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Effects of the antidepressant/antipanic drug phenelzine on GABA concentrations and GABA-transaminase activity in rat brain

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Abstract—The effects of long-term (28-day) administration of several antidepressant/antipanic drugs [imipramine, desipramine, tranylcypromine and phenelzine (PLZ)] on γ-aminobutyric acid-transaminase (GABA-T) activity and GABA levels were investigated in rat frontal cortex. Of the drugs investigated, only PLZ inhibited GABA-T and elevated GABA levels. Additional short-term experiments were conducted with PLZ, and they demonstrated a dose-dependent inhibition of GABA-T in rat whole brain. Time–response studies on inhibition of GABA-T in whole brain demonstrated that at a dose of PLZ of 15 mg/kg i.p. inhibition of GABA-T remained relatively constant from 1 to 8 hr and that the enzyme was still inhibited by 23% at 24 hr after PLZ administration.

In recent years there has been considerable interest in the possible role of the putative amino acid neurotransmitter γ-aminobutyric acid (GABA*) in the etiology and pharmacotherapy of affective disorders. Chronic administration of antidepressants of every class [tricyclics (imipramine [IMI], desipramine [DMI], amitriptyline), monoamine oxidase (MAO) inhibitors (pargyline), novel antidepressants (mianserin, zimelidine, maprotiline, nomifensine, fluoxetine)] as well as repeated electroshocks have been reported to result in an upregulation of $GABA_B$ receptors in rat cortex [see Ref. 1 for review] but this effect has been disputed by others [2–6]. More recently, there has been a great deal of interest in the effects of antidepressants on $GABA_A$ receptors and the intimately associated benzodiazepine receptors and chloride ion channel, with chronic administration of antidepressants reported to result in a decrease in the number of all three binding sites [7–9]. Antidepressant effects have been observed in animal models after administration of the GABA agonists progabide, baclofen, muscimol and fengabine [1].

Reduced levels of CSF GABA have been reported in depressed patients [see Ref. 1 for review], and Petty and coworkers [10] have found that patients with primary unipolar affective disorder, bipolar disorder, mania or secondary depression have plasma GABA levels significantly lower than control values. The MAO inhibitors pargyline and phenelzine (PLZ) and the tricyclic antidepressant DMI when administered acutely at high doses have been reported to produce an elevation of rat brain GABA [11–14], and recent experiments in our laboratories indicate that this is a rather dramatic effect with PLZ, remaining for extended periods after a single dose [15]. Despite the interesting results of acute studies with antidepressants, there appears to be a paucity of information available on the effects of longer term administration of antidepressants on brain concentrations of GABA; such details are important, considering that antidepressants often must be administered for 2 weeks or longer before improvement is noted in the clinical situation. We have conducted experiments on the effects of tricyclic antidepressants and MAO-inhibiting antidepressants (one hydrazine-type and one non-hydrazine-type) on GABA levels and activity of the catabolic enzyme GABA transaminase (GABA-T) in rat brain after acute and chronic administration, and some of those results are reported here.

Materials and Methods

Male Sprague–Dawley rats (275 ± 50 g) were chronically administered drugs or the distilled water vehicle via osmotic minipumps (Alzet 2ML4) implanted s.c. in the dorsal thoracic region. Drug treatments were as follows: PLZ sulfate (10 mg/kg/day); (±)-tranylcypromine (TCP) hydrochloride (1 mg/kg/day); IMI hydrochloride (30 mg/kg/day); DMI hydrochloride (10 mg/kg/day). On day 28, the rats were killed by decapitation, and the frontal cortex (a brain area often used for studying receptor binding after long-term administration of antidepressants) was dissected out immediately and frozen in isopentane on solid carbon dioxide. The samples were then removed from the isopentane and frozen at −80° until the time of analysis.

For the dose–response study on the effects of PLZ on GABA-T activity, rats were injected i.p. with a variety of doses of PLZ and killed 4 hr after injection. In the time–response study, rats were injected with a dose of PLZ of 15 mg/kg (based on free base weight) and groups were killed at 1, 4, 8, 16, or 24 hr after injection. This dose of PLZ is one which is frequently used for studying effects of PLZ on brain amines and which has been shown to cause a marked increase in whole brain levels of GABA [15].

At the time of analysis, the tissue was homogenized in ice-cold 0.32 M sucrose and aliquots were taken for analyses of GABA levels and/or GABA-T activity. For measurement of GABA, the samples were homogenized in ice-cold 0.1 M perchloric acid and centrifuged to precipitate protein; the supernatant was used to analyze for GABA according to the electron-capture gas chromatographic procedure of Wong *et al.* [16]. For the measurement of GABA-T activity, the samples were homogenized in a modification of the medium described by Palfreyman *et al.* [17]. Composition of the medium was: glycerol (20%, v/v), Triton X-100 (0.13%, v/v), reduced glutathione (100 μM), pyridoxal 5′-phosphate (1 μM), Na_2EDTA (1 mM), dipotassium monophosphate (5 mM), and sufficient acetic acid to bring the pH value to 7.2–7.4. An aliquot of this homogenate was utilized to assay for GABA-T activity using the radiochemical procedure described by Sterri and Fonnum [18].

Data were analyzed by analysis of variance followed by the Newman–Keuls multiple comparison test. A two-tailed

* Abbreviations: GABA, γ-aminobutyric acid; DMI, desipramine; Na_2EDTA, ethylenediamine tetraacetic acid, disodium; GABA-T, GABA-transaminase; IMI, imipramine; MAO, monoamine oxidase; PLZ, phenelzine; and TCP, tranylcypromine.

probability distribution was used and the general convention of a probability value of $P < 0.05$ was used to establish statistical significance.

Results and Discussion

The drug doses utilized in the chronic study described here were chosen because they have been shown in other studies to result, after long-term administration, in a down-regulation of β-adrenergic receptors, a characteristic of many antidepressants [for review, see Ref. 19]. Levels of GABA were studied in frontal cortex because in a previous report from our laboratories and those of others [1, 6], this brain area was used to study possible changes in $GABA_B$ binding sites after chronic administration of antidepressants. Aliquots of the same tissue suspension used to study $GABA_B$ binding [6] were employed in the present study for measurement of GABA levels and GABA-T activity. In the acute studies on inhibition of GABA-T reported here, whole brain was utilized since previous research demonstrating elevated GABA levels after PLZ administration [e.g. Refs. 11, 13, and 15] had employed whole brain. PLZ and TCP were studied because they are the two most frequently prescribed MAO inhibitors and represent MAO inhibitors of the hydrazine (PLZ) and non-hydrazine (TCP) chemical classes. A comparison between the two drugs is important since the hydrazine moiety of PLZ has been proposed as being important for its interaction with pyridoxal-dependent enzymes such as GABA-T.

As shown in Table 1, of the four antidepressants tested, only PLZ produced a significant increase in frontal cortex levels of GABA after chronic administration. This increase was 54% above control levels. Similarly, only PLZ caused a significant inhibition of GABA-T; the degree of inhibition was 28%. Perry and Hansen [13] noted significant elevations in whole brain GABA levels after feeding rats a variety of doses (12–27 mg/kg) of PLZ for time periods ranging from 5 to 54 days; these authors did not measure GABA-T activity and did not investigate other antidepressants in that study.

The results of our study reported here, combined with our previous findings on the effects of PLZ on GABA levels in whole brain up to 24 hr after drug injection [15], led us to investigate the inhibition of GABA-T in rat brain by PLZ more extensively. Experiments in whole brain (at 4 hr post-injection, a time at which PLZ was shown previously in our laboratories to produce a maximal increase in GABA levels [15]) showed a dose-dependent inhibition of GABA-T by PLZ (Table 2). A time study (Table 2) at a dose of PLZ of 15 mg/kg showed that the maximum inhibition of GABA-T (30–35%) was attained by 1 hr and did not increase significantly from 1 to 8 hr. By 24 hr after PLZ injection, 20% inhibition was still evident; it is of interest that this is similar to the percent inhibition observed in the chronic study, and that under the same circumstances (24 hr after injection), our previously reported percent increase in GABA was 50% [15], also similar to the increase in GABA levels observed in the chronic study reported here. The findings after acute administration of PLZ are similar to those of Popov and Matthies [11], who also reported marked increases of GABA levels in brain at relatively low levels of inhibition of GABA-T; these authors did not conduct comprehensive dose–response and time–response studies on activity of the enzyme. Thus, the relationship between GABA levels and inhibition of GABA-T appears to be quite different from that of levels of catecholamines and 5-hydroxytryptamine (5-HT) and degree of inhibition of MAO. It has been reported that MAO must be inhibited by >85% before elevation of brain levels of catecholamines and 5-HT is evident in rat brain [20].

We have assumed that inhibition of GABA-T is important in the elevation of GABA levels by PLZ, but an indirect effect related to inhibition of MAO cannot be ruled out. Popov and Matthies [11] reported that pretreatment of rat brain tissue with the MAO inhibitors phenylisopropylhydrazine or TCP (a hydrazine and non-hydrazine MAO inhibitor, respectively) inhibits the effects of PLZ on GABA and GABA-T activity. These same two drugs do not, however, influence the effects of aminooxyacetic acid (a drug which is known to inhibit GABA-T and elevate brain GABA levels). TCP sulfate administered alone at a dose of 5 mg/kg was found by those authors [11] to produce no change in rat brain GABA levels or GABA-T activity. Similarly, Wong [21] studied TCP at doses of 1–20 mg/kg, at 4 hr after drug administration, and found no change in rat brain GABA levels from control levels, and Yu and Boulton [22] recently reported that TCP has no effect on GABA-T. PLZ is an unusual drug in that not only is it an inhibitor of MAO, but it is also a substrate for that enzyme [23, 24]. The fact that the MAO inhibitor TCP, a drug which does not inhibit GABA-T or elevate GABA itself, can block these effects of PLZ suggests that a metabolite of PLZ may be responsible for the actions on GABA-T and GABA.

PLZ is frequently employed clinically as an antipanic drug as well as an antidepressant [25], and it is feasible

Table 1. GABA concentrations and GABA-T activity in rat frontal cortex after chronic (28 day) administration of antidepressants

Drug	GABA Concentrations (μg/g)	GABA-T activity (μmol/g tissue/hr)
Vehicle	238 ± 10	26.6 ± 2.9
Desipramine	248 ± 17	27.4 ± 1.6
Imipramine	224 ± 13	28.3 ± 1.4
Phenelzine	366 ± 30*	19.2 ± 1.2*
Tranylcypromine	254 ± 14	28.5 ± 1.2

Doses are described in the text. Values are means ± SEM, N = 7.
* $P < 0.05$, compared to vehicle-treated levels.

Table 2. Effects of PLZ on GABA-T activity in rat whole brain

Dose of PLZ (mg/kg, i.p.)	Time after PLZ administration (hr)	% inhibition of GABA-T activity
(A) 2.5	4	7.6 ± 6.7
7.5	4	15.5 ± 3.6*
15	4	32.0 ± 3.2*
30	4	44.5 ± 6.0*
(B) 15	1	30.3 ± 1.1*
15	4	32.0 ± 3.2*
15	8	34.3 ± 4.9*
15	16	20.4 ± 2.5*
15	24	23.3 ± 2.6*

Enzyme values are expressed as percent inhibition (mean ± SEM) compared to vehicle-treated controls (N = 6 at each dose and time). Control values in studies A (dose–response study) and B (time–response study) were 28.5 ± 2.2 μmol/g tissue/hr (N = 6) and 33.4 ± 1.1 μmol/g tissue/hr (N = 30), respectively.
* $P < 0.05$ compared to controls.

that its effects on GABA-T and on GABA levels may contribute to its efficacy in treating panic disorder since, as reviewed by Breslow *et al.* [26], there is now substantial evidence indicating that GABA may play an important role in the actions of antipanic drugs. The present results indicate that in the cases of IMI, DMI and TCP, all three of which have also been used to treat panic disorder, inhibition of GABA-T and elevation of brain GABA levels are not important components of such a GABAergic mechanism.

Behavioural experiments and receptor binding studies in our laboratories [5, 6] indicate that PLZ does not cause an upregulation of $GABA_B$ receptors. Considering that PLZ actually elevates brain GABA, such a finding is not unexpected. However, experiments on the effects of PLZ on $GABA_A$ receptors (and the associated benzodiazepine receptor and chloride ionophore [27]) may be warranted. Suzdak and Gianutsos [7] and Suranyi-Cadotte *et al.* [8] reported that long-term administration of antidepressants results in down-regulation of $GABA_A$ receptors and benzodiazepine receptors, respectively, but Pilc and Lloyd [28] and Kimber *et al.* [29] were unable to find such effects. To our knowledge, PLZ has not been investigated in such systems, and these studies, as well as experiments on binding to the chloride ionophore, are now underway in our laboratory.

In summary, the results of the present study indicate that long-term administration of PLZ, but not of TCP, IMI or DMI, at doses which cause down-regulation of β-adrenergic receptors, produces significant inhibition of GABA-T and elevation of GABA in rat frontal cortex. This represents, to our knowledge, the first time that a chronic comparative study of the effects of these antidepressants on brain GABA levels and GABA-T activity has been reported. Results from long- and short-term studies indicate that inhibition of GABA-T of only 20% is sufficient to cause a marked elevation of brain GABA levels. These effects on GABA may play a role in the antipanic effects of PLZ, and the results reported here suggest that studies on the effects of long-term administration of PLZ on components of the $GABA_A$ receptor/benzodiazepine receptor/chloride ionophore complex are warranted. Although speculation on the effects of drugs in humans based on findings in rats should be approached with caution, investigations of the effects of PLZ on GABA in humans would also seem to be warranted. Petty and coworkers [10] have reported low levels of GABA in plasma of depressed patients, and an examination of PLZ on these plasma levels would be of interest.

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Induction of propranolol metabolism by the azo dye sudan III in rats

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Abstract—Effects of the azo dye sudan III, an inducer of cytochrome P450 isozymes belonging to the CYP1A subfamily, on propranolol (PL) *in vitro* and *in vivo* metabolism were investigated in rats. The kinetic parameters of the activity for each metabolic pathway were determined in liver microsomes from control and sudan III-treated rats. Sudan III pretreatment increased extensively PL 4-hydroxylase, 5-hydroxylase and *N*-desisopropylase activities at high but not at low PL concentrations. On the other hand, kinetic parameters of 7-hydroxylase activity were not affected by sudan III pretreatment. Sudan III pretreatment decreased blood concentrations of PL after intraportal infusion of PL at high doses (12.5 and 20 mg/kg), but not at a low dose (5 mg/kg). These observations were consistent with data obtained from the *in intro* studies showing that sudan III pretreatment induced low-affinity but not high-affinity cytochrome P450 isozymes involved in PL metabolism in rat liver microsomes.

Major metabolic pathways of propranolol (PL*) in rat liver microsomes are hydroxylations at the 4-, 5- and 7-positions of the naphthalene ring and *N*-dealkylation of the propanolamine side chain [1, 2]. Of these pathways 4-hydroxylation is common to rats, dogs and humans [3]. Previous studies have shown that PL metabolism in rat liver subcellular fractions is induced by phenobarbital [4, 5], 3-MC [5] or sudan III [5]. The pretreatment of rats with 3-MC or sudan III increased markedly the formation rate of ND-PL in liver microsomes, but phenobarbital treatment caused a less marked induction [5]. None of the inducers, however, affected the PL 4-hydroxylase activity.

In connection with the regioselective induction of PL metabolism, cigarette smoking has been shown to increase the oral clearance of PL in human subjects selectively by induction of side-chain oxidation without affecting the aromatic ring oxidation of PL [6]. The mechanism for this selective effect is assumed to involve differential induction of the P450 monooxygenase system by PAHs, which are components of cigarette smoke.

Sudan III has been shown in rats to induce P450 isozymes which are very similar to those induced by a PAH, 3-MC, with regard to their substrate specificity, electrophoretic pattern and immunochemical inhibition [7–9]. This indicates that sudan III as well as 3-MC is an inducer of P450 isozymes belonging to the CYP1A subfamily [7, 10]. Since sudan III is not a carcinogen, it may be safer than carcinogenic 3-MC as a CYP1A subfamily inducer for use in the laboratory. Therefore, in this study we present *in vitro* and *in vivo* inducing effects of sudan III on PL metabolism in rats.

* Abbreviations: PL, propranolol; 3-MC, 3-methylcholanthrene; X-HO-PL, X-hydroxy propranolol; ND-PL, *N*-desisopropyl propranolol; PAH, polycyclic aromatic hydrocarbon.