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γ-AMINOBUTYRIC ACID$_A$ (GABA$_A$) RECEPTOR MODULATION OF MORPHINE INHIBITION OF NOREPINEPHRINE RELEASE

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Abstract—Agents that enhance γ-aminobutyric acid (GABA) neurotransmission can modulate certain effects of opioids, such as analgesia. In this study, the interaction between morphine and GABAergic agents on the release of [^{3}H]norepinephrine ([^{3}H]NE) from rat frontal cortical slices was examined. GABA (10^{-4} M), enhanced potassium-stimulated [^{3}H]NE release and reversed the inhibitory effect of 10^{-6} M morphine. GABA and muscimol modulated the inhibitory effect of morphine in a noncompetitive manner. Bicuculline methiodide (10^{-4} M) reduced the effect of GABA in the absence of morphine, and appeared to reduce the effect of GABA in the presence of morphine, although the latter effect was not statistically significant from the controls. While the GABA$_A$ agonist muscimol mimicked the effect of GABA, the GABA$_B$ agonist baclofen did not affect the release of [^{3}H]NE in the absence or the presence of 10^{-6} M morphine. These results support the involvement of GABA$_A$ receptors in modulating the action of opioids on the noradrenergic system in the cerebral cortex of the rat.

Previous studies have demonstrated that γ-aminobutyric acid (GABA†) and GABAergic agents can alter the analgesic effect of morphine and other opioids. This interaction between GABAergic agents and opioids appears to vary depending upon the route of administration of the GABAergic agents [1]. Thus, while systemic administration of GABA$_A$ agonists, the GABA$_B$ agonist baclofen, or GABA transaminase inhibitors enhances opioid analgesia, central administration of GABA, GABA$_A$ agonists, or GABA uptake inhibitors reverses opioid analgesia [1–6].

Although this interaction between GABAergic agents and opioids has potential clinical relevance, its neurochemical basis has not been elucidated. A central neurotransmitter that may be involved in this interaction is the catecholamine norepinephrine (NE). An involvement of NE in the forebrain in mediating the effects of opioids, including analgesia, has been demonstrated [7–11]. In addition, the stimulated release of [^{3}H]NE in the rat cerebral cortex *in vitro* is inhibited by morphine and other μ-opioid agonists [12, 13], and enhanced by GABA [14, 15]. Thus, GABA could oppose the analgesic effect of opioids by an action on the noradrenergic terminals in the forebrain. The aim of the current study was to determine if GABA would reverse the inhibitory effect of morphine on [^{3}H]NE release in slices of rat frontal cortex, and to characterize the GABA receptor responsible for the effect.

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† Abbreviations: GABA, γ-aminobutyric acid; NE, norepinephrine; MKB, modified Krebs buffer; and HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

MATERIALS AND METHODS

Assay of [^{3}H] *release*. Measurement of [^{3}H]NE release from brain slices was performed by the method of Werling *et al.* [13], with minor modifications. Briefly, 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. Coronal slices of frontal cortex (0.3 × 2 × 2 mm) were prepared using a McIlwain tissue chopper, and were dispersed immediately in ice-cold oxygenated modified Krebs buffer [MKB; composition, in mM: NaCl, 127; KCl, 5; Na_2HPO_4, 1.3; $MgSO_4$, 1.2; $CaCl_2$, 2.5; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 15; and glucose, 10]. Brain slices were rinsed three times in oxygenated MKB and incubated for 15 min in a solution of 100 nM [^{3}H]NE in MKB at 37° under 95% O_2/5% CO_2 with gentle agitation. Brain slices were then rinsed twice for 5 min each in MKB and once for 5 min in MKB containing 10^{-6} M desipramine and 10^{-5} M phentolamine (to block NE reuptake and α_2-adrenergic receptors; these drugs were present in all subsequent steps), all at 37° under 95% O_2/5% CO_2. Slices were then transferred to a set of 24 nylon mesh baskets resting in a 24-well tissue culture plate containing 2 mL of oxygenated MKB per well. The tissue culture plate was placed in a shaking water bath and incubated for 10 min at 37° under 95% O_2/5% CO_2 with gentle shaking. Slices were exposed to drug treatments by transferring the set of baskets to a second 24-well plate containing MKB with the desired treatments arranged randomly among wells, and incubating as before. Release of [^{3}H]NE was evoked by transferring the set of baskets to a third 24-well plate containing the desired treatments in high-potassium MKB (in which KCl was substituted for NaCl on an equimolar basis) and incubating as before. After the third incubation, the

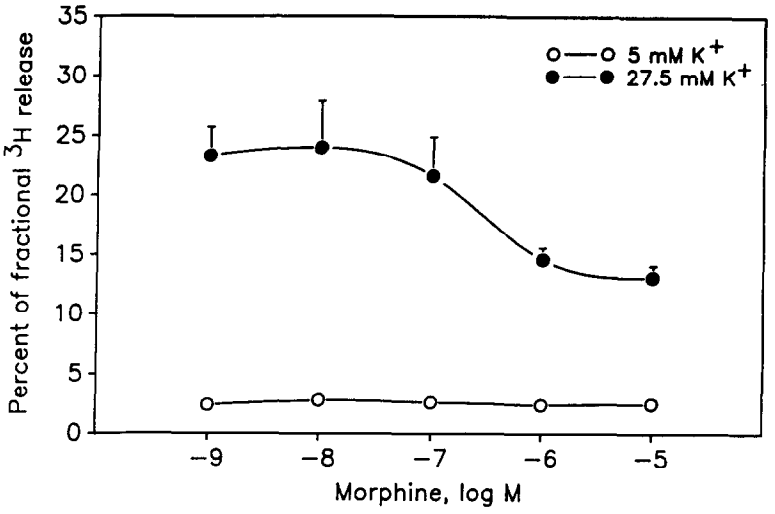

Fig. 1. Effect of morphine on basal (○) and potassium-stimulated (●) [^{3}H]norepinephrine release from rat frontal cortical slices. Values are the means ± SEM of three rats.

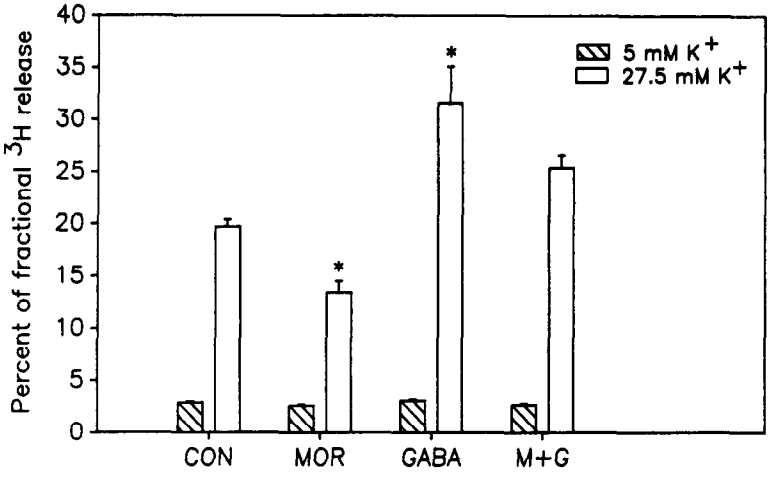

Fig. 2. Effect of GABA on basal (hatched bars) and potassium-stimulated (open bars) [^{3}H]-norepinephrine release from rat frontal cortical slices in the absence and the presence of morphine. CON, control; MOR, morphine (10^{-6} M); GABA (5×10^{-4} M); M + G, morphine (10^{-6} M) + GABA (5×10^{-4} M). Values are the means ± SEM of six brain slices. Repeat experiments gave similar results. Key: * significantly different from control ($P < 0.05$).

radioactivity remaining in the slices was extracted by transferring the set of baskets to a 24-well plate containing 0.2 N HCl at 37° 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.

Drugs and chemicals. The following drugs and chemicals were used in this study: GABA and muscimol (Sigma Chemical Co., St. Louis, MO); (±)-baclofen and 1(*S*),9(*R*)-(−)-bicuculline methiodide (Research Biochemicals Inc., Natick, MA); picrotoxin (Nutritional Biochemicals Corp., Cleveland, OH); morphine sulfate (Penick Chemical Co., Newark, NJ); naloxone HCl (DuPont Pharmaceuticals, Wilmington, DE); desipramine HCl (Merrell Dow Inc., Cincinnati, OH); 1-[7-^{3}H]-norepinephrine, 14.2 Ci/mmol (New England Nuclear Research Products, Boston, MA); phentolamine HCL (Ciba-Geigy Corp., Summit, NJ); and pargyline HCl (Abbott Laboratories, North Chicago, IL).

Statistical analysis. Results were subjected to one- or two-way analysis of variance and differences among means were determined by the Newman–

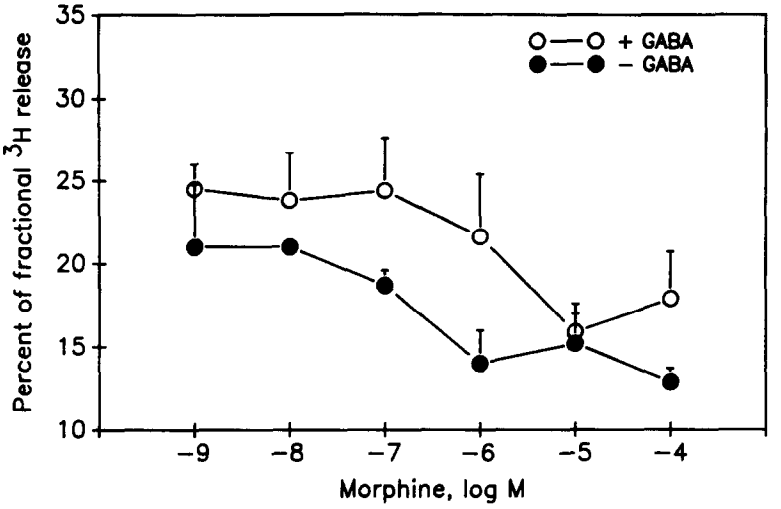

Fig. 3. Concentration–response for morphine inhibition of potassium-stimulated [^{3}H]norepinephrine release from rat frontal cortical slices in the absence (●) and the presence (○) of GABA (10^{-4} M). Values are the means ± SEM of four rats (two brain slices per treatment from each rat). The morphine concentration–response curve determined in the absence of GABA was significantly different ($P < 0.05$) from the curve determined in the presence of GABA.

Keuls range test at the 0.05 significance level. Concentration–response data were analyzed using the nonlinear curve-fitting program ALLFIT. Data are expressed as means ± SEM; in figures where error bars are absent, they are contained within the symbol.

RESULTS

Morphine inhibited potassium-stimulated [^{3}H]NE release from cortical slices (Fig. 1), as has been reported previously [12–14, 16]. The EC_{50} for morphine in the present study was approximately 2×10^{-7} M, and the maximal inhibition produced by morphine was approximately 45% of control. GABA (10^{-4} M) did not affect the basal efflux of [^{3}H]NE, but enhanced potassium-stimulated [^{3}H]-NE release [17] and reversed the effect of morphine (Fig. 2). GABA (10^{-4} M) shifted the morphine concentration–response curve along the vertical axis (Fig. 3), as evidenced by a significant increase in both the E_{min} and E_{max} of morphine ($E_{min} = 14$ and $E_{max} = 21$ in the absence of GABA vs $E_{min} = 17$ and $E_{max} = 24$ in the presence of GABA; ANOVA, $P < 0.05$). The EC_{50} for morphine appeared to increase in the presence of GABA (1.1×10^{-6} M in the presence of GABA vs 1.3×10^{-7} M in the absence of GABA); however, this apparent difference was not statistically significant (ANOVA, $P = 0.12$).

Experiments using selective agonists and antagonists supported a role of $GABA_A$, but not $GABA_B$, receptors in mediating the effect of GABA. The $GABA_A$ agonist muscimol at 10^{-4} M enhances potassium-stimulated [^{3}H]NE release [17] and reversed the inhibition produced by 10^{-6} M morphine (Fig. 4), while the $GABA_B$ agonist baclofen (10^{-6}–10^{-4} M) did not affect potassium-stimulated [^{3}H]NE release or its inhibition by 10^{-6} M morphine (Fig. 4B). In concentration–response studies (Fig. 5), muscimol produced a shift in the morphine concentration–response curve along the vertical axis similar to that observed with GABA. The $GABA_A$ antagonist bicuculline methiodide (10^{-4} M) reduced the effect of GABA in the absence of morphine, and appeared to reduce the effect of GABA in the presence of 10^{-6} M morphine, although the latter effect was not statistically significant from the control (Fig. 6).

DISCUSSION

Previous studies have demonstrated that stimulated release of [^{3}H]NE in the cerebral cortex of the rat is inhibited by stimulation of μ-opioid receptors [12, 13] and enhanced by stimulation of GABA receptors [14, 15]. In the present study the inhibitory effect of morphine on potassium-stimulated [^{3}H]NE release in the rat frontal cortex was reversed by GABA.

It should be pointed out that the concentrations of GABAergic agents used were relatively high. In a previous study, EC_{50} values for enhancement by GABA or muscimol of potassium-stimulated [^{3}H]-NE release from rat cortical slices were determined to be 5×10^{-4} M and 1.5×10^{-4} M, respectively [17]. These high EC_{50} values may result from poor penetration of the brain slices by the GABAergic agents [18]. Nevertheless, these concentrations are similar to those that were effective in other studies using *in vitro* tissue preparations. For example, Brown and Scholfield [19] reported that GABA-induced depolarization of guinea pig olfactory cortex slices occurs at minimal effective concentrations of 5×10^{-5} to 2×10^{-4} M. Similarly, Fung and Fillenz [20] found that 10^{-5} to 10^{-3} M GABA stimulates

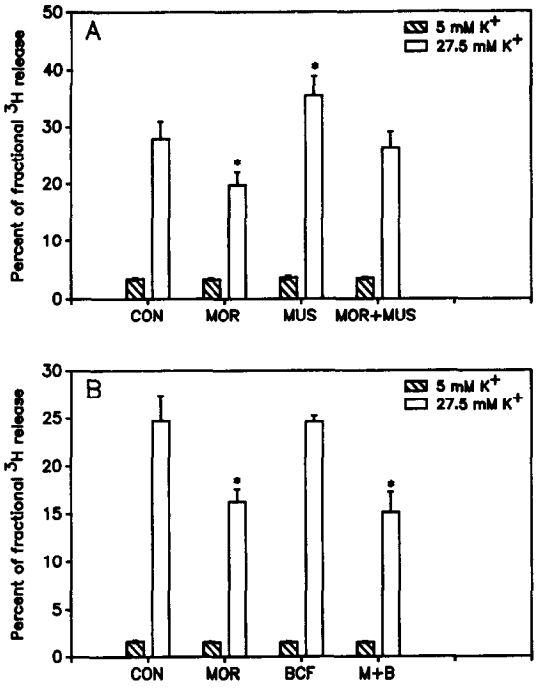

Fig. 4. (A) Effect of muscimol on basal (hatched bars) and potassium-stimulated (open bars) [^{3}H]norepinephrine release from rat frontal cortical slices in the absence and the presence of morphine. CON, control; MOR, morphine (10^{-6} M); MUS, muscimol (10^{-4} M); MOR + MUS, morphine (10^{-6} M) + muscimol (10^{-4} M). (B) Effect of baclofen on basal (hatched bars) or potassium-stimulated (open bars) [^{3}H]NE release in the absence or the presence of morphine. CON, control; MOR, morphine (10^{-6} M); BCF, baclofen (10^{-4} M); M + B, morphine (10^{-6} M) + baclofen (10^{-4} M). Values are the means ± SEM of three rats (six brain slices per treatment from each rat). Key: * significantly different from control ($P < 0.05$).

the release of [^{3}H]NE from rat hippocampal synaptosomes in a concentration-dependent manner, and that the effect of GABA is reversed by picrotoxin or bicuculline methobromide.

Characterization of the GABA response using selective agonists and antagonists supported a role for $GABA_A$ receptors. The effect of GABA in the absence of morphine was reduced by the $GABA_A$ antagonist bicuculline methiodide and mimicked by the $GABA_A$ agonist muscimol, while the $GABA_B$ agonist baclofen was without effect. Therefore, although both $GABA_A$ and $GABA_B$ receptors are present at high density in the frontal cortex of the rat [21], and $GABA_B$ receptors appear to be located on noradrenergic terminals in the frontal cortex [22, 23], only $GABA_A$ receptors appear to regulate potassium-stimulated release of [^{3}H]NE in this brain region. In other areas of the brain, presynaptic $GABA_B$ receptors regulating NE release have been demonstrated [20, 24]. Use of the newly developed $GABA_B$ antagonists would give more conclusive support to the role of $GABA_A$ receptors.

In addition to an action mediated by $GABA_A$ receptors, GABA appears to enhance potassium-stimulated [^{3}H]NE release from cortical slices by an action involving GABA transport [17]. Other investigators have demonstrated the involvement of a GABA uptake mechanism in the stimulation by GABA of spontaneous [^{3}H]NE release from cortical and hippocampal synaptosomes [25–27]. In these studies this additional action of GABA may have been reflected in the failure of bicuculline methiodide or picrotoxin to block the effect of GABA completely.

The interaction between GABA and morphine may involve opposing effects on the membrane potential of the noradrenergic terminals. Opioids, through an action upon μ-opioid receptors, have been shown to hyperpolarize the cell bodies of

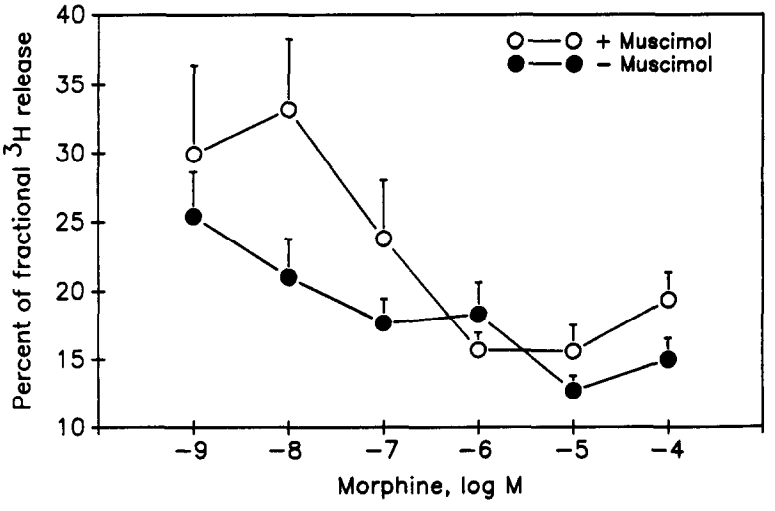

Fig. 5. Concentration–response for morphine inhibition of potassium-stimulated [^{3}H]norepinephrine release from rat frontal cortical slices in the absence (●) and the presence (○) of muscimol (10^{-4} M). Values are the means ± SEM of four rats (2–3 brain slices per treatment from each rat). The morphine concentration–response curve determined in the absence of muscimol was significantly different ($P < 0.05$) from the curve determined in the presence of muscimol.

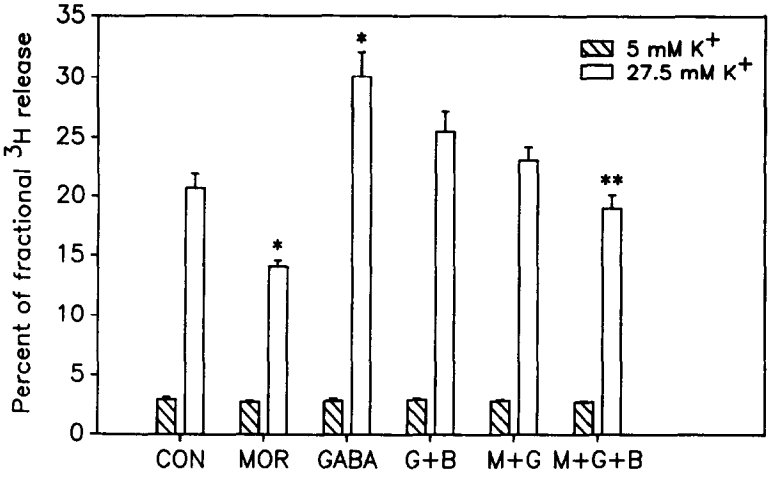

Fig. 6. Effect of bicuculline methiodide on the enhancement by GABA of potassium-stimulated [^{3}H]-norepinephrine release from rat frontal cortical slices in the absence and the presence of morphine. CON, control; MOR, morphine (10^{-6} M); GABA (10^{-4} M); G + B, GABA (10^{-4} M) + bicuculline methiodide (10^{-4} M); M + G, morphine (10^{-6} M) + GABA (10^{-4} M); M + G + B, morphine (10^{-6} M) + GABA (10^{-4} M) + bicuculline methiodide (10^{-4} M). Values are the means ± SEM of three rats. Key: * significantly different from control ($P < 0.05$); ** significantly different from the G + B treatment group.

noradrenergic neurons in select brain areas by opening potassium channels [28, 29]. Similarly, opioids appear to hyperpolarize the noradrenergic terminals in the cortex by increasing potassium conductance, as the inhibitory effect of opioids on [^{3}H]NE release in this tissue decreases with increasing concentrations of extracellular potassium [13]. GABA probably enhances potassium-stimulated [^{3}H]NE release by inducing a depolarization of the noradrenergic terminals. Although $GABA_A$ receptors are generally associated with hyperpolarization, $GABA_A$ receptor-mediated depolarization has been demonstrated both in peripheral neurons [30–32] and in central neurons [19, 33]. *In vivo*, the depolarization produced by GABA would be expected to inhibit the release of norepinephrine in the frontal cortex by reducing action potential height, but it would still be expected to reverse the hyperpolarizing effect of opioids, which act by preventing action potential invasion of the terminals [32, 34].

This study may provide an explanation for the observed *in vivo* interactions between opioids and centrally-administered GABAergic agents. GABAergic agents, by acting upon $GABA_A$ receptors and possibly GABA uptake, could reverse the analgesic effect of opioids by reversing opioid inhibition of norepinephrine release in the forebrain.

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