Evidence that GABA augmentation of norepinephrine release is mediated by interneurons

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Abstract

GABA_A receptor activation augments stimulated release of ^3_H-norepinephrine (NE) in brain slices from female rats. This effect is not blocked by acetazolamide or MK-801, indicating that permeability of the GABA_A chloride channel to bicarbonate ions and NMDA receptor activation do not mediate GABA-induced NE release. Furthermore, GABA augments ^3_H-NE release from slices, but not from isolated nerve terminals (synaptosomes), indicating that interneurons mediate GABA effects on ^3_H-NE release.

Keywords: Preoptic area; Hypothalamus; Frontal cortex; Brain slices; Synaptosome; NMDA

Both norepinephrine (NE) and GABA in the hypothalamus (HYP) and preoptic area (POA) play critical roles in the expression of female reproductive behavior and in regulation of gonadotropins [2,8,11,15]. Previously, we showed that the inhibitory amino acid neurotransmitter GABA, acting through GABA_A receptors, facilitates electrically evoked release of ^3_H-NE in slices from the cortex, HYP and POA of female rats [4]. The mechanism of this paradoxical effect is unknown. Recently, it has been shown that GABA can depolarize neurons because of the permeability of the GABA_A chloride channel to bicarbonate ions; the ensuing depolarization then facilitates NMDA receptor stimulation [16]. These mechanisms could underlie GABA augmentation of ^3_H-NE release [6]. Alternatively, because there are no NE cell bodies in these brain regions, GABA could act directly on NE terminals to augment release. However, it is also possible that GABA acts on inhibitory interneurons to enhance NE release by disinhibition. The present study determined whether any of these mechanisms are responsible for GABA facilitation of NE release from the cortex, HYP and POA of female rats.

Female Sprague–Dawley rats (150–175 g, Taconic Farms, Germantown, NY) were bilaterally ovariohysterectomized 10–14 days prior to killing. There is no effect of gonadal steroids on GABA facilitation of electrically evoked NE release [4]. Therefore, experiments reported here were performed on ovariohysterectomized rats only. After decapitation, brains were rapidly removed and placed in ice-cold medium containing 124 mM NaCl, 5 mM KCl, 1.2 mM KHPO_4, 1.3 mM MgSO_4, 2.4 mM CaCl_2, 26.2 mM NaHCO_3 and 10.0 mM glucose [18], previously saturated with O_2:CO_2 95:5 and at pH 7.4. For experiments using acetazolamide, 10 mM HEPES (pH 7.4) replaced NaHCO_3, and the medium was saturated with 100% O_2 [16]. The HYP, POA and frontal/parietal cortex were dissected over ice, removed as a block and 350 mm slices were preloaded with ^3_H-NE (0.1 μM; specific activity 58.1 Ci/mmol) as previously described [4].

Slices were placed into a Brandel SF2000 automated superfusion apparatus and perfused for a 70-min washout period at a flow rate of 1.5 ml/4.5 min. The medium contained 1.0 μM desmethylimipramine (DMI; Sigma, St. Louis, MO), a NE uptake inhibitor, and was saturated with O_2:CO_2 (95:5). For electrical stimulation, 1 preconditioning stimulus (18 pulses, 18 mA, 0.3 Hz) was delivered to slices with a Brandel electrical stimulator after 35 min. Following the washout period, up to 20 consecutive 4.5 min fractions were collected for each slice. After collection of 3 baseline samples, slices were stimulated (72 pulses, 18 mA, 3 Hz) at 15 min (S1), 50 min (S2) and 75 min (S3) from the start of the 100-min collection period. A mini-
imum of 3 baseline samples were collected between each electrical stimulation. In independent experiments, either MK-801 (10 μM, dissolved in water; RBI, Natick, MA), a specific NMDA antagonist, acetazolamide (10 μM, dissolved in dimethylsulfoxide; RBI, Natick, MA), a carbogenic anhydrase inhibitor, tetrodotoxin (TTX; 1–2 μM, dissolved in water; RBI, Natick, MA) or vehicle was introduced 10 min prior to S2. This was followed by the addition of 100 μM GABA or vehicle simultaneously with S2. All drugs were removed 5 min later. Calculations for fractional release, S2:S1 ratios and S3:S1 ratios have been previously described [4].

For KCl stimulation, slices were perfused as described above for a 40-min washout period at a flow rate of 1 ml/3 min. Following the washout period, up to 20 consecutive 3.0 min fractions were collected for each slice. After collection of 3 baseline samples, 20 mM KCl with either 100 μM GABA or vehicle was introduced for 3 min (S1). Following the first stimulation, 5 baseline samples were collected, then a second 20-mM KCl stimulation (S2) was delivered without GABA. At the end of the superfusion period, each slice was dissolved in 1.0 M NaOH. The 3H content of each superfusion sample and 3H remaining in the slice were determined using a Beckman scintillation counter. Fractional release in each sample for each slice were determined using a Beckman scintillation counter. Using this preparation, 1 ml 3H-NE uptake, and 10-counted. Using this preparation, 1 ml

Following the first stimulation, 5 baseline samples were collected, then a second 20-mM KCl stimulation (S2) was delivered without GABA. At the end of the superfusion period, each slice was dissolved in 1.0 M NaOH. The 3H content of each superfusion sample and 3H remaining in the slice were determined using a Beckman scintillation counter. Fractional release in each sample for each slice was determined after calculating the 3H content of each slice at the start of sample collection as described previously [4]. We have previously determined using high-pressure liquid chromatography that stimulated 3H release represents at least 65% authentic NE [9].

Synaptoneurosomes (crude synaptosomes) were prepared by homogenization of each brain region from 1 rat in 3 ml ice cold modified Krebs medium (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 1.2 mM KH2PO4, 1.3 mM MgSO4, 0.8 mM CaCl2, 26 mM NaHCO3 and 10.0 mM glucose, previously saturated with O2:CO2 (95:5) with 20 strokes of a hand-held Teflon homogenizer. Homogenate was passed through a 20-μm pore nylon mesh filter and centrifuged at 20,000 × g for 20 min at 4°C. Pellets containing synaptoneurosomes were resuspended in 3 ml Krebs and equilibrated to 37°C with shaking for 10 min. 3H-NE (0.1 μM) was added and allowed to incubate for 15 min, followed by centrifugation at 20,000 × g for 20 min at 4°C. Pellets were washed and resuspended 2 additional times as previously described [5]. Aliquots of 200 μl were then incubated in medium containing 5, 20 or 50 mM KCl, with or without 100 μM GABA, at 30°C for 10 min, or on ice (control). The suspension was centrifuged and medium collected to determine 3H-NE released from each pellet. Each pellet was dissolved in 750 μl of 1 M NaOH and counted. Using this preparation, 1 μM DMI blocks 60–75% of 3H-NE uptake, and 10 μM DMI blocks 77–81% of 3H-NE uptake into synaptoneurosomes, demonstrating that 3H-NE was taken up via the NE transporter and not leaking in or out of the synaptoneurosomes. Percent release of 3H-NE from synaptoneurosomes was determined by dividing the dpm of 3H-NE in the medium by the dpm of 3H-NE in the pellet plus medium and multiplying by 100. The percent of 3H-NE released in the ice control aliquots was subtracted from percent released in samples incubated at 30°C.

Differences between groups were determined using a 1-way analysis of variance (ANOVA) for independent samples. Each brain region was analyzed separately. For experiments using synaptoneurosome preparations, differences between groups were determined using a 2-way ANOVA, with GABA as one between-groups variable, and KCl concentration as the second. Groups were considered to be different from each other if p < 0.05. Post-hoc analysis was performed using a Student Newman–Keuls test.

In cortical, HYP and POA slices, 100 μM GABA introduced simultaneously with S2 increases electrically stimulated 3H-NE release above that of vehicle-perfused controls (Fig. 1). The S2:S1 ratios are greater in tissue perfused with 100 μM GABA than those of vehicle controls (p < 0.0005; Fig. 1A). In addition, GABA increases the duration of the 3H-NE efflux during S2 (p < 0.005; Fig. 1B). GABA also has long-lasting effects on 3H-NE release. GABA significantly increases the S3:S1 ratios, although GABA is washed out immediately following S2 (p < 0.0001; Fig. 1C). Acetazolamide, a carbogenic anhydrase inhibitor, was used to test the role of bicarbonate regeneration in the augmentation of stimulated 3H-NE release by GABA. Acetazolamide alone (10 μM) has no effect on the S2:S1 ratio, the duration of the 3H-NE efflux during S2, or the S3:S1 ratio. Furthermore, it does not attenuate GABA enhancement of the S2:S1 ratio, the duration of the S2 or the S3:S1 ratio (Fig. 1). The data in Fig. 1 show results from slices perfused with 10 mM HEPES-buffered medium. Acetazolamide also failed to modify GABA facilitation of 3H-NE release in slices perfused with sodium bicarbonate-buffered medium (data not shown).

MK-801, a specific NMDA antagonist, was introduced 10 min before S2 to determine whether GABA augments electrically stimulated release of 3H-NE by facilitating NMDA receptor activity. The S2:S1 ratios are greater in tissue perfused with 100 μM GABA than those of vehicle controls (p < 0.01; Fig. 2A). GABA also increases the duration of the 3H-NE efflux during S2 (p < 0.005; Fig. 2B) and increases the S3:S1 ratios (p < 0.05; Fig. 2C). MK-801 alone has no effect on the S2:S1 ratio, S2 duration or S3:S1 ratio and fails to attenuate the GABA augmentation of S2:S1 ratio, S2 duration or S3:S1 ratio (Fig. 2).

To determine whether GABA acts via an interneuron to augment 3H-NE release, TTX was introduced prior to and during S2 using the electrical and KCl stimulation paradigms. TTX inhibited electrically stimulated and KCl-stimulated release of 3H-NE by more than 90% in both the presence and absence of GABA (data not shown). Other

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Fig. 1. Acetazolamide (ACET) has no effect on GABA enhancement of electrically stimulated 3H-NE release. Values are means ± S.E.M. for each group (n = 4 for cortex; n = 7–8 for hypothalamus [HYP]; n = 5–8 for preoptic area [POA]). HEPES = 10 mM HEPES-buffered artificial cerebrospinal fluid, pH 7.4. (A) ACET (10 μM) did not attenuate enhancement of electrically stimulated 3H-NE release (S2:S1) by 100 μM GABA. *Significantly greater than slices perfused without GABA, p < 0.0005. (B) ACET did not attenuate GABA augmentation of the duration of S2. *Significantly greater than slices perfused without GABA, p < 0.005. (C) ACET had no effect on GABA enhancement of S3:S1 ratios. *Significantly greater than slices perfused without GABA, p < 0.0001.

Researchers have also observed this effect of TTX in slice preparations [3]. Thus, TTX could not be used to evaluate the role of interneurons in GABA enhancement of 3H-NE release. Therefore, synaptoneurosomes were prepared from cortex, HYP and POA and depolarized with KCl in order to address this question. Additional experiments confirmed that GABA augments KCl-stimulated release of 3H-NE from slices (p < 0.01; Fig. 3A). In synaptosomes, however, GABA at 100 μM has no effect on basal (5 mM KCl) or stimulated (20 mM KCl) release of 3H-NE (Fig. 3B). Stimulated release of 3H-NE (20 mM KCl) is greater than basal (5 mM KCl) release of 3H-NE (p < 0.005; Fig. 3B). Raising the KCl concentration to 50 mM also increases 3H-NE release compared to 5 mM KCl in all three brain regions (p < 0.0005; data not shown).

These data show that GABA augments electrically and KCl-stimulated 3H-NE release from HYP, POA and frontal cortex slices. However, permeability of the GABA chloride channel to bicarbonate ions is not responsible for GABA facilitation of stimulated 3H-NE release, because acetazolamide did not attenuate the GABA enhancement of 3H-NE release. GABA facilitation of NMDA receptor activity following bicarbonate ion-induced depolarization [16] is also not responsible for GABA facilitation of

Fig. 2. MK-801 has no effect on GABA enhancement of electrically stimulated 3H-NE release. Values are means ± S.E.M. for each group (n = 4 for cortex; n = 7–8 for hypothalamus [HYP] and preoptic area [POA]). ACSF = artificial cerebrospinal fluid, pH 7.4. (A) MK-801 (10 μM) did not attenuate enhancement of electrically stimulated 3H–NE release (S2:S1) by 100 μM GABA. *Significantly greater than slices perfused without GABA, p < 0.01. (B) MK-801 did not attenuate GABA augmentation of the duration of S2. *Significantly greater than slices perfused without GABA, p < 0.005. (C) MK-801 had no effect on GABA enhancement of S3:S1 ratios. *Significantly greater than slices perfused without GABA, p < 0.05.
stimulated \(^3\)H-NE release from slices as evidenced by the failure of MK-801 to block GABA-augmented \(^3\)H-NE release. In addition, GABA augments KCl-stimulated release of \(^3\)H-NE from slices, but not from synaptoneurosomes, indicating that GABA is acting on an interneuron and not on the noradrenergic terminals in these brain regions.

GABA augmentation of stimulated \(^3\)H-NE release has previously been shown to be mediated through the GABA\(_A\) receptor [4,13]. Because the present study shows that the facilitatory action of GABA is not mediated by bicarbonate ion-induced depolarization, it is likely that GABA is acting through inhibitory GABA\(_A\) chloride channels located on interneurons. This interpretation is supported by the finding that GABA fails to modify basal or evoked \(^3\)H-NE release from isolated nerve terminals (synaptoneurosomes). Thus, GABA indirectly augments stimulated \(^3\)H-NE release through GABA\(_A\) receptors on interneurons.

It is unknown what inhibitory neurotransmitters or neuromodulators might be localized within these GABA-receptive interneurons; however, there is anatomical and neurochemical evidence that enkephalin and beta-endorphin are inhibited by GABA in other brain regions [10,17]. These opioid peptides inhibit stimulated \(^3\)H-NE release in the POA [1]. Furthermore, GABA can reverse inhibitory effects of morphine on \(^3\)H-NE release from frontal cortex slices [14]. Hence, it is possible that GABA disinhibits \(^3\)H-NE release via modulation of endogenous opioids. Alternatively, GABA may disinhibit a tonic GABAergic inhibition of NE release. GABA–GABA disinhibitory circuits have been proposed as a mechanism for other neural functions [7,12]. Further investigation is necessary to determine what neurotransmitters might be regulated by GABA to influence NE release in these brain regions.

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References


