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Differential Effects of Nialamide and Clomipramine on Serotonin Efflux and Autoreceptors

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OFFORD, S J AND R O WARWICK, JR *Differential effects of nialamide and clomipramine on serotonin efflux and autoreceptors* PHARMACOL BIOCHEM BEHAV **26**(3) 593–600, 1987 —Serotonin (5-HT) activity *in vivo* and *in vitro* was evaluated in rats following acute and chronic administration of the antidepressants nialamide (NMD) and clomipramine (CMI) The 5-HT motor syndrome was used as an index of *in vivo* serotonergic function *In vitro*, ^{3}H-5-HT uptake, potassium-evoked ^{3}H-5-HT release and 5-HT autoreceptor activity were evaluated as measures of presynaptic function Repeated injections of NMD abolished the 5-methoxy-N, N-dimethyltryptamine (5-MeODMT)-induced motor syndrome and the ability of 5-methoxytryptamine (5-MEOT) to attenuate the potassium-evoked release of ^{3}H-5-HT Autoreceptor subsensitivity was associated with a marked increase in basal and potassium-evoked ^{3}H-5-HT release In contrast, acute NMD, and acute and chronic CMI did not affect the expression of the motor syndrome or alter ^{3}H-5-HT release or autoreceptor activity Acute and chronic injections of NMD enhanced ^{3}H-5-HT uptake The results suggest that the antidepressant efficacy of monoamine oxidase inhibitor (MAOI) antidepressants may be related to their ability to increase endogenous levels of 5-HT and thereby produce a subsensitivity of 5-HT$_1$ type receptors This subsensitivity is reflected both by attenuation of the motor syndrome and enhanced 5-HT neurotransmission resulting in part from autoreceptor down-regulation

5-HT autoreceptors 5-HT uptake and release Motor syndrome
Acute and chronic nialamide and clomipramine pretreatment

REPEATED treatment with various classes of antidepressant drugs has been shown to alter the number and sensitivity of serotonin (5-HT) receptors [1, 12, 28, 29, 31–33] Furthermore, antidepressant drugs may affect the two major classes of 5-HT receptors differently. For example, clomipramine (CMI), a tricyclic (TCA) type antidepressant, and atypical antidepressants decrease the number of 5-HT$_2$ type receptors [28]. These compounds also reduce the number of head shakes induced by 5-HTP [13], a behavioral syndrome thought to be mediated by 5-HT$_2$ receptors [19]. Conversely, various monoamine oxidase inhibitors (MAOI's), including nialamide, are reported to reduce the Bmax of both 5-HT$_2$ and 5-HT$_1$ receptors [28, 31–33] and abolish the 5-HT motor syndrome, a behavioral syndrome mediated via the 5-HT$_1$ type receptor [18].

When activated, presynaptic autoreceptors exert an inhibitory influence over neurotransmitter efflux. It is generally accepted that the 5-HT autoreceptor belongs to the 5-HT$_1$ receptor class This hypothesis is based upon the affinity of various serotonergic agonists and antagonists for 5-HT$_1$ and 5-HT$_2$ binding sites and the relative potency of these compounds to inhibit evoked efflux of ^{3}H-5-HT [10,22] Recent reports suggest this receptor is a 5-HT$_{1B}$ subtype [9,30] It has been further suggested that autoreceptors mediate an important homeostatic influence over the synaptic microenvironment and may be a potential therapeutic target in various disease states [17]. Affective disorders are thought to be a result of an abnormality in the neurotransmission of various biogenic amines including 5-HT [5]. An alteration in autoreceptor activity may be one mechanism by which some antidepressant drugs alleviate depression. The major purpose of this investigation was to compare the effects of acute and chronic administration of the 5-HT uptake inhibitor clomipramine and the MAOI nialamide on 5-HT release and presynaptic autoreceptor activity These compounds were chosen because of their above cited effects on 5-HT$_1$ and 5-HT$_2$ receptors, respectively. Because the pretreatments may affect the uptake of 5-HT, and hence tissue

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loading, the uptake process was also assessed. The 5-HT motor syndrome was evaluated as a behavioral correlate for the effects of the pretreatments on 5-HT_1 receptor activity.

METHOD

Animals and Drug Treatments

Male Sprague-Dawley rats (250–300 g, Hilltop Labs, Scottdale, PA) were housed two per cage, maintained on a 12 hour light cycle (0600–1800) and had free access to Purina rat chow and tap water

Control animals received injections of 0 9% saline; treated animals received clomipramine HCl (CMI) (10 mg/kg, IP) or nialamide base (NMD) (40 mg/kg; IP). The injection volume for all treatments was 0.2 ml per 100 g body weight. In acute studies, animals were sacrificed one hour after a single injection, and in the chronic studies, animals were sacrificed 24 hours after the last of 13 twice daily injections The acute sacrifice time period was chosen to allow adequate blood levels of drug to be achieved. In the chronic studies, twenty-four hours was allowed to elapse after the last injection, a protocol identical to that reported by Savage *et al* [31–33]

Serotonin Motor Syndrome

Serotonin receptor-mediated stereotyped behavior was studied employing previously characterized methods [15,18]. Animals were examined for signs of the 5-HT motor syndrome in clear Plexiglas cages (34 l × 30 w × 17 h, cm), the floor of which was covered with fresh bedding chips, for a 10 minute period after administration of 5-MeODMT (5 mg/kg, IP) or saline (2 ml/kg, IP).

The following signs of the 5-HT motor syndrome were recorded individually for each animal (1) repetitive dorsal-ventral treading of the forepaws; (2) abduction of the hind limbs; (3) side-to-side head weaving; (4) resting tremor, (5) rigidity; and (6) straub tail For a positive response to be recorded, four of the six signs had to appear in a single animal during the observation period. Data were expressed quantally as the number of animals exhibiting the syndrome per total number of animals in a pretreatment group.

Determination of ^{3}H-5-HT Uptake

Animals were decaptiated, their brains removed and the hypothalamus dissected over ice. Coronal slices of the hypothalamus (0.25 mm thick) were prepared with a McIlwain tissue chopper Four slices (tissue wet weight=9.6±1 2 mg, mean±S.D for 36 samples) were transferred into 25 ml beakers containing 5 ml oxygenated Krebs-Henseleit bicarbonate buffer (K-H). The composition of the buffer (mM) was; NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, glucose 11.1, ascorbic acid 1 14, $CaCl_2$ 1 2, $MgSO_4$ 1 2, KH_2PO_4 1 0 NMD was included at a concentration of 10 μM to prevent the deamination of ^{3}H-5-HT Tissue was preincubated for 10 minutes in a metabolic shaking incubator at 37°C, under an atmosphere of 95% O_2-5% CO_2 and at 100 oscillations per minute Following preincubation, ^{3}H-5-HT creatinine sulfate (20 0–29.8 Ci/mmol; New England Nuclear, Boston, MA) was added, so as to produce a final concentration range of 0 005–0.1 μM, and incubation was continued for an additional 35 minutes. This incubation time was chosen to ensure that the high affinity reuptake process was at equilibrium (data not shown). Following incubation, the tissues were rinsed on glass fiber filters with 5 ml of chilled saline The slices were then solubilized with NCS tissue solubilizer (Amersham/Searle, Arlington Hts., IL) and the total tritium content was determined by liquid scintillation spectrometry The tritium content of the incubation medium was also determined. Uptake data were calculated as tissue/medium (T/M) ratios (dpm per g tissue/dpm per ml medium) Quenching was determined by external standardization (counting efficiency, 35%)

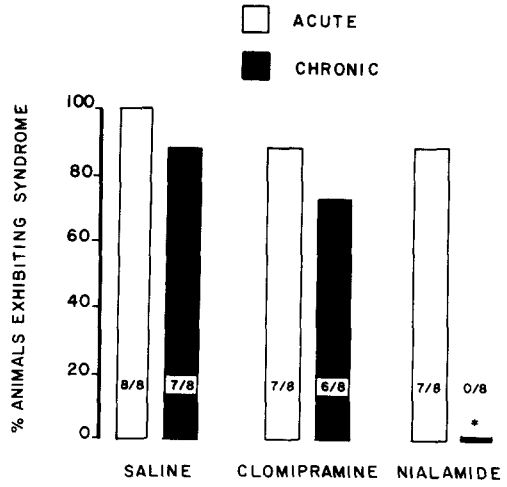

FIG 1 Effect of acute and chronic antidepressant treatment on the 5-methoxy-N, N-dimethyl-tryptamine-induced serotonin motor syndrome Rats were given single or repeated injections of saline (2 ml/kg, IP), clomipramine (CMI, 10 mg/kg, IP) or nialamide (NMD, 40 mg/kg, IP) and the direct-acting serotonin agonist 5-methoxy-N, N-dimethyltryptamine was administered (5 mg/kg, IP) 1 hr after acute treatment or 24 hr following repeated treatment The motor syndrome was evaluated as described in the Method section Each bar represents the percentage of animals in each group exhibiting the syndrome The number of animals responding over the number tested is indicated in each column Statistical significance ($p<0$ 05) from respective saline control is indicated by the asterisk (*)

Superfusion Studies

The measurement of ^{3}H-5-HT release was similar to that reported previously by Cox and Ennis [6]. In release experiments, the dissection, preparation and incubation of the tissue was similar to that described above, except 16 slices (tissue wet weight=36 3±4 9 mg, mean±S D. for 26 samples) were incubated in 5 ml of buffer containing 0.1 μM ^{3}H-5-HT

After the 30 minute tissue labeling process, groups of four slices were rinsed with one ml of K-H buffer and positioned into Plexiglas superfusion chambers (internal volume=0 25 ml) Tissues were superfused at 37°C with buffer which was continuously bubbled with 95% O_2-5% CO_2 Following an initial 42 minute period of superfusion (0 5 ml/minute), 15 successive four minute fractions were collected directly into scintillation vials A stable baseline of tritium efflux was established following the initial 42 minute superfusion period. Depolarization-induced release was evoked by superfusing the slices with two six-minute periods of K-H buffer containing 25 mM potassium at 44 minutes (S1) and 74 minutes (S2) after the initiation of superfusion The elevated potassium concentration was made by the isomolar replacement of

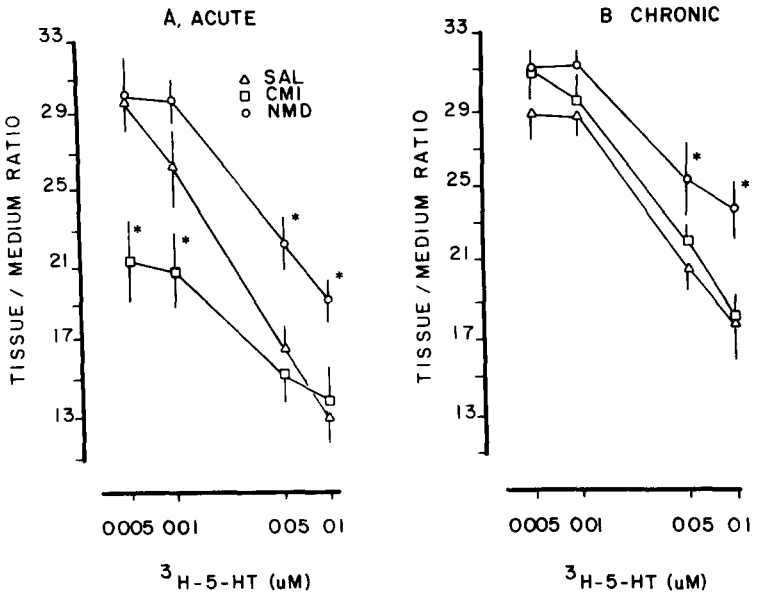

FIG 2 Effect of acute (A) and chronic (B) *in vivo* clomipramine (CMI) or nialamide (NMD) administration on ^{3}H-5-HT uptake in the rat hypothalamus Rats were sacrificed 1 hour after acute treatment or 24 hr following repeated treatment Hypothalamus slices were incubated in buffer with ^{3}H-5-HT concentrations varying from 5 0 nM to 0.1 μM Tritium accumulation is expressed as the mean dpm/g tissue (T) per dpm/ml medium (M) ±S E M for seven animals The asterisks (*) indicate values statistically significant ($p<0$ 05) from saline (SAL) pretreatment at the respective ^{3}H-5-HT concentration

sodium chloride. The autoreceptor agonist 5-MEOT (0.32–3.2 μM) was superfused immediately after the S1 stimulation period (t=50 min) and continuously for the remainder of the experiment NMD (10 μM) and CMI (5.0 μM) were routinely added to the superfusion buffer to prevent the oxidative deamination and reuptake of released ^{3}H-5-HT, respectively.

Following each release experiment, the tissues were solubilized with NCS and the tritium content of the superfusate and solubilized tissue was determined by scintillation spectrometry as previously described. Radioactivity in each superfusate sample was expressed as the percent of tritium remaining in the tissue prior to collection of that fraction The percentage of tritium released above basal efflux in response to the two potassium periods was expressed as an S2/S1 ratio, using a previously described method [6]

5-HT Metabolism Experiments

Modifications of previously described extraction methods [7,18] were used to determine the percent of tritium represented by ^{3}H-5-HT and ^{3}H-5-hydroxyindoleacetic acid (5-HIAA) in brain tissue and release effluent To extract tritiated indoles from tissue, slices of hypothalamus were prepared and incubated in 0.1 μM ^{3}H-5-HT as described above. After tritium loading, the tissue samples were placed into 10 ml conical glass centrifuge tubes and ice-cold saline (5 ml) was added The tubes were vortexed and centrifuged at 1,000 rpm for two minutes The saline solution was discarded and 3 ml of ice-cold acidified butanol (0.86 ml of 11 N HCl/l butanol) was added to each tube. The tissue was homogenized and then centrifuged for five minutes at 2,000 rpm The butanol supernate was combined with 5 ml of n-heptane followed by the addition of 0 1 N HCl (400 μl). The mixture was shaken for 5 minutes and then centrifuged for 5 minutes at 2,000 rpm. The organic layer was saved and 400 μl of 0.33 N $NaHCO_3$ was added to extract the 5-HIAA. This mixture was shaken and centrifuged as described above A 100 μl aliquot of the organic, acidic and basic aqueous phases was sampled and the tritium content determined.

^{3}H-5-HT and ^{3}H-5-HIAA were separated and extracted from the release effluent by adding a 900 μl sample of the release effluent to a conical glass centrifuge tube containing 100 μl of 1.0 N HCl. Butanol and heptane were added as before and the mixture was vortexed and centrifuged as previously described For extraction into the basic medium, a 1 ml aliquot of 0.033 N $NaHCO_3$ was added to the organic layer. Tritium contained in the organic phase during extraction of ^{3}H-indoles from tissue or release effluent represented less than 3% of the total extracted radioactivity.

Following the extraction of the radiolabeled indoles from the tissue or release effluent, samples (four μl) of the acidic and basic aqueous fractions were spotted on thin layer chromatography plates (250 μ, Silica Gel G, Analtech) to confirm that ^{3}H-5-HT and ^{3}H-5-HIAA were the major constituents in each respective fraction. The organic layer was also chromatographed on TLC plates. Samples of unlabeled 5-HT creatinine sulfate and 5-HIAA (10 μg each) were chromatographed in parallel with the extracted samples The plates were scored at one cm intervals, scraped into scintillation vials and counted for tritium. The Rf values for the radioactive peaks were determined. For plates containing cold compounds, the migrated spot was visualized using ninhydrin reagent spray Rf values for 5-HT (0.52) and

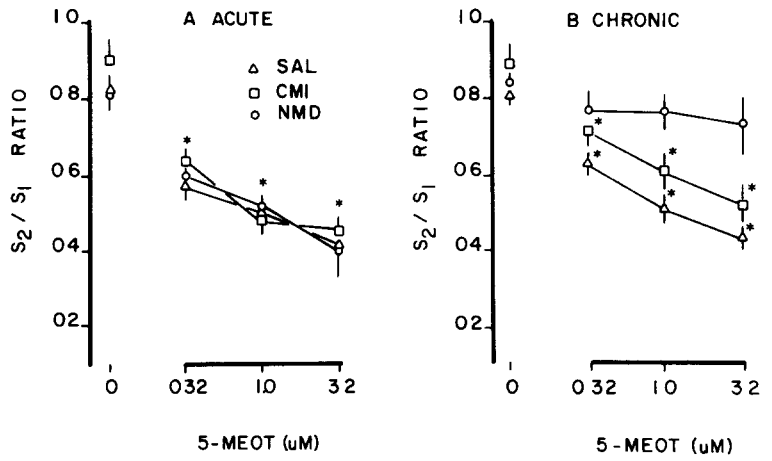

FIG 3 Effect of superfused 5-methoxytryptamine (5-MEOT) on the tritium stimulation ratios (S2/S1) from slices of rat hypothalamus following acute (A) and chronic (B) clomipramine (CMI) and nialamide (NMD) pretreatments Hypothalamus slices were incubated in buffer containing 0 1 μM ^{3}H-5-HT, positioned into superfusion chambers and superfused with K-H buffer as described in the Method section Two 6 minute periods of 25 mM K+ were superfused at 44–50 (S1) and 74–80 (S2) minutes after the initiation of superfusion The S2/S1 ratios were calculated as described in the Method section 5-MEOT, at the indicated concentrations, was superfused from minute 50 of superfusion until the end of the experiment Each data point represents the mean±S E M of 5–10 animals The asterisks (*) signify statistical significance ($p<0$ 05) from the respective control values (i e , no 5-MEOT present)

TABLE 1

RECOVERY OF ^{3}H-5-HT AND ^{3}H-5-HIAA FROM SLICES OF THE RAT HYPOTHALAMUS FROM UPTAKE EXPERIMENTS FOLLOWING ACUTE AND CHRONIC *IN VIVO* CLOMIPRAMINE AND NIALAMIDE PRETREATMENTS†

Treatment	% of Total Tritium Recovered as‡ ^{3}H-5-HT	^{3}H-5-HIAA
A Acute Treatment		
Saline	73 8 ± 0 5	19 9 ± 0 3
Clomipramine	77 6 ± 1 3	16 4 ± 0 4*
Nialamide	81 6 ± 1 9*	13 9 ± 1 6*
B Chronic Treatment		
Saline	72 9 ± 0 4	21 7 ± 0 4
Clompiramine	74 7 ± 0 3	19 7 ± 0 5*
Nialamide	91 4 ± 0 3*	4 4 ± 0 2*

*$p<0$ 05 (compared to respective saline control values)

†Rats were treated with saline or antidepressants, sacrificed and slices of hypothalamus were incubated in buffer containing 0 1 μm ^{3}H-5-HT as described in the Method section Following incubation, ^{3}H-indoleamines were extracted and expressed as a % of total tritium recovered from the tissue

‡Each value represents the mean ± S E M of 4 animals

5-HIAA (0 86) were identical for both unlabeled and labeled samples

Statistical Analysis

A one way analysis of variance (ANOVA) was calculated on the T/M ratios of the uptake experiments and on the S2/S1 ratios of release experiments When the ANOVA F value was significant at $p<0$ 05, Dunnett's multiple range test [8] was used to determine significant differences between control and individual treatments

The Chi-Square test was used for determining significance in the 5-HT motor syndrome

Drugs and Chemicals

All chemicals were of reagent grade quality and obtained from Sigma Chemical Co (St Louis, MO) as was the nialamide, 5-hydroxy-tryptamine creatinine sulfate, 5-hydroxyindoleacetic acid, 5-methoxy-N, N-dimethyltryptamine, and 5-methoxytryptamine Clomipramine HCl was generously donated by Ciba-Geigy (Summit, NJ)

RESULTS

Serotonin Motor Syndrome

As shown in Fig 1, the 5-MeODMT-induced 5-HT motor syndrome was produced in all animals pretreated with a single injection of saline, and in 88% of those animals pretreated with a single injection of CMI or NMD. Similarly, following chronic administration of saline or CMI, 88 and 75% of the pretreated animals exhibited the syndrome, respectively. The slight decreases between the respective acute and chronic saline or CMI pretreatments were not sig-

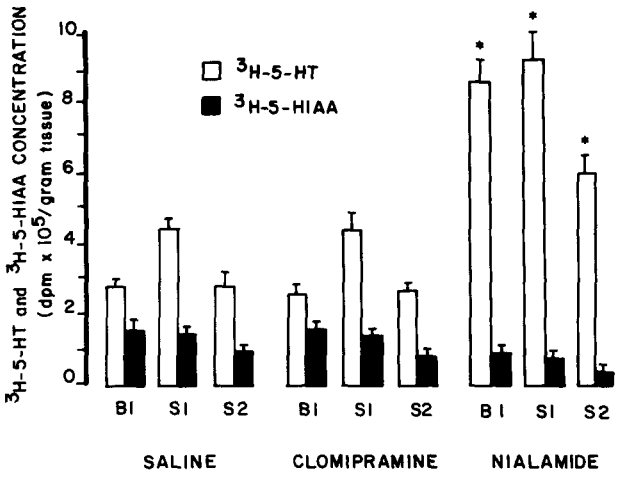

FIG 4 Effect of chronic clomipramine (CMI) and nialamide (NMD) pretreatments on basal and potassium-evoked ^{3}H-5-HT and ^{3}H-5HIAA release 5-MEOT (3 2 μM) was superfused from minute 50 of superfusion until the end of the experiment Four minute effluent fractions were collected as in previous experiments Aliquots of the release samples collected during baseline (B1) and the two potassium stimulation pulses (S1 and S2) were sampled and the ^{3}H-5-HT and ^{3}H-5-HIAA content was determined as described in the Method section The data were expressed as the dpm of ^{3}H-5-HT (open bars) and ^{3}H-5-HIAA (solid bars) released per gram of tissue Each bar represents the mean±S E M for 6 animals Asterisks signify statistical significance ($p<0$ 05) in ^{3}H-5-HT levels between saline and nialamide treated rats

nificant In contrast, 5-MeODMT failed to evoke the motor syndrome in all animals treated repeatedly with NMD, an effect significantly different from chronic saline

Tritium Uptake Following Acute and Chronic In Vivo *Antidepressant Treatment*

There was an inverse relationship between T/M ratios and media ^{3}H-5-HT concentrations among all treatment groups (Fig 2 A,B). Acute and chronic NMD pretreatment significantly enhanced the accumulation of tritium at respective media ^{3}H-5-HT concentrations of 0.05 and 0.1 μM, as compared to respective acute and chronic saline pretreatments (Fig. 2 A,B). Tritium uptake was increased 30% and 39% following acute NMD treatment, and 21% and 35% following repeated NMD at the respective ^{3}H-5-HT concentrations

Hypothalamic slices from acute CMI treated rats accumulated a significant 26 and 20% less neurotransmitter compared to saline controls at media ^{3}H-5-HT concentrations of 5 0 nM and 0.01 μM ^{3}H-5-HT, respectively (Fig 2A). Chronic administration of CMI did not alter T/M ratios compared to repeated saline at any ^{3}H-5-HT concentration evaluated (Fig 2B).

Tissue ^{3}H-5-HT and ^{3}H-5-HIAA Following Antidepressant Treatment

In all tissue samples, the major percentage of extracted tritium was contained in the acidic aqueous phase representing ^{3}H-5-HT (Table 1 A,B) Acute and repeated CMI pretreatment produced significant decreases of 18 and 19%, respectively, in the level of extracted ^{3}H-5-HIAA (Table 1 A,B)

Acute NMD administration produced a significant increase in ^{3}H-5-HT (11%) and a significant decrease in ^{3}H-5-HIAA (30%), as compared to acute saline. Following repeated NMD treatment, 91.4% of accumulated tritium was associated with the acidic 5-HT phase, a significant 20% increase over saline Consistent with this observation, the level of ^{3}H-5-HIAA from chronic NMD treated animals was a significant 80% less than that extracted out of slices from respective saline control animals (Table 1B).

Autoreceptor Activity Following Acute and Chronic Antidepressant Treatment

None of the acute antidepressant pretreatments affected autoreceptor activity (Fig 3A) Superfusion of 5-MEOT produced a consistent pattern of significant concentration-dependent decreases in S2/S1 ratios of 30, 40 and 50% at 5-MEOT concentrations of 0 32, 1 and 3.2 μM, respectively, for saline, CMI and NMD treated animals. A similar concentration-dependent decrease in S2/S1 ratios induced by 5-MEOT was observed after chronic pretreatment with saline or CMI (Fig 3B). The autoreceptor agonist significantly decreased tritium release by 22, 37 and 47% as 5-MEOT concentrations increased from 0 32 to 3.2 μM. However, following chronic NMD treatment there was complete abolition of the autoreceptor-induced modulation of tritium release (Fig 3B).

^{3}H-5-HT and ^{3}H-5-HIAA Determination in Release Effluent Following 5-MEOT Superfusion

To determine whether the above described alterations in tritium efflux induced by the chronic pretreatments represented a change in actual 5-HT efflux, the concentration (dpm/g tissue) of ^{3}H-5-HT and ^{3}H-5-HIAA released during baseline (B1) and the first (S1) and second (S2) stimulation periods was measured (Fig 4).

Following repeated NMD treatment, the basal efflux of ^{3}H-5-HT and ^{3}H-5-HIAA represented 90.1 and 8 7%, respectively, of the total amount of radioactivity released. This was a significant (70%) increase in ^{3}H-5-HT release over saline or CMI treated rats.

Compared to basal efflux, potassium stimulation (S1, i.e., no 5-MEOT present) produced a significant increase in ^{3}H-5-HT efflux from saline or CMI treated rats. In contrast, there was only a slight (n.s.) increase (7%) in ^{3}H-5-HT release during S1 from NMD treated rats However, despite this modest increase, total ^{3}H-5-HT released from NMD animals during S1 was 53% greater than that released from saline or CMI treated rats.

In the presence of 3 2 μM 5-MEOT, potassium depolarization (S2) produced a significant enhancement of ^{3}H-5-HT efflux from NMD treated rats as compared to saline or CMI treated animals.

Following the analysis of release effluent for ^{3}H-5-HT, the data illustrated in Fig. 4 were used to calculate a stimulation ratio. This ratio was termed S2(5-HT)/S1(5-HT) and was calculated similarly to the S2/S1 ratio described in the Method section, the only difference being that the level of ^{3}H-5-HT (not total tritium) was used in the calculation (Table 2) The S2(5-HT)/S1(5-HT) ratio in the presence of 5-MEOT for NMD treated rats was significantly greater than those for saline or CMI. The latter two values were not significantly different from each other. Although direct statistical comparison was not possible, ratios calculated by this method for chronic NMD were similar in comparison to S2/S1 tritium

TABLE 2

EXTRACTED ^{3}H-5-HT STIMULATION RATIOS FROM ANIMALS CHRONICALLY TREATED WITH CLOMIPRAMINE OR NIALAMIDE

Treatment	S2(5-HT)/S1(5-HT)†
Saline	0 65 ± 0 04
CMI	0 62 ± 0 02
Nialamide	0 75 ± 0 02*

*$p<0$ 05 (compared to saline control values)

†S2(5-HT)/S1(5-HT) ratios were calculated from the extracted indoleamine data illustrated in Fig 4 and as described in the Method section Each value represents the mean ± S E M of 6 animals

ratios obtained routinely in control (no 5-MEOT) superfusion experiments (refer to Fig. 3). These data further suggest that chronic NMD, but not CMI, downregulates the 5-HT autoreceptor.

DISCUSSION

The most important findings in this investigation were that repeated treatment of rats with NMD significantly increased both basal and potassium-evoked ^{3}H-5-HT efflux and abolished the 5-MEOT-induced inhibition of evoked tritium release from slices of rat hypothalamus. The latter finding suggests that 5-HT autoreceptors are functionally down-regulated by NMD. The NMD-induced decrease in autoreceptor activity was further suggested by the calculation of an S2(5-HT)/S1(5-HT) ratio based on the efflux of extracted ^{3}H-5-HT. In the extraction experiment (Table 2), 5-MEOT (3.2 μM) was superfused immediately after S1 and the S2(5-HT)/S1(5-HT) stimulation ratio from NMD treated rats was significantly increased compared to the respective saline and CMI ratios This result suggested that 5-MEOT did not inhibit the evoked release of ^{3}H-5-HT from NMD treated rats and lends further support for the tritium overflow data illustrated in Fig. 3 In Fig. 3, the tritium S2/S1 ratios, for the NMD treated group, indicated that a complete disinhibition of the autoreceptor had occurred throughout the range of 5-MEOT concentrations as compared to the NMD control (no 5-MEOT present). Although we did not attempt to calculate S2(5-HT)/S1(5-HT) ratios in the absence of 5-MEOT, there is close similarity between the NMD extraction ratios in the presence of 5-MEOT and the S2/S1 tritium ratios in the absence of 5-MEOT. The tritium and extraction S2/S1 ratios provide strong evidence for NMD-induced autoreceptor down-regulation and loss of inhibitory control over 5-HT efflux. Acute CMI, NMD or chronic CMI pretreatment did not alter autoreceptor activity or modify basal or stimulated ^{3}H-5-HT release (Fig 3 and Table 2).

Converging lines of evidence support our hypothesis that the ability of NMD to desensitize 5-HT autoreceptors is related to a reduction in the number of 5-HT$_1$ type receptors Repeated, but not acute, NMD administration blocked the precipitation of the 5-HT motor syndrome and elimination of this syndrome has been shown to parallel a reduction in brainstem 5-HT$_1$ type receptors [18], the receptor type thought to mediate the 5-HT motor syndrome [19] The autoreceptor is generally thought to be a member of the 5-HT$_1$ receptor class [10,22]. In light of the results from the present study, it is likely that repeated NMD pretreatment reduces the number of 5-HT$_1$ receptors in the hypothalamus, as functionally reflected by decreased autoreceptor responsiveness. The desensitization of the autoreceptor is probably related to exposure of the receptor to enhanced levels of 5-HT. Savage and coworkers [33] report that the same NMD dosing protocol as used in this study produces a 165% increase in whole brain 5-HT concentration Consistent with that observation, we have reported a 70% increase in basal efflux and a 53% increase in stimulated ^{3}H-5-HT efflux following NMD pretreatment. Collectively, these two findings indicate that NMD dramatically increases the synaptic pool of 5-HT The profound increase in basal ^{3}H-5-HT efflux would result in a persistent high level of synaptic 5-HT despite the modest (7%) increase in stimulation evoked efflux The increase in endogenous whole brain 5-HT, as reported by Savage *et al* [33], and the high level of ^{3}H-5-HT efflux reported in this study, would presumably result in repeated occupancy of the autoreceptor by the endogenous ligand Persistent stimulation of central serotonin receptors would then lead to down-regulation of post- and presynaptic 5-HT$_1$ type receptors as demonstrated by the blockade of the motor syndrome and loss of autoreceptor activity, respectively.

The 5-HT$_1$ receptor has been subdivided into three main homogeneous populations termed 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ [27] The 5-HT autoreceptor, as defined by release studies, appears to be a member of the 5-HT$_{1B}$ class [9,30], although an earlier report suggests it may be a 5-HT$_{1A}$ receptor [14]. A second type of 5-HT autoreceptor, which should be differentiated from the presynaptic autoreceptor, is located on dendrites and cell bodies in the raphe and has been studied electrophysiologically by measuring firing rates [3] However, its subtype classification is unclear A behavioral measure, forepaw treading, and one sign of the 5-HT motor syndrome, has been linked to activation of the 5-HT$_{1A}$ receptor [35,37]. In light of these reports, it may be asked whether NMD has a differential effect on 5-HT receptor subtypes We observed that chronic NMD pretreatment completely abolished the motor syndrome, including forepaw treading (quantitation of individual signs not shown), and caused an apparent down-regulation of the 5-HT presynaptic autoreceptor. Assuming the presynaptic autoreceptor is of the 5-HT$_{1B}$ class, these results suggest that NMD reduces indiscriminantly the functions of both 1A and 1B receptors.

The observations reported here are consistent with those of other groups that report MAOIs, but not TCAs, selectively down-regulate central presynaptic autoreceptors. For example, autoreceptor sensitivity in perfused hypothalamic synaptosomes [23] is reduced in rats given 15 daily injections of clorgyline plus CGP 6085A, a selective inhibitor of 5-HT uptake [38]. Pretreatment with clorgyline alone decreases the sensitivity of central alpha$_2$ autoreceptors [4]. Also, clorgyline, but not deprenyl, desensitizes somatodendritic 5-HT autoreceptors to microiontophoretically applied 5-HT [3] In contrast, chronic pretreatment with TCAs is without effect on 5-HT$_1$ receptor binding, induction of the 5-HT motor syndrome [18] or, as reported here, on hypothalamic 5-HT autoreceptors Blier and de Montigny [2] previously report that a 14 day pretreatment period with imipramine has no effect on 5-HT autoreceptors in the rat dorsal raphe nucleus Although these investigators have previously reported MAOI-induced down-regulation of central autoreceptors [3], our report directly compares a representative TCA and MAOI

The primary process by which the brain slices were loaded with ^{3}H-5-HT was by the high affinity 5-HT reuptake mechanism, the usual method for loading tissues to study neurotransmitter release *in vitro* [6, 10, 25]. Therefore, it was

important to determine whether the loading process was altered after drug pretreatment. Tritium loading was consistent with an active uptake process following acute and chronic *in vivo* antidepressant pretreatment (as illustrated by T/M ratios greater than 1; Fig. 2). Further, accumulated tritium was represented primarily by ^{3}H-5-HT. The percentage of tissue tritium recovered as ^{3}H-5-HT ranged from 73–78% in acute and chronic saline and CMI treated rats. These values increased to 81–91% for the respective NMD treatments.

Acute CMI administration significantly decreased T/M ratios when tissue was incubated at the lower medium ^{3}H-5-HT concentrations of 5.0 nM and 0 01 μM (Fig. 2). This effect is most likely related to a partial inhibition of high affinity [34] ^{3}H-5-HT uptake by CMI remaining in the hypothalamic slices. Such a finding is consistent with a previous report on the *ex vivo* effects of other TCAs [21] and with the pharmacokinetics of CMI [11]. Since hypothalamus slices used in the present release studies were incubated with 0.1 μM ^{3}H-5-HT, a concentration at which T/M ratios were unaffected by CMI *ex vivo*, uptake inhibition at the lower ^{3}H-5-HT concentrations was not considered a major factor in the interpretation of the release data. Repeated CMI treatment did not alter T/M ratios as compared to respective uptake control values. The elimination rate of CMI from brain regions following chronic administration is increased 12 fold over acute pretreatment, and the plasma half-life of CMI is reduced from 6 to 1.2 hr [11]. Therefore, it can be reasonably hypothesized that negligible concentrations of CMI were left in the brain tissue 24 hr following CMI withdrawal in our study. Simultaneously with the decreases in tritium accumulation, acute and chronic CMI produced significant decreases in tissue levels of ^{3}H-5-HIAA and a trend toward enhanced ^{3}H-5-HT (Table 1) TCAs are weak inhibitors of MAO, with Ki values for type A MAO in the range of 100 μM [24]. Such inhibitory activity, albeit small, may have contributed to the observed slight decreases in tissue ^{3}H-5-HIAA content (Table 1).

Acute and chronic NMD pretreatment significantly enhanced the percentage (11 and 20%, respectively) of tissue tritium extracted as ^{3}H-5-HT, and markedly decreased the percentage (30 and 80%, respectively) of tritium represented as ^{3}H-5-HIAA (Table 1). These findings would be expected following inhibition of MAO by NMD In addition, both NMD pretreatments significantly increased the level of tritium extracted from hypothalamus slices, an unexpected finding. It is hypothesized that the enhanced T/M ratios may be related to inhibition of MAO. The decreased oxidative deamination of ^{3}H-5-HT would allow the amine to remain in the nerve ending and presumably be contained within both cytoplasmic and vesicular storage sites. A larger pool of intraneuronal ^{3}H-5-HT would account for the significantly higher T/M ratios. An augmented cytoplasmic pool of ^{3}H-5-HT was supported by the observation of a marked increase in basal ^{3}H-5-HT efflux (Fig. 4), which we have previously reported to be calcium-independent [26].

Finally, it may be speculated that the NMD-induced increase in neurotransmission reported in this study may provide insight into the clinical sequelae of affective disorders. Autoreceptors may exert a physiologic role by providing local negative feedback control on neurotransmitter release [16,36]. It can be proposed, albeit indirectly, that in patients suffering from unipolar depression an overly stringent feedback system impairs the relay of information across the 5-HT synapse. This impairment may be an integral factor associated with depression. The NMD-induced down-regulation of autoreceptors suggests that monoamine oxidase inhibitors may uncouple the "braking" mechanism on 5-HT release and provide an important role in the pharmacologic process of mood elevation in depressed individuals.

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