Effects of Maprotiline, Nomifensine and Trazodone on Gamma Amino Butyric Acid Turnover in the Limbic System

M.S. Abdel Halim, A.H. Abdel Fattah, S. Abdel Hafize, S. Mahmoud, A. Okasha, and Khadiga Ragheb

The effects of chronic treatment with 3 antidepressant drugs: Maprotiline, Nomifensine and Trazodone on the turnover rate of gamma amino butyric acid were studied in 4 rat brain areas related to the limbic system. Trazodone markedly increased the Gamma amino butyric acid level in the hypothalamus and hippocampus but lowered its level in the amygdala and olfactory tubercle. Nomifensine increased its level in the hypothalamus and hippocampus and markedly decreased it in the olfactory tubercle. Maprotiline on the other hand consistently lowered its level in all 4 areas. These results indicate the involvement of gamma amino butyric acid in the mechanism of action of antidepressant drugs. Its possible role in modulating other neurotransmitters and production of antidepressant manifestations are discussed.

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Introduction

The mechanism by which antidepressant drugs exert their effect on brain neurotransmitters is not fully elucidated. The absence of correlation between their effects on brain monoamine turnover and the onset of their antidepressant action (Surgue1980,1983) raised the concept of complex pattern mechanism in which more than one transmitter may interact; with the possibility that one of them modulates the effect of the other. The role of gamma aminobutyric acid (GABA) in the mediation of antidepressant drugs action is not thoroughly investigated. Earlier studies indicated its direct or indirect involvement; thus chronic treatment of rats with imipramine was shown to increase the release of GABA in the hippocampal area (Sherman and Petty 1980). In the "learned helplessness" model in rats, antidepressant drugs produce a mitigating effect which appear to depend on a GABA-ergic mechanism, since only intrahippocampal injection of GABA produce effects similar to those produced by the drugs (Jalfre and Prosolt 1985).

The present work was undertaken to evaluate the possible role with which GABA may participate in the mechanism of antidepressant drugs. We choose

M.S. Abdel Halim, Department of Pharmacology, Tanta University.

A.H. Abdel Fattah, Department of Pharmacology, Ain Shams University.

S. Abdel Hafize, Department of Pharmacology, Ain Shams University.

S. Mahmoud, Department of Pharmacology, Ain Shams University.

A.Okasha,Professor and Chairman, Department of Neuropsychiatry, Ain Shams University.

Khadiga Ragheb, Department of Psychiatry, Al-Azhar University.

3 antidepressant drugs famous for their selectivity on neurotransmitters. These drugs are: Maprotiline (MAP), Trazodone (TRAZ) and Nomifensine (NOM) which selectivity inhibit the re-uptake of Norepinephrine, Serotonin, and Dopamine respectively.

Material and Method

A) Test Drugs Maprotiline hydrochloride (Ludiomil) was obtained from Ciba-Giegy, Egypt, Nomifensine Maleate (Merital) from Hoechst, and Trazodone hydrochloride (Trittico) from Epico, Egypt.

B) Chemicals Gamma Amino Butyric Acid (GABA) supplied in powder form obtained from Sigma. Ethyl Alcohol Chromatography grade from (Merck). Ethyl Alcohol 70% from (EL Nasr Chemicals).

C) Animals Male Sprague Dawley Albino rats weighing 100-150 gms were kindly offered by the animal facility department, Namru-3, Abbasia, Cairo. Diet was offered so as to contain protein and vitamin requirements, room temperature and number of animals per cage were adequately considered.

Drug Administration:

Thirty two rats were divided into 4 equal groups. One group served as a control. The animals in the other three groups each one received either: Maprotiline hydrochloride, Nomifensine Maleate or Trazodone hydrochloride in a dose of 20 mg/kg/day (i.p.) injection at 08:00 am for 14 days. The dose was chosen according to those used by Miyauchi et al (1981) and Nielsen (1986).

Sample Preparation

Animals were sacrificed by decapitations 24 hours after the last injections. Brains were rapidly dissected out, washed thoroughly and the hypothalamus, hippocampus, amygdala and olfactory tubercle were rapidly dissected according to Konig and Klippel (1983). Each brain region was blotted and weighed, then 4 ml of ethyl alcohol 70% was added. The mixture was homogenized then centrifuged for 40 minutes at 3000 xg. The supernatant was aspirated and filtered in a millipore filter (size 0.45 um mesh).

The filtrate was dried under nitrogen stream. The dried residue was dissolved in 10 ul ethanol chromatography grade and vortexed, to be ready for injection in the gas chromatography system.

Gas Liquid Chromatography:

The system used was Perkin Elmer Model 8310 gas chromatograph with a built-in data handling facility and Perkin Elmer nitrogen phosphorus detector with nitrogen and phosphorus modes. The system was supplied by an Epson GP 100 chart recorder.

Column used was Carbowax 240 M+ 2% KOH on chromosorb WAW 80-100 mesh with dimensions of 1/8 inch x 6 feet, metal with glass lining (GLT). Gases used were hydrogen and air. The carrier gas was nitrogen at a flow rate of 50 ml/min. oven temperature was set at 190 C and injector temperature at 250 C. The NPD was set on the N mode at a temperature 300 C. Sensitivity was set high using bead 5. The procedures for separation were modified after Saeki et al (1982) using the NPD instead of the flame ionization detector (FID). The former being 15 times as sensitive as the latter for nitrogen containing compounds (Burtis et al., 1987). A standard curve for GABA was done. The recovery of the extraction was determined by adding 10 ug

of standard GABA to brain tissue and the procedures for extraction were done. The amount of GABA recovered after extraction was calculated after injecting the samples in the GC system and comparing the peak surface areas of the extracted samples to the standard recovery. It was found to be 45%.

The authentic samples, three injections per sample, were injected, 1 ul per injection and peak surface areas were compared to those of the standard curve and were corrected for recovery.

Statistical Analysis

Data were analyzed by the computer package "stat graphics" statistical graphics system version 2 copyright 1987 STSL, Inc. and Statistical Graphics Corp.

The following tests were used according to Kleinbaum and Kupper (1987).

1. Student's t-test (unpaired) was used to compare between mean levels of GABA in drug treated animals and control.

2. One way analysis of variance (ANOVA) was used for comparison between means of more than 2 sets of data e.g. to compare between the mean levels of GABA in the control group and those after the effects of the three tested drugs in every area of the four limbic areas.

3. The "Scheffe's method" for multiple range analysis was used to determine which of the components of the ANOVA test was different from the others and responsible for the statistical significance.

Results

A. Effects of Antidepressants on GABA Levels:

The effects of the three drugs on GABA levels in the four limbic areas are illustrated in Table (1). Trazodone induced a very highly significant ($P<0.001$) increase in GABA levels in the hypothalamus and hippocampus (+ 205.44% and+ 97.83%) respectively, and decrease in the amygdala and olfactory tubercle (- 19.94% and - 55.86%) respectively. Nomifensine also exerted a very highly significant ($P<0.001$), increase in GABA levels in the hypothalamus (+197.38), and hippocampus (+89.97%). However, it induced a very highly significant ($P<0.001$) decrease in the olfactory tubercle. Maprotiline, on the other hand, induced a consistent decrease in GABA levels in all areas studied. This decrease was very highly significant ($P<0.001$) in the hypothalamus, hippocampus, and olfactory tubercle (-38.84%, - 51.10% and - 48.80%) respectively. Both Maprotiline and Nomifensine exerted insignificant ($P>0.05$) decrease in GABA levels in the amygdala (-7.82% and - 4.75%) respectively.

B. Comparison of Percentage Changes In GABA Levels:

The percentage changes in GABA levels was compared in the four limbic areas in each of the 3 treated groups.

In Maprotiline treated group there was a very highly significant difference ($P<0.001$) in GABA% changes in the four limbic areas. Multiple range analysis revealed that the hippocampus and olfactory tubercle were similar in their response which was different from the

Table 1

Compiled Data Showing the Effect of Maprotiline Nomifensine and Trazodone on GABA Content of Specific Limbic Areas of Brains of 20 Albino Rats Elicited After 14 Days i.p. Daily Injections.

Specified Limbic Areas of Rat Brain	Mean Values (x)±SD For GABA (ug/gm Tissue), Level of Significance & %change			
	Control	Maprotiline (20 mg/kg/day)	Nomifensine (20 mg/kg/day)	Trazodone (20 mg/kg/day)
Hypothalamus	814.38±23.29	499.13±50.81*** -38.74%	2421.88±200.20*** +197.38%	2487.50±150.00*** + 205.44%
Hippocampus	617.00±22.93	301.75±19.91*** -51.10%	1172.13±52.78*** +89.97%	1220.63±125.92*** + 97.83%
Amygdala	358.00±99.41	330.00±39.15# -7.82%	341.00±21.52# - 4.75%	286.63±38.66*** - 19.94%
Olfactory Tubercle	315.50±25.20	163.13±5.17*** - 48.40%	176.88±7.72*** - 43.94%	139.25±7.11*** 55.86%

* p<0.05 ** (p<0.01)
# Non-significant (P>0.05) *** (p<0.001)

Table 2

Multiple Range Analysis for GABA Percentage Change after Maprotiline Medication.

Method: 95 Percent Scheffé:

Level	Count	Average	Homogeneous Groups
HIP	8	-51.05904	*
OLF	8	-46.296375	*
HYP	8	-36.742625	*
AMY	8	-7.821250	*

*= $p^{(F\ (K-1;\ N-K))}$ K= Number of Groups
F= F-ratio N= Total number of data

Table 3

Multiple Range Analysis for GABA Percentage Change after Nomifensine Medication

Method: 95 Percent Scheffé:

Level	Count	Average	Homogeneous Groups
OLF	8	-43.93825	*
AMY	8	-4.74850	*
HIP	8	89.97175	*
HYP	8	197.38139	*

*= $p^{(F\ (K-1;\ N-K))}$ K= Number of Groups
F= F-ratio N= Total number of data

response of the hypothalamus and amygdala, the latter being the least affected (Table 2). With regards the Nomifensine and the Trazodone treated groups, the four limbic areas were found to be very highly significantly different (P<0.001) in their response. The multiple range analysis demonstrated that the hippocampus, and olfactory tubercle were similar in their response which was differen

from the response of the hypothalamus and amygdala, the latter being the

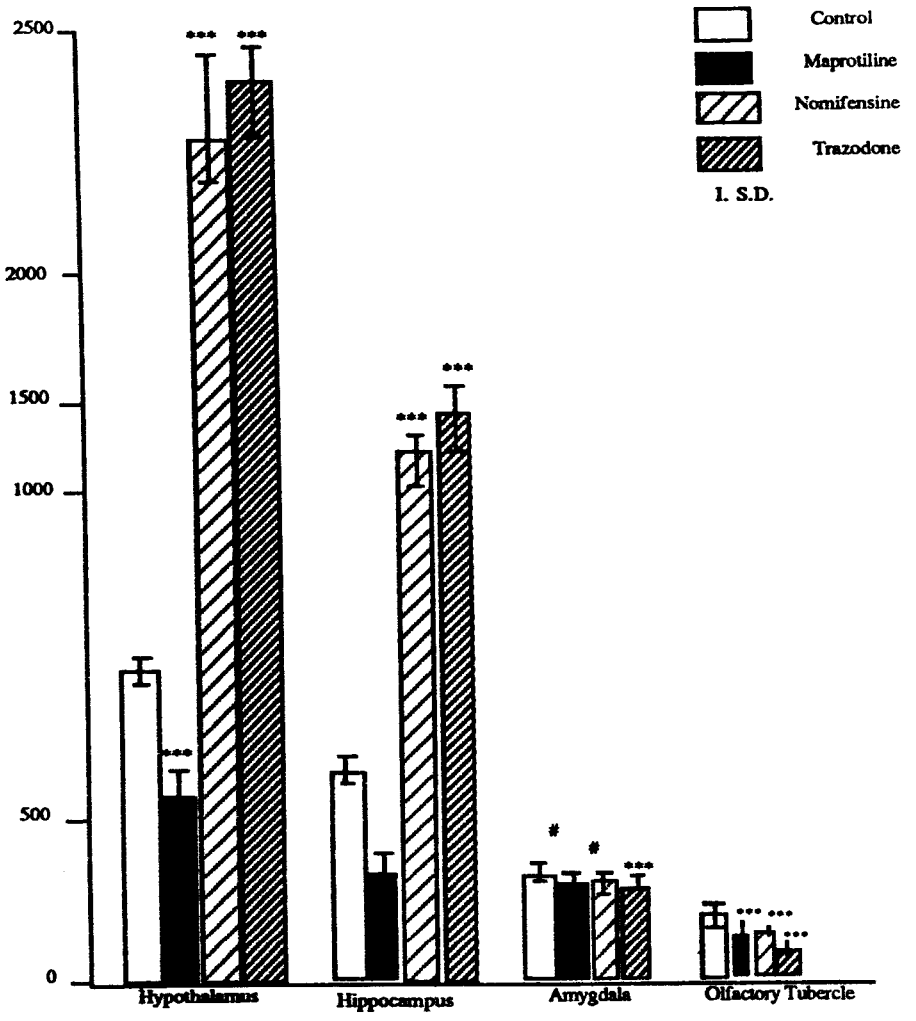

Fig. 1 Bar-chart showing mean GABA levels +S.D. (ug/gm tissue) in the different albino rat brain limbic areas, in control group and in response to 14 days treatment with Maprotiline, Nomifensine and Trazodone.

Table 4
Multiple Range Analysis for GABA Percentage Change after Trazodone Medication

Method: 95 Percent Scheffé:

Level	Count	Average	Homogeneous Groups
OLF	8	-55.86363	*
AMY	8	-19.93713	*
HIP	8	97.83230	*
HYP	8	205.43950	*

*= $p^{(F\ (K\text{-}J;\ N\text{-}K)}$ K= Number of Group
F= F-ratio N= Total number of data

least affected (Table 2). With regards the Nomifensine and the Trazodone treated

groups, the four limbic areas were found to be very highly significantly different ($P<0.001$) in their response. The multiple range analysis demonstrated that the four areas are different from each other and the hypothalamus is the area mostly affected (Tables 3 & 4).

C. Comparison of GABA Mean Levels:

The mean levels of GABA were compared in the control and the three drug treated groups. In the hypothalamus and hippocampus, the 3 drug groups showed highly significant difference from the control group. Nomifensine and Trazodone groups were similar while Maprotiline was different from them and having the lowest GABA level. In amygdala, only Trazodone group showed significant lowering from the control and other drug groups. In the olfactory tubercle, the drug treated groups showed significant lowering from control and Trazodone had the lowest level.

Discussion

The present work compares the effects of 14 days treatment with 3 antidepressant drugs on GABA level in discrete areas of the limbic system.

Maprotiline induced a decrease in GABA levels in all the limbic areas studied i.e. hypothalamus - 39% ($P<0.001$), hippocampus - 51% ($P<0.001$), and olfactory tubercle - 48% ($P<0.001$). There was a slight reduction in GABA levels in the amygdala by - 8%, that proved to be insignificant. These observations of the marked reduction of the GABA in all areas of the limbic system are somehow perplexing. Jalfre and Porsolt (1985) pointed out that the effects of antidepressants on the learned helpless animal model necessitated an enhancing effect of the drug on both serotoninergic and GABA-ergic transmission in the hippocampus. However, the most relevant to the effect of Maprotiline on GABA in limbic areas was the relatively high incidence of Maprotiline induced convulsions (Lipka & Lathers, 1987).

Luchins et al (1984) mentioned that increased spike activity in hippocampal slices was implicated in Maprotiline induced convulsions. Meldrum (1985) suggested that any compound that blocks GABA ergic inhibition, or leads to GABA-ergic insufficiency in selected neuronal cell groups in the hippocampus, might lead to seizure production. It is interesting here to mention that Handley and Brown (1982) stressed on the importance of enhancing limbic serotoninergic transmission together with GABA-ergic insufficiency in eliciting tremors. head jerks and sometimes myoclonus in rats. This observation could be considered as an explanation to the Maprotiline-induced seizures, as Maprotiline also induces 5-HT enhancing effects in the limbic areas.

Nomifensine in the present investigation was found to affect the GABA levels in a highly significant ($P<0.001$) way in most limbic areas. The changes in GABA levels in the hypothalamus, hippocampus, and olfactory tubercle were + 197%, +90% and - 44% respectively On the other hand, the change in GABA levels in the amygdala was insignificant. No previous reports on GABA changes by Nomifensine were available. However, reports on the effect of chronic imipramine medication in naive rats, supported the possibility that this drug increased hippocampal GABA release

(Sherman and Petty, 1982). It is worth mentioning here that hippocampal release of GABA is diminished in the "helpless" rats in the "Learned helplessness" models (Sherman and Petty, 1980). This latter decrease in hippocampal GABA release was reported to be reversed by chronic treatment with imipramine. Moreover, the mitigating effects of antidepressants on "Learned helplessness" appear to depend on the GABA-ergic mechanism because only intrahippocampal injections of GABA produce effects similar to those observed with tricyclic antidepressants (Jalfre and Prosolt 1985).

It is interesting here to mention the inhibitory role of GABA on long-term potentiation (LTP) in the hippocampus, a widely used mode of learning and memory (Collingridge, 1985). According to Collingridge (1985), this inhibitory effect of GABA plays an important role in preventing LTP from occurring, until the necessary period of high frequency activation occurs. The effect of Nomifensine on hypothalamic GABA levels in the present study was very pronounced.

Trazodone was found to affect the levels of GABA in the different limbic areas in a very highly significant ($p<0.001$) way. Thus it increased GABA levels in the hypothalamus and hippocampus (+205% and +98% respectively). Meanwhile, it induced a decrease in its level in the amygdala and olfactory tubercle by - 20% and - 56% respectively. The pronounced effect of the drug on the GABA level in the hippocampus, and the previously assumed post-synaptic 5-HT receptor antagonism, could explain the sedative effect of Trazodone. This could be compared to the effects of anxiolytics (i.e. Benzodiazepines) on these particular neurotransmitters. Iversen (1986) reported that benzodiazepines interact with GABA-ergic receptors in the hippocampus together with their inhibitory effect on 5-HT neurones of the raphe which innervate limbic structures, and these effects were found to correlate with the anxiolytic effects of the drugs.

It is interesting here to mention the study of Scatton et al (1982) on the GABA receptor stimulant progabide which is considered by the authors to have an antidepressant potential. In their study, Scatton et al (1982) demonstrated a profile for the drug effects on NE and 5-HT turnover. So, progabide which enhance GABA-ergic transmission is reported to enhance the turnover of NE and 5-HT (i.e. increasing the levels of MHPG and 5-HIAA) in the mesolimbic areas.

The present work positively indicates the involvement of GABA in the modulation of action of antidepressant drugs. The tested antidepressants produced variable effects on its level in the different limbic areas. More work is certainly needed to elucidate its role in the central action of antidepressant drugs.

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Les Effets de Maprotiline,Nomifensine et Trazodone sur le Metabolisme de L'Acide Gamma Amino Butyrique dans le Systéme Limbique

Les effets de traitement chronique par 3 médicaments antidépresseurs: Maprotiline, Nomifensine et Trazodone sur le taux de la métabolisme de l'acide Gamma amino butyrique dans 4 régions de cerveau de rat, régions liées au systéme limbique. Le Trazodone a remarquablement augmenté le niveau du gamma acide amino-butyrique dans l'hypothalamus et l'hippocampus mais l'en a diminué dans les tubercules amygdales et olfactifs. Le Nomifensine en a augmenté le niveau dans l'hypothalamus et l'hippocampus et l'en a remarquablement diminué dans le tubercule olfactif. D'autre part, le Maprotiline en a reguliérement diminué le niveau dans toutes les 4 régions. Ces résultats indiquent l'implication du gamma acide amino-butyrique dans le mécanisme de l'action des antidépresseurs. Son rôle possible dans la modulation d'autres neurotransmetteurs et la production de manifestations antidépressives est discutée.

تأثير المابروتيلين والنوميفينزين والترازودون على تحويل حامض الجاما أمينو بيوتيريك فى الجهاز الطرفى

تم فى هذا البحث دراسة تأثير ثلاثة من مضادات الاكتئاب على نسبة حامض أمينوبيوتيريك فى أربعة مناطق من المخ لها علاقة بالجهاز الطرفى فى الفئران. وتشمل هذه العقاقير: نوميفينزين – ترازودون – مابروتيلين. ولقد أثبتت الدراسة أن الترازودون يزيد من نسبة هذه المادة فى المنطقتين المعروفتين باسم تحت المهاد (الهيبوثالاموس) "والجسم الشبية بحصان البحر (الهيبوكامبوس). بينما يسبب هذا العقار نقصا فى نسبة هذه المادة فى المنطقتين المعروفتين باسم اللوزة" (الأميجدالا) "والدرنة الشمية" ومن ناحية أخرى أظهرت الدراسة ان نوميفينزين يزيد من نسبة هذه المادة فى منطقتى تحت المهاد "والجسم الشبية بحصان البحر فى حين يسبب نقصها بصورة ملحوظة فى الدرنة الشمية" أما عقار مابروتيلين فإنه يسبب نقصا فى نسبة هذه المادة فى المناطق الأربعة سالفة الذكر. وتشير نتائج هذه الدراسة إلى دور تلك المادة (حامض الجاما أمينو بيوتيريك) فى الكيفية التى تؤدى بها مضادات الإكتئاب عملها. ويناقش البحث تفسير ذلك متضمنا تأثيرها على الموصلات العصبية الأخرى.