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Possible Noradrenergic Involvement in Naloxone Potentiation of Apomorphine-Induced Stereotypic Climbing in Mice

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QUOCK, R. M., A. S. BLOOM AND J. A. SADOWSKI. *Possible noradrenergic involvement in naloxone potentiation of apomorphine-induced stereotypic climbing in mice.* PHARMACOL BIOCHEM BEHAV 21(5) 733–736, 1984.— Apomorphine-induced stereotypic climbing behavior in mice was significantly potentiated by pretreatment with the opiate receptor blocker naloxone. In animals additionally pretreated with α-methyl-*p*-tyrosine, brain levels of norepinephrine (NE) and dopamine (DA) were markedly reduced and naloxone potentiation of apomorphine-induced stereotypic climbing was blocked. In mice pretreated with diethyldithiocarbamic acid, brain NE was slightly reduced, brain DA was slightly elevated but naloxone potentiation of apomorphine-induced stereotypic climbing was unaltered. In animals pretreated with reserpine, both brain NE and DA were significantly reduced by naloxone potentiation of apomorphine-induced stereotypic climbing was not affected. In other experiments, pretreatment with low doses of the α-adrenergic receptor blocker BE-2254 failed to suppress climbing activity induced by apomorphine alone but did successfully prevent naloxone potentiation of apomorphine-induced stereotypic climbing. These findings suggest the possibility that NE and α-adrenergic receptors might play a role in the potentiating influence of naloxone upon apomorphine-induced stereotypic climbing activity in mice.

Naloxone Apomorphine Stereotypic climbing activity Brain catecholamines

IN previous research, we have investigated the drug interaction between opiate receptor blockers and dopaminergic receptor agonists. We have reported that pretreatment with naloxone and other narcotic antagonists can potentiate apomorphine-induced hyperthermia in rabbits [17], apomorphine-induced rotational behavior in rats with unilateral nigral lesions [21], apomorphine-induced stereotypic climbing behavior in mice [19], l-dopa reversal of reserpine-induced catalepsy in mice [15], and l-dopa suppression of oxotremorine-induced tremors in mice [20].

In more recent studies, we have attempted to elucidate the mechanism by which naloxone can potentiate the effects of dopaminergic agonists. We have found that pretreatment with opiate drugs selective for μ- or σ-opiate but not κ-opiate receptors competitively reversed naloxone potentiation of apomorphine-induced stereotypic climbing in mice [18]. A more recent investigation has found that selective δ-opiate receptor blockade also potentiated this behavioral effect of apomorphine [6]. One hypothesis for the potentiating effect of naloxone suggests that naloxone might block presynaptic opiate receptors and reduce the inhibitory influence of these receptors upon dopaminergic neuronal activity.

In the present investigation, we pretreated different groups of mice with the tyrosine hydroxylase-inhibitor α-methyl-*p*-tyrosine, the dopamine-β-hydroxylase-inhibitor diethyldithiocarbamic acid, the depleting agent reserpine and the α-adrenergic receptor blocker BE-2254 and assessed their influence upon naloxone potentiation of apomorphine-induced stereotypic climbing, an activity thought to be associated with stimulation of striatal dopaminergic receptors [16]. We also confirmed alteration of brain catecholamine concentrations by both spectrofluorimetric as well as high performance liquid chromatographic techniques. We will report herein evidence of a possible involvement of noradrenergic mechanisms in the potentiating influence of naloxone upon apomorphine-induced stereotypic climbing in mice.

METHOD

Stereotypic Climbing Experiments in Mice

Male ICR mice (King Animal Laboratories, Oregon, WI), 20–25 g, were used in these experiments. The animals were acclimatized for at least 30 min to individual circular cages (12 cm in diameter and 14 cm in height, surrounded by 1-mm metal bars spaced 1 cm apart) separated by cardboard screens. Following intraperitoneal administration of a standard challenge dose of 2.0 mg/kg of apomorphine, the mice

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were returned to their cages and observed for a 20-min period. Stereotypic climbing behavior was quantified according to a previously reported method [16]: 0 points, the mouse sits with all four paws on the floor of the cage; 1 point, the mouse persistently stands up against the wall of the cage with its forepaws grasping the bars; and 2 points, the mouse persistently climbs on the walls of the cage with all four paws grasping the bars. Climbing scores were assigned to each mouse during 5-min observation periods ending 10 and 20 min after the apomorphine challenge and the two scores were then averaged to yield a mean climbing score for each animal.

Drugs

Drugs used in these experiments included: apomorphine hydrochloride (Merck); naloxone hydrochloride (DuPont); DL-α-methyl-*p*-tyrosine methyl ester hydrochloride (α-MT) (Sigma); sodium diethyldithiocarbamic acid (DDC) (Sigma); reserpine (CIBA); and 2-[β-(4-hydroxyphenyl)ethylaminomethyl] tetralone (BE-2254) (Beiersdorf). Apomorphine, naloxone, α-MT and BE-2254 were all prepared in aqueous solution. One drop of 0.1-N hydrochloric acid was added to each 10 ml of apomorphine solution to retard oxidation. DDC was prepared as an aqueous suspension and reserpine was supplied in Serpasil® multiple dose vials with appropriate dilutions made in double distilled water. All drugs were administered intraperitoneally in injection volumes of 0.1 ml per 10 g body weight. Pretreatment times were 5 min for naloxone, 30 min for BE-2254, 2 hr for α-MT and DDC and 24 hr for reserpine.

Quantitative Analysis of Brain Catecholamines

The efficacy of drug pretreatments in altering brain levels of the catecholamines norepinephrine (NE) and dopamine (DA) was assessed by both spectrofluorimetry and high performance liquid chromatography (HPLC). Control mice and mice pretreated with α-MT, DDC and reserpine were sacrificed by rapid decapitation following their respective pretreatment times. Their brains were removed and homogenized in 1:10 w/v 0.1-M perchloric acid, using a Brinkmann polytron. The homogenates were centrifuged at 28,000×G for 10 min and the supernatants were stored at −70°C until assayed. Whole brain NE and DA concentrations were determined in 4 ml of tissue supernatant after alumina extraction by the spectrofluorimetric method of Shellenberger and Gordon [25].

Additional analyses were performed by HPLC to verify that there was no fluorimetric interference in the catecholamine determinations by any of the pretreatment drugs. Whole brain levels of NE and DA from other groups of control and drug-pretreated animals were determined in 3 ml of tissue supernatant after alumina extraction by HPLC, using a Waters microprocessor-controlled HPLC system consisting of a Model 6000A pump, WISP automatic sample injector, data module and systems controller. Separation was conducted on a Bio-Rad ODS-5S column (150×4 mm) with a solvent flow rate of 0.7 ml/min (0.1-M KH_2PO_4 in 10% methanol at pH 3.0 with 0.19 mM sodium octyl sulfonate and 0.1 mM Na_2-EDTA). NE and DA were detected in the column effluent, using a Bioanalytical System LC-4 amperometric detector set at +0.72 volts with 0.5 amps/volt, giving a full scale response from an oil-based carbon paste electrode.

TABLE 1

INFLUENCE OF VARIOUS DRUG PRETREATMENTS UPON NALOXONE POTENTIATION OF APOMORPHINE-INDUCED STEREOTYPIC CLIMBING IN MICE

Treatment (mg/kg)	No Naloxone	Naloxone (1.0 mg/kg)	Naloxone (5.0 mg/kg)
APO (2.0)	1.26	1.50	1.83
α-MT (50) + APO (2.0)	1.25	1.17*	1.19*
DDC (400) + APO (2.0)	1.35	1.39	1.40
Reserpine (1.0) + APO (2.0)	1.39	1.60	1.68
BE-2254 (0.03) + APO (2.0)	1.22	1.08*	1.28*
Kruskal-Wallis one-way ANOVA	$p<0.05$	$p<0.01$	$p<0.001$

Figures represent the mean stereotypic climbing scores of at least 20 mice per group. *Significantly different from appropriate APO control group, $p<0.05$ (Mann-Whitney U test).

Statistical Analysis of Data

In assessing the influence of these drug pretreatments upon the naloxone/apomorphine drug interaction, the mean climbing scores of variously treated groups of animals were compared, using the Kruskal-Wallis one-way analysis of variance (ANOVA) and the Mann-Whitney U test for nonparametric data [26]. In determining the neurochemical effects of these drug pretreatments, the mean brain NE and DA concentrations of variously treated groups of animals were compared, using ANOVA and the multiple comparison test of Dunnett [7].

RESULTS

The administration of neither distilled water nor naloxone in doses as great as 5.0 mg/kg evoked stereotypic climbing in acclimatized animals. However, treatment with 2.0 mg/kg of apomorphine typically produced climbing activity equivalent to a mean climbing score of 1.26. Pretreatment with 1.0 and 5.0 mg/kg of naloxone just 5 min prior to the apomorphine challenge increased the mean climbing scores to 1.50 and 1.83, respectively ($p<0.001$, ANOVA) (Table 1). The mean score of the high dose naloxone pretreatment group was significantly greater than those of the control ($p<0.01$, Mann-Whitney U test) or the low dose naloxone pretreatment groups ($p<0.01$). Pretreatment doeses of α-MT, DDC, reserpine and BE-2254 that did not interfere with apomorphine-induced stereotypic climbing were identified in preliminary dose-response experiments. Of these additional drug pretreatments, only α-MT and BE-2254 were capable of blocking the potentiating influence of both doses of naloxone. Pretreatments with DDC and reserpine were both ineffective in altering the naloxone/apomorphine drug interaction.

The extent of brain catecholamine depletion produced by these drug pretreatments was initially determined by spectrofluorimetric assay and later by HPLC. Pretreatment with α-MT consistently reduced both brain NE and DA levels by

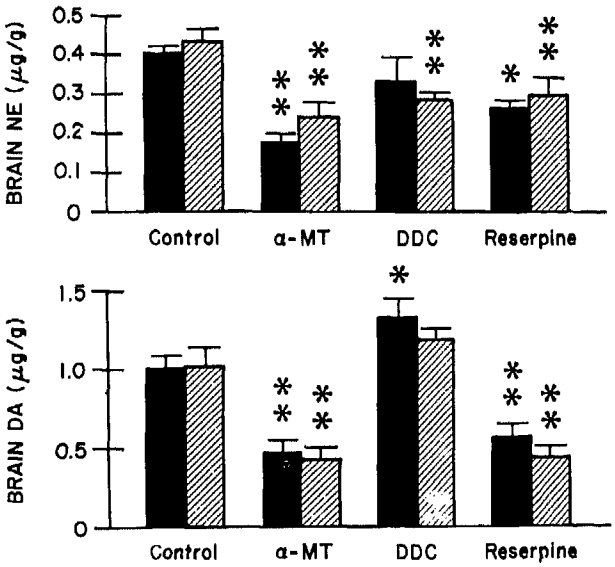

FIG. 1. Influence of various drug pretreatments upon brain NE and DA concentrations as determined by spectrofluorimetry (solid bars) and HPLC (cross-hatched bars). Bars represent the mean brain catecholamine concentration±s.e.m. (μg/g) of at least 10 mice per group. Refer to text for doses and pretreatment times of various groups. Significance of difference: $*p<0.05$ and $**p<0.01$, compared to control groups.

55–60% (Fig. 1). Pretreatment with DDC reduced brain NE while slightly elevating brain DA content. Endogenous NE concentrations were reduced 35% in experiments using HPLC, while there was significantly less DDC-induced depletion of brain NE according to spectrofluorimetric assays. Both methods also found central DA levels increased by 15–20% in DDC-treated mice. Pretreatment with reserpine consistently lowered brain NE content by 30–35%, however the effects of reserpine upon brain DA were somewhat more variable. Using the HPLC assay, we detected a 55% depletion, whereas using the spectrofluorimetric assay, we measured a 40% depletion of brain DA content.

DISCUSSION

We and others [1, 6, 8, 10, 11, 12] have reported that pretreatment of animals with narcotic antagonists can potentiate the effects of dopaminergic agonists in a variety of pharmacological paradigms. The mechanism of this drug interaction remains undetermined.

In the present investigation, we demonstrate that there is a tendency towards a dose-related increase in the mean climbing scores of groups of mice pretreated with two doses of naloxone, although only the mean score of the high dose naloxone pretreatment group was significantly different from the control group. We attribute this finding to a previously reported observation that greater doses of narcotic antagonists are required to block the effects of endogenous opioid peptides than are needed to reverse the effects of exogenous opiate drugs [13]. But it must also be acknowledged that such greater doses of narcotic antagonists may be capable of nonspecific drug effects as well [22].

We also discovered in this study that the potentiating effect of naloxone upon apomorphine-induced stereotypic climbing in mice was reversed in animals with reduced brain NE and DA levels as a consequence of pretreatment with α-MT. This finding implicates brain catecholamines in the mechanism of the naloxone/apomorphine drug interaction. It might be expected that reserpine, which also depletes both brain NE and DA albeit via a different mechanism than α-MT, would replicate this finding, however, reserpine proved ineffective in reversing naloxone potentiation of apomorphine-induced stereotypic climbing. We attribute this failure of reserpine to a possible involvement in the naloxone/apomorphine drug interaction of a pool of newly synthesized transmitter that is resistant to depletion by reserpine [14].

DDC selectively reduces brain NE content and it was thought that DDC would indicate whether NE or DA played the more important role in mediating the naloxone/apomorphine drug interaction. However, the results of the DDC experiments were ambiguous. Although the mean climbing scores of the DDC/naloxone/apomorphine groups were much less than those of the naloxone/apomorphine control groups, these differences were not considered significant when analyzed by nonparametric statistics. While DDC pretreatment may have significantly reduced the whole brain content of NE (as determined by HPLC assay) it is possible that there was insufficient depletion of NE in some vital, specific brain region to produce a greater interference with the naloxone effect. Accordingly, we continued to pursue the issue of a possible noradrenergic contribution to the drug interaction by pretreating animals with BE-2254, an antagonist with a high specificity for α-adrenergic receptors and poor antidopaminergic capability [4]. BE-2254 proved to be extremely potent and produced profound sedation and locomotor inactivity capable of suppressing apomorphine-induced stereotypic climbing. A dose of 0.03 mg/kg of BE-2254 was found not to affect the climbing activity induced by apomorphine alone, yet this same pretreatment dose also effectively prevented naloxone potentiation of apomorphine-induced stereotypic climbing behavior.

It has been reported that the α-adrenergic receptor blockers yohimbine and SKF 64139 can accelerate striatal DA turnover, thereby suggesting a possible role of NE and α-adrenergic receptors in the regulation of dopaminergic neuronal activity [2,3]. If this is indeed the case, then it is possible that naloxone might directly or indirectly interfere with this inhibitory noradrenergic mechanism and enhance dopaminergic drug effect. However, more recent investigations re-examining this issue have now proposed that the increased striatal DA turnover may be a response to a hitherto undescribed dopaminergic receptor blocking action of yohimbine rather than to its α-adrenergic receptor antagonist action [23,24]. On the other hand, new research suggests that DA release in the rat hypothalamus may be regulated by presynaptic α_2-adrenergic receptors as well as dopaminergic autoreceptors on dopaminergic nerve terminals [27]. Whether this also holds true for dopaminergic neuronal function in the striatum remains to be seen.

Apomorphine-induced stereotypic climbing behavior in mice is thought to result from the activation of striatal dopaminergic mechanisms [16]. Since the corpus striatum contains a low concentration of endogenous NE [9] and a low density of α-adrenergic receptors [28], it is possible that the noradrenergic mechanisms possibly involved in naloxone potentiation of apomorphine-induced stereotypic climbing behavior may reside outside the striatum. That noradrener-

gic mechanisms might mediate dopaminergic drug effects was demonstrated earlier by disruption of apomorphine-induced stereotypy in rats by electrolytic lesions of the predominantly noradrenergic nucleus amygdaloideus lateralis [5]. Hence it is possible that BE-2254 and possibly α-MT blockade of the naloxone effect observed in this study may originate from an extrastriatal site of action.

Our investigation also demonstrates that spectrofluorimetric and HPLC assay methods are generally consistent in determining neurochemical effects of various drug pretreatments. Although we did not employ these techniques to quantify catecholamine concentrations in brain tissues from the same animals, groups of mice treated with identical doses of drug at the same pretreatment times were used. With the exception of α-MT-induced effects on brain NE and reserpine-induced effects on brain DA, the differences determined by the two assays between control and drug-pretreated groups of mice were generally within 10%. Despite large variances in some groups of animals, the directions of drug-induced changes in brain catecholamine content detected by the two techniques were consistent. But it must be acknowledged that alterations in drug effect caused by such drug pretreatments are likely due to changes in catecholamine levels in specific brain regions and not whole brain content as determined in this investigation.

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