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γ-AMINOBUTYRIC ACID ENHANCEMENT OF POTASSIUM-STIMULATED RELEASE OF [^{3}H]NOREPINEPHRINE BY MULTIPLE MECHANISMS IN RAT CORTICAL SLICES

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Abstract—γ-Aminobutyric acid (GABA) and $GABA_A$ agonists enhance stimulated release of [^{3}H]norepinephrine ([^{3}H]NA) in several regions of the rat brain. In this study, the mechanisms by which GABA and GABAergic agonists augment potassium-stimulated release of [^{3}H]NA from rat frontal cortical slices were examined. GABA enhanced potassium-stimulated [^{3}H]NA release, but did not alter release of [^{3}H]NA evoked by the calcium ionophore A23187, 10^{-5} M, either in the presence or the absence of extracellular calcium. The effect of GABA on potassium-stimulated [^{3}H]NA release was apparently reduced by the $GABA_A$ antagonist bicuculline methiodide, 10^{-4} M, and by the selective inhibitor of GABA uptake SKF 89976A, 10^{-5} M, but was abolished only when bicuculline methiodide and SKF 89976A were present in combination. The $GABA_A$ agonist muscimol enhanced potassium-stimulated release of [^{3}H]NA in a manner similar to GABA. In addition, nipecotic acid, a substrate for GABA uptake, enhanced potassium-stimulated [^{3}H]NA release. Thus, GABA appears to enhance potassium-stimulated [^{3}H]NA release by acting upon both GABA uptake and $GABA_A$ receptors. The $GABA_A$ receptors involved in this effect may be a subtype of $GABA_A$ receptors since they are not modulated by benzodiazepines. These results support the involvement of the GABA uptake carrier and the $GABA_A$ receptor in mediating the enhancement by GABA of potassium-stimulated [^{3}H]NA release in the cortex of the rat.

γ-Aminobutyric acid (GABA) enhances the spontaneous and stimulated release of [^{3}H]norepinephrine ([^{3}H]NA) in several regions of rat brain including cerebral cortex and hippocampus [1–4]. In rat cerebellar slices and hippocampal synaptosomes, GABA modulates potassium-stimulated release of [^{3}H]NA by two opposing mechanisms: GABA enhances [^{3}H]NA release by stimulating $GABA_A$ receptors and inhibits [^{3}H]NA release by stimulating $GABA_B$ receptors [3, 5]. Spontaneous release of [^{3}H]NA from rat hippocampal synaptosomes differs from stimulated [^{3}H]NA release in that it is enhanced by $GABA_A$ receptor stimulation, but is not inhibited by $GABA_B$ receptor stimulation [1, 3]. In addition, GABA enhances spontaneous release of [^{3}H]NA through an action mediated by the GABA uptake carrier [2, 6]. The influence of this action of GABA upon the GABA transport carrier in regulating the stimulated release of [^{3}H]NA in the rat CNS has not been determined. These studies were performed to characterize the mechanisms by which GABA enhances potassium-stimulated [^{3}H]NA release in rat frontal cortical slices.

MATERIALS AND METHODS

Assay of [^{3}H]*NA release.* Measurement of [^{3}H]NA release from brain slices was performed as described by Werling *et al.* [7], with minor modifications. Male Sprague–Dawley rats weighing 180–250 g were anesthetized with ether and decapitated, and the brains were removed and dissected on an ice-cooled plate to yield strips of frontal cortex approximately 2 × 2 × 4 mm. Coronal slices of frontal cortex 0.3 × 2 × 2 mm were prepared using a McIlwain tissue chopper, and were immediately dispersed in ice-cold oxygenated modified Krebs buffer (NaCl, 127 mM; KCl, 5 mM; Na_2HPO_4, 1.3 mM; $MgSO_4$, 1.2; $CaCl_2$, 2.5; HEPES, 15 mM; and glucose, 10 mM). Brain slices were rinsed three times in oxygenated modified Krebs buffer and incubated for 15 min in a solution of 100 nM [^{3}H]NA in modified Krebs buffer at 37° under 95% O_2/5% CO_2 with gentle agitation. Brain slices were then rinsed twice for 5 min each in modified Krebs buffer and once for 5 min in modified Krebs buffer containing 10^{-6} M desipramine and 10^{-5} M phentolamine (to block reuptake of released [^{3}H]NA and stimulation of α_2 autoinhibitory receptors by released [^{3}H]NA respectively; these drugs were present in all subsequent steps), all at 37° under 95% O_2/5% CO_2. After the final rinsing step, slices were transferred to a set of 24 nylon mesh baskets resting in a 24-well tissue culture plate containing 2 mL of oxygenated modified Krebs buffer per well, and incubated for 10 min at 37° under 95% O_2/5% CO_2 with gentle shaking. Slices were exposed to drug treatments during a subsequent incubation in a 24-well plate containing modified Krebs buffer with the desired treatments arranged randomly among wells.

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The effects of the various treatments on potassium-stimulated release of [^{3}H]NA were determined by transferring the set of baskets to a third 24-well plate containing the desired treatments in high-potassium modified Krebs buffer and incubating as before. After the final incubation, the radioactivity remaining in the slices was extracted with 0.2 N HCl for 45 min. The amount of radioactivity released during each incubation was determined by liquid scintillation counting, and expressed as the percent of tissue radioactivity present at the beginning of the incubation period. Three to six brain slices were used for each experimental treatment per day, and each experiment was repeated from one to three times.

Experiments in which release of [^{3}H]NA from frontal cortical slices was stimulated with the calcium ionophore A23187 were performed similarly, except that cortical slices were prepared in calcium-free modified Krebs buffer containing 10^{-5} M ethylene glycolbis(aminoethyl ether)tetra-acetic acid (EGTA). A23187, 10^{-5} M, was added during the second incubation step, and modified Krebs buffer containing calcium was added during the third incubation step to stimulate release of [^{3}H]NA.

Materials. The following drugs and chemicals were used in this study: GABA, muscimol, and calcium ionophore A23187 (Sigma Chemical Co., St. Louis, MO); 1(*S*),9(*R*)-(−)-bicuculline methiodide (Research Biochemicals Inc., Natick, MA); desipramine HCl (Merrell Dow Inc., Cincinnati, OH); phentolamine HCl (Ciba-Geigy Corp., Summit, NJ); chlordiazepoxide HCl and diazepam HCl (Roche Laboratories, Nutley, NJ); *l*-[7-^{3}H]norepinephrine, 14.2 Ci/mmol (New England Nuclear Research Products, Boston, MA); pargyline HCl (Abbott Laboratories, North Chicago, IL); and *N*-(4,4-diphenyl-3-butenyl) nipecotic acid (SKF 89976A; Nova Pharmaceutical Co., Baltimore, MD).

Statistical analysis. Results were subjected to one- or two-way analysis of variance, and differences among means were determined by the Newman–Keuls range test at the 0.05 significance level.

RESULTS

GABA, 5×10^{-4} to 10^{-3} M, enhanced potassium-stimulated [^{3}H]NA release (Fig. 1). Spontaneous release of [^{3}H]NA was not affected by GABA. To determine if GABA was acting at a step in the neurotransmitter release process prior to the entry of calcium, its effect on [^{3}H]NA release stimulated by the calcium ionophore A23187 was assessed. A23187, 10^{-5} M, produced a slight stimulation of [^{3}H]NA release in the absence of extracellular calcium, and an approximately 2-fold stimulation of [^{3}H]NA release in the presence of extracellular calcium (Fig. 2). GABA, 5×10^{-4} M, failed to enhance A23187-stimulated [^{3}H]NA release in the absence or the presence of extracellular calcium, suggesting that the effect of GABA upon potassium-stimulated [^{3}H]NA release involves enhancement of calcium entry rather than enhancement of sensitivity to calcium.

Experiments performed to characterize the response to GABA indicated that multiple actions

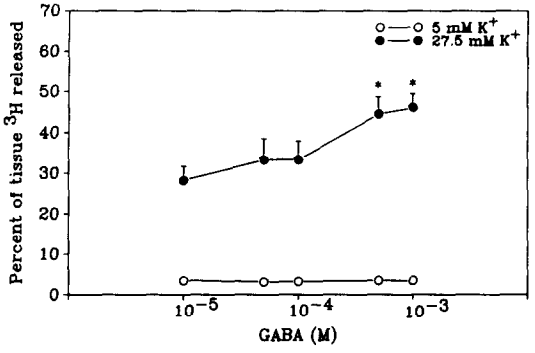

Fig. 1. Concentration–response for GABA enhancement of potassium-stimulated [^{3}H]NA release from rat frontal cortical slices. Values are the means ± SE of three rats (four brain slices per treatment from each rat). The control values were: 3.2 ± 0.1% of fractional ^{3}H released under basal conditions (9.3 ± 0.4 fmol/slice); 27.7 ± 0.5% of fractional ^{3}H released following potassium stimulation (81.3 ± 7.5 fmol/slice). Key: *significantly different from control ($P < 0.05$).

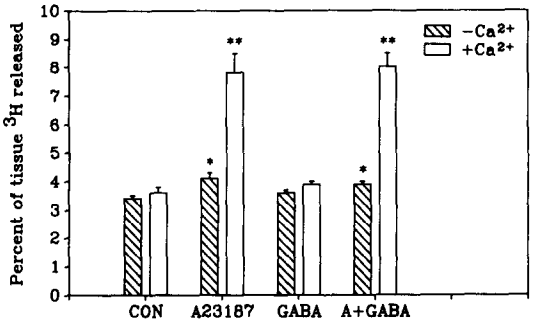

Fig. 2. Effect of GABA on A23187-stimulated release of [^{3}H]NA from rat frontal cortical slices in the absence (hatched bars) and the presence (open bars) of extracellular calcium. CON, control; A23187 (10^{-5} M); GABA (5×10^{-4} M); and A + GABA, A23187 (10^{-5} M) + GABA (5×10^{-4} M). Values are the means ± SE of six brain slices. The control values were: 3.4 ± 0.1% of fractional ^{3}H released in calcium-free buffer (26.6 ± 3.1 fmol/slice); in calcium-containing buffer, 3.6 ± 0.2% of fractional ^{3}H released (27.3 ± 3.3 fmol/slice). Key: *significantly different from control [^{3}H]NA release in the absence of calcium ($P < 0.05$); and **significantly different from control [^{3}H]NA release in the presence of calcium ($P < 0.05$).

of GABA were responsible for its effects. The effect of GABA, 5×10^{-4} M, was reduced slightly but not significantly by the $GABA_A$ antagonist bicuculline methiodide or by the selective inhibitor of GABA uptake SKF 89976A, but was abolished in the presence of both bicuculline methiodide and SKF 89976A (Fig. 3). In addition, potassium-stimulated release of [^{3}H]NA was enhanced by the $GABA_A$ agonist muscimol (Fig. 4), which is a poor substrate for GABA uptake [8], and by the GABA uptake inhibitor nipecotic acid, which is a substrate for GABA uptake (Fig. 5).

Previous studies have demonstrated that the

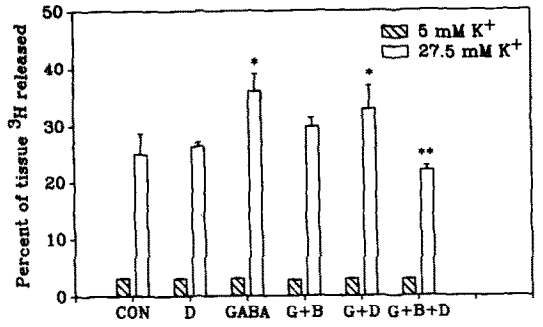

Fig. 3. Effect of SKF 89976A and bicuculline methiodide on the enhancement by GABA of potassium-stimulated [^{3}H]NA release in rat frontal cortical slices. CON, control; D, SKF 88976A (10^{-5} M); GABA (5×10^{-4} M); G + B, GABA (5×10^{-4} M) + bicuculline methiodide (10^{-4} M); G + D, GABA (5×10^{-4} M) + SKF 89976A (10^{-5} M); G + B + D, GABA (5×10^{-4} M) + bicuculline methiodide (10^{-4} M) + SKF 89976A (10^{-5} M). Values are the means ± SE of three rats (four brain slices per treatment from each rat). Basal release (5 mM K$^+$) of NA in controls was 3.1 ± 0.1% of fractional ^{3}H released (12.1 ± 1.3 fmol/slice) and potassium-stimulated release (27.5 mM K$^+$) in controls was 24.9 ± 3.8% of fractional ^{3}H released (98.4 ± 14.5 fmol/slice). Key: *significantly different from control ($P < 0.05$); and **significantly different from the GABA treatment group ($P < 0.05$).

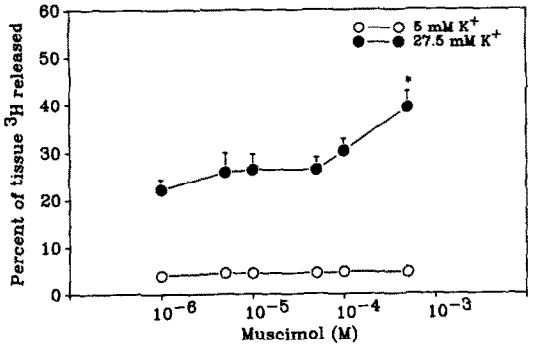

Fig. 4. Concentration–response for muscimol enhancement of potassium-stimulated [^{3}H]NA release from rat frontal cortical slices. Values are the means ± SE of three rats (four brain slices per treatment from each rat). The control values were: 4.5 ± 0.4% of fractional ^{3}H released under basal conditions (6.9 ± 2.4 fmol/slice); 21.2 ± 2.5% of fractional ^{3}H released following potassium stimulation (39.7 ± 16.4 fmol/slice). Key: *significantly different from control ($P < 0.05$).

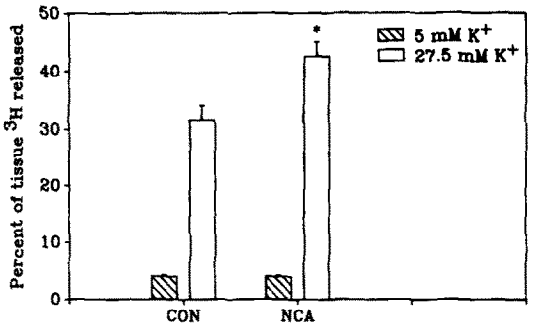

Fig. 5. Effect of nipecotic acid on potassium-stimulated [^{3}H]NA release from rat frontal cortical slices. CON, control; NCA, nipecotic acid (10^{-4} M). Values are the means ± SE of two rats (six brain slices per treatment from each rat). Basal release (5 mM K$^+$) in controls was 4.0 ± 0.2% of fractional ^{3}H released (6.0 ± 0.2 fmol/slice), and potassium-stimulated release (27.5 mM K$^+$) in controls was 31.5 ± 2.5% of fractional ^{3}H released (45.0 ± 3.2 fmol/slice). Key: *significantly different from control ($P < 0.05$).

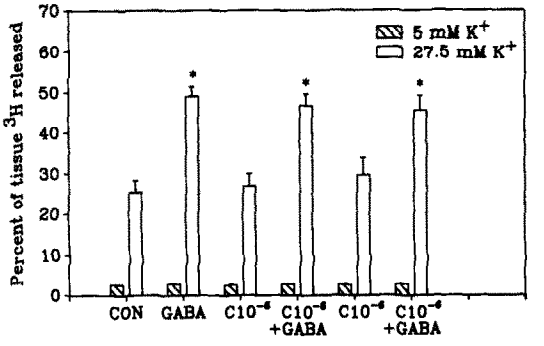

Fig. 6. Effect of chlordiazepoxide on potassium-stimulated [^{3}H]NA release in the absence and the presence of GABA. CON, control; GABA (10^{-4} M); C10^{-8}, chlordiazepoxide (10^{-8} M); C10^{-8} + GABA, chlordiazepoxide (10^{-8} M) + GABA (10^{-4} M); C10^{-6}, chlordiazepoxide (10^{-6} M); C10^{-6} + GABA, chlordiazepoxide (10^{-6} M) + GABA (10^{-4} M). Values are the means ± SE of four brain slices. Basal release (5 mM K$^+$) in controls was 2.7 ± 0.1% of fractional ^{3}H released (7.8 ± 0.8 fmol/slice), and potassium-stimulated release (27.5 mM K$^+$) in controls was 25.5 ± 2.9% of fractional ^{3}H released (74.3 ± 15.6 fmol/slice). Key: *significantly different from control ($P < 0.05$).

GABA$_A$ receptor is allosterically regulated by benzodiazepines at nanomolar concentrations [9]. In contrast to the results that would be predicted for an event involving GABA$_A$ receptors, however, chlordiazepoxide at 10^{-8} M or 10^{-6} M did not enhance the effect of 10^{-4} M GABA (Fig. 6). Chlordiazepoxide at 10^{-4} M inhibited potassium-stimulated [^{3}H]NA release (results not shown), probably by decreasing calcium influx, as has been demonstrated for benzodiazepines in other preparations [10]. Similar results were obtained with diazepam (not shown).

DISCUSSION

In these studies, potassium-stimulated release of [^{3}H]NA was enhanced by GABA. The enhancement by GABA of potassium-stimulated [^{3}H]NA release was reduced, but not completely blocked, by the GABA$_A$ antagonist bicuculline methiodide. In addition, potassium-stimulated release of [^{3}H]NA was enhanced by the GABA$_A$ agonist muscimol.

The failure of GABA to augment release of [^{3}H]NA stimulated by the calcium ionophore A23187

indicates that its effect did not involve increasing the sensitivity of the neurotransmitter release process to calcium. The GABA receptor mediating this effect thus appears to act by enhancing the influx of calcium into the noradrenergic terminals, most probably by producing depolarization. Although $GABA_A$ receptors in the cell bodies of CNS neurons usually mediate hyperpolarization, the effect of $GABA_A$ receptor stimulation in the neuronal terminals may differ. The effect of $GABA_A$ receptor stimulation on membrane potential in a particular neuron, or a given region of a neuron, can vary depending upon the resting membrane potential and the chloride gradient [11, 12]. $GABA_A$ receptor-mediated depolarization in peripheral neurons is well established [13–16], and has also been demonstrated recently in CNS neurons [12, 17].

In addition to an action at $GABA_A$ receptors, GABA also enhances potassium-stimulated [^{3}H]NA release by a process involving the GABA uptake carrier. This conclusion is based on two observations. First, a component of the [^{3}H]NA release enhanced by GABA was not reduced by bicuculline methiodide; this component was blocked by the selective inhibitor of GABA uptake SKF 89976A. Second, potassium-stimulated release of [^{3}H]NA was enhanced by nipecotic acid, which does not stimulate $GABA_A$ receptors but is a substrate for GABA uptake, but not by the nipecotic acid analogue SKF 89976A, which is not a substrate for GABA uptake. In other preparations GABA has been demonstrated to produce a depolarization mediated by the GABA uptake carrier [18–20]. In addition, other investigators have reported that GABA or nipecotic acid enhances basal release of [^{3}H]NA from cerebrocortical and hippocampal synaptosomes in a manner reversible by SKF 89976A [1, 6].

Although results of studies using selective GABA agonists and antagonists are consistent with an involvement of $GABA_A$ receptors in mediating the response to GABA, the lack of modulation by benzodiazepines is not characteristic of the classical $GABA_A$/benzodiazepine receptor complex. However, multiple subtypes of $GABA_A$ receptors have been identified, and $GABA_A$ receptors possessing the benzodiazepine binding site may represent one or more of these subtypes [21]. $GABA_A$ receptors that are not modulated by benzodiazepines have been observed in mouse spinal cord neurons [22] and in *Xenopus* oocytes injected with mRNA coding for a $GABA_A$ receptor type from bovine brain [21]. The benzodiazepine binding site on the $GABA_A$ receptor has been shown recently to reside on a subunit distinct from those possessing the GABA and barbiturate binding sites [23]; the absence of this subunit in a subclass of $GABA_A$ receptors could account for benzodiazepine-insensitive $GABA_A$ receptor-mediated responses.

Various investigators have reported previously that GABA and $GABA_A$ agonists enhance spontaneous release of [^{3}H]NA from slices or synaptosomes prepared from a number of brain regions [1–3, 24, 25], although this was not observed in all studies. In the present study, GABA did not increase spontaneous release of [^{3}H]NA at concentrations as high as 1 mM. The reason for the effect of GABA upon potassium-stimulated, but not spontaneous, release of [^{3}H]NA in these studies is not clear. One explanation could be that in this preparation GABA produces a slight depolarization that in itself is not sufficient to induce [^{3}H]NA release, but that contributes to the depolarization, and therefore the [^{3}H]NA release, induced by high potassium.

Whether the effect of GABA on release of [^{3}H]NA by high potassium reflects a physiological role of GABA in the cortex is not clear. The effect of GABA on potassium-stimulated [^{3}H]NA release occurs at relatively high concentrations that may not be attained *in vivo*. However, the high concentrations of GABAergic agents required may reflect their limited penetration of the tissue, as has been suggested previously by Wong *et al.* [26], who observed similar potencies for GABAergic agents in stimulating chloride flux in rat hippocampal slices. In addition, although the presence of GABA receptors on the noradrenergic terminals could be considered to indicate a GABAergic innervation of the terminals, this has not been demonstrated conclusively. An alternative explanation for the presence of GABA receptors and GABA uptake carriers in the noradrenergic terminals is that GABA is a cotransmitter with NA in these neurons [2]. If GABA is indeed released from these terminals along with NA, it could modulate its own release as well as that of NA. Although *in vitro*, the depolarization produced by GABA enhances potassium-stimulated release of [^{3}H]NA by increasing the degree of depolarization of the terminals, *in vivo* the depolarization produced by GABA would be expected to inhibit release of NA by decreasing action potential height [27]. Thus, GABA could provide an additional mechanism for the regulation of NA release in the cortex.

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