence of \(\beta_1\)--\(\beta_3\) subunits (Pirker et al. 2000). Our data provide functional confirmation that \(\beta_2/\beta_3\) subunit-containing GABA\(_A\) receptors are widely expressed in the hypothalamic areas.

**Analysis of \(\gamma\) \(\delta\) and \(\epsilon\) subunits**

In vivo, \(\alpha\) and \(\beta\) subunits generally combine with either a \(\gamma\) or \(\delta\) subunit (Hevers and Lüddens 1998). However, binary receptors of only \(\alpha\) and \(\beta\) subunits can readily form functional GABA\(_A\) receptors, and they are known to exist in specific brain regions (Brickley et al. 1999; Kawahara et al. 1993). None of our experiments suggested the existence of binary \(\alpha\beta\) receptors in hypothalamic neurons. Moreover, our pharmacological analyses favor expression of \(\gamma\) over \(\delta\) subunits in hypothalamic GABA\(_A\) receptors. For instance, the IC\(_{50}\) for Zn\(^{2+}\) is significantly greater in \(\alpha\beta\gamma\) receptors (\(\sim 50\) \(\mu\)M) than in \(\alpha\beta\delta\) receptors (\(\sim 1\) \(\mu\)M) (Gingrich and Burkat 1998; Smart et al. 1991). We obtained in hypothalamic neurons a value consistent with the former (70.5 \(\mu\)M). In addition, neurons in the present investigation were sensitive to diazepam and zolpidem, which requires the presence of a \(\gamma_2\) subunit in combination with an \(\alpha\) and a \(\beta\) subunit (Pritchett et al. 1989). The maximal efficacy of diazepam to enhance an EC\(_{20}\) [GABA] in \(\alpha_1\beta_1\gamma_2\) receptors was 86–135% (similar to the maximal efficacy of 106% we recorded in hypothalamic neurons), whereas only approximately a 25–58% potentiation was observed in \(\alpha_2\beta_1\gamma_1\beta_3\) receptors (Ducic et al. 1993; Wafford et al. 1993a).

Responses to the neurosteroid 3a-\(\alpha\)-OH-DHP also favor expression of the \(\gamma\) subunit over expression of the \(\delta\) subunit. In the present studies, we observed 323% potentiation of GABA-activated current in response to 1 \(\mu\)M 3a-\(\alpha\)-OH-DHP. The maximal potentiation is similar to values reported for \(\alpha_1\beta_2\gamma_2\) receptors (Maitra and Reynolds 1998; Zhu et al. 1996) and is several-fold less than observed with \(\alpha_1\beta_2\) receptors (Maitra and Reynolds 1998). Finally, the maximal efficacy we noted for 3a-\(\alpha\)-OH-DHP also suggests the \(\delta\) subunit does not co-express with the \(\alpha\), \(\beta\), and \(\gamma\) subunits (thus forming an \(\alpha\beta\gamma\) receptor), as the presence of \(\delta\) along with these other subunits diminishes the efficacy of 3a-\(\alpha\)-OH-DHP several-fold below that which we observed in the present investigation (Zhu et al. 1996; but see Mihailek et al. 1999). As with our investigation of which \(\alpha\) subunits may be functional in the hypothalamus, our data confirm and expand the in situ hybridization studies of Wisden et al. (1992) and immunohistochemical studies of Peng et al. (2002). They found no evidence for the existence of the \(\delta\) subunit, while mRNA for all \(\gamma\) subunits (although mainly \(\gamma_1\) and \(\gamma_2\)) was present. Our functional studies revealed no evidence for \(\delta\) subunit expression and are most consistent with the suggestion that the \(\gamma\) subunit expressed in the hypothalamus is likely the \(\gamma_2\) isoform.

The \(\epsilon\) subunit has been reported to express in the arcuate-ventromedial region of the hypothalamus via immunohistochemistry and in situ hybridization (Whiting et al. 1997). GABA\(_A\) receptors expressing \(\alpha\), \(\beta\), and \(\epsilon\) subunits have high sensitivity to GABA (average EC\(_{50}\) of 4 \(\mu\)M in \(\alpha_1\beta_1\epsilon\) recep-
tors) and are insensitive to stimulation by benzodiazepines (Kasparov et al. 2001; Whiting et al. 1997). In the present report, no hypothalamic neurons had a GABA EC$_{50}$ <11 μM, and all were responsive to stimulation by diazepam. Thus functional expression of neurons with the αβγ configuration may be minimal.

In summary, results of pharmacological analysis of hypothalamic GABA$_A$ receptors have provided insight about likely subunit configurations expressed in these neurons. The predominant α and β isoforms are likely α1 and/or α2 and β2 and/or β3, respectively. Our data suggest these subunits generally coassemble with the γ2 subunit. We further suggest that α3, α4, α5, α6, β1, and δ subunits express minimally in functional receptors. Given that the GABA$_A$ receptor CI$^-$ channels participate in many hypothalamic functions, modulation of GABA$_A$ receptor function by both endogenous and exogenous modulators may have a profound influence on synaptic transmission in this region. In addition, the knowledge we obtained here should prove to be useful in predicting and explaining effects on various hypothalamic functions as newer therapeutics with greater subunit specificity continue to be developed.

This work was supported by a grant from the American Heart Association and in part by National Institute of Environmental Health Services Grant ES-07904.

REFERENCES

AMIN J, BROOKS-KAYAL A, AND WEISS DS. Two tyrosine residues on the α subunit are crucial for benzodiazepine binding and allosteric modulation of γ-aminobutyric acid $\alpha_1$ receptors. Mol Pharmacol 51: 833–841, 1997.


DRECAVE C AND VAN DEN POL AN. GABA: a dominant transmitter in hypo-


MAJWICZ MD, DEMBROGIO S, SPIVAK CE, AND LONDON ED. The neuroste-


MEISTER B. Gene expression and chemical diversity in hypothalamic neuro-

MIHALEK RM, BANERJEE PK, KORPI ER, QUINLAN JJ, FIRESTONE LL, MI Z-P, LAGENACUR C, TREITTER V, SEIGHART W, ANAGNOSTARAS G, SAGE JR, FANESLO MS, GUIDOTTI A, SPEIGELMAN I, LI Z, DELOREY TM, OLSEN RW, AND HOMANICS GE. Attenuated sensitivity to neuroactive steroids in γ-ami-


OBRIETAN K AND VAN DEN POL AN. GABA neurotransmission in the hypo-
thalamic: developmental reversal from Ca$^{2+}$ elevating to depressing. J Neuro-
PROPERTIES OF HYPOTHALAMIC GABA_A RECEPTORS


WINGROVE PB, WАFFORD KA, BAIN C, AND WHITING PJ. The modulatory action of loreclezole at the γ-aminobutyric acid type A receptor is determined by a single amino acid in the β2 and β3 subunit. Proc Natl Acad Sci USA 91: 4560–4573, 1994.
