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Effects of the antidepressant/antipanic drug phenelzine and its putative metabolite phenylethylidenehydrazine on extracellular γ-aminobutyric acid levels in the striatum

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Abstract

Phenelzine (PLZ) is a non-selective monoamine oxidase inhibitor (MAOI) commonly used to treat depression and panic disorder. As expected, PLZ increases brain levels of dopamine, norepinephrine, and serotonin. Interestingly, PLZ also elevates brain levels of γ-aminobutyric acid (GABA), and previous studies have suggested that these increases may also contribute to the anxiolytic effects of PLZ. Using *in vivo* microdialysis in conscious, freely moving rats, combined with high performance liquid chromatography, the present experiments determined that PLZ (15 or 30 mg/kg, free base weight) increases extracellular levels of GABA in the caudate-putamen and nucleus accumbens. The results also indicated that phenylethylidenehydrazine (PEH; 29.6 mg/kg, free base weight), a putative intermediate metabolite of PLZ that is not an MAOI, also significantly increases extracellular GABA levels in the caudate-putamen. These findings provide further evidence that GABA may play an important role in the actions of PLZ and suggest that PEH should be pursued further as a GABAergic drug in its own right.

Keywords: Phenelzine; Phenylethylidenehydrazine; GABA; Striatum; *In vivo* microdialysis; HPLC

1. Introduction

PLZ (Fig. 1) is a potent, long-term inhibitor of MAO-A and -B [1–3]. It is used clinically in the treatment of panic disorder, social phobia, and depression, particularly depression associated with anxiety [4–8]. In rodents, PLZ has antipanic and anxiolytic effects in the mouse defense battery, the elevated plus maze, and in an operant conflict procedure [9–11].

As expected, PLZ increases brain levels of the monoamines DA, NE, and 5-HT [2,3,10]. Interestingly, PLZ also produces large increases in brain levels of the amino acid GABA [1–3,12,13], an effect that appears to be related to the anxiolytic properties of PLZ [9]. The exact mechanisms underlying the effects of PLZ on GABA are not understood completely. Evidence suggests that PLZ-induced inhibition of the degradative enzyme GABA-T is involved [1,14–16]. However, other mechanisms may also be implicated because PLZ produces a 3- to 4-fold increase in GABA levels while only inhibiting GABA-T by less than 50% [1,15].

Evidence suggests that a metabolite of PLZ produced by MAO may mediate the effect of PLZ on GABA. In addition to being an MAO inhibitor, PLZ is also a substrate for MAO [17–20], and PLZ-induced increases in GABA levels are blocked by prior treatment with other MAO inhibitors [1,16, 21]. One putative metabolite of PLZ that could contribute to the effects of PLZ on GABA is PEH [22] (Fig. 1). Like PLZ, PEH increases whole brain levels of GABA and inhibits GABA-T [23]. Unlike PLZ, the effects of PEH on GABA are not prevented by pretreatment with an MAO inhibitor [23], suggesting that it is unlikely that MAO me-

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Abbreviations: DA, dopamine; GABA, γ-aminobutyric acid; GABA-T, GABA transaminase; 5-HT, 5-hydroxytryptamine or serotonin; MAO, monoamine oxidase; N^2-acetylPLZ, N^2-acetylphenelzine; NE, norepinephrine; PEH, phenylethylidenehydrazine; and PLZ, phenelzine, 2-phenylethylhydrazine.

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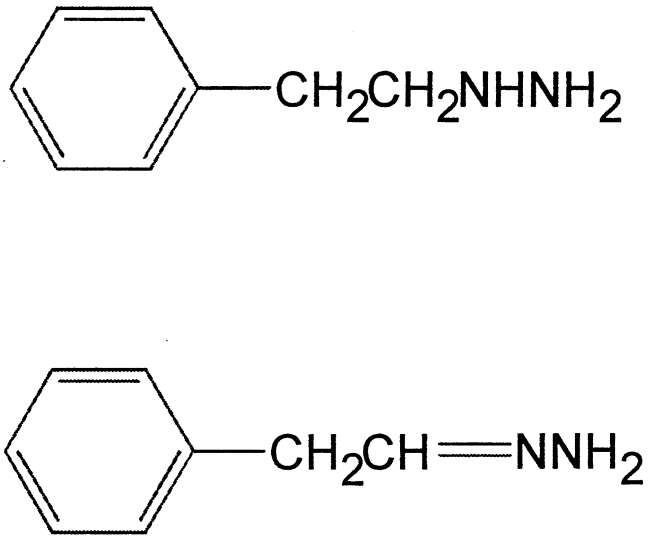

Fig. 1. Chemical structures of PLZ (top) and PEH (bottom). Like PLZ, PEH retains a free hydrazine group, but in contrast to the parent drug, it has a double bond in its side chain.

tabolizes PEH to another active metabolite in order to mediate the effects of PEH on GABA.

Converging evidence suggests that the therapeutic effects of PLZ and other antipanic drugs are mediated, in part, through an increase in GABAergic neurotransmission [24]. The benzodiazepine anxiolytics, which act through a binding site on the $GABA_A$ receptor [25], are also used in panic disorder. The anticonvulsants vigabatrin and valproic acid, both GABAergic drugs, have been reported to have anxiolytic and antipanic properties [26–28]. In rats, only doses of PLZ that increase whole brain levels of GABA have anxiolytic effects in the elevated plus maze [9]. Importantly, the *N*-acetylated metabolite of PLZ, N^2-acetylPLZ, which increases brain levels of monoamines but does not increase brain GABA levels [2,9], also does not mimic the anxiolytic effects of PLZ in the plus maze [9].

In the present experiments, to examine the effects of PLZ on extracellular levels of GABA in the dorsal (caudate-putamen) and ventral striatum (nucleus accumbens), we combined the technique of *in vivo* microdialysis in conscious, freely-moving rats with that of HPLC. In addition, we examined the effects of PEH, a putative metabolite of PLZ, on extracellular levels of GABA in the caudate-putamen. We chose to examine the striatum because it has high levels of GABA-containing cell bodies and terminals [29, 30], because it has been implicated in affect, social withdrawal, anxiety, and depression [31–35], and because PLZ is known to affect other neurotransmitters in this region [36–39].

2. Materials and methods

All animal procedures were approved by the University of Alberta Biosciences Animal Policy and Welfare Committee and were carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

2.1. Animals

Male Sprague–Dawley rats (Ellerslie; 250–350 g) were used. Each rat was individually housed in a polycarbonate cage and maintained on a 12-hr light–dark cycle (lights on at 7:00 a.m.), with water and food *ad lib.* (Purina Rat Chow).

2.2. Surgery

At least 1 week after arrival at the animal facility, the rats were given atropine sulfate (0.4 mg/kg, i.p.; American Pharmaceutical Partners) and were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Abbott Laboratories). When necessary, supplemental doses of sodium pentobarbital were given. The rats were hydrated with saline (3.0 c.c., s.c.; 0.9%) and given penicillin (4300 U/kg, i.m.; Rhône Mérieux).

Stereotaxic surgical procedures were used to implant a permanent intracerebral guide cannula [Bioanalytical Systems (BAS)] into the region immediately dorsal to either the caudate-putamen (right hemisphere; 0.2 mm anterior to bregma [AP]; 3.0 mm lateral to bregma [ML]; -2.7 mm ventral to dura [DV]) or the nucleus accumbens (right hemisphere; 1.6 mm AP; 2.2 mm ML; -5.5 mm DV) [40]. The nucleus accumbens cannula was angled at 10° from the mid-sagittal plane to avoid the ventricular system. The cannula was secured to the skull with four jeweler's screws and cranioplastic cement (Plastics One). In addition, a wire loop was embedded in the cement to be used later to attach the rat to the tether during the microdialysis procedures. A dummy cannula was inserted into the guide cannula to keep it free of debris. The skin was sutured rostral and caudal to the cemented area (5–0 braided silk, Ethicon Sutures), and the incision was sealed with an adhesive (Vetbond; 3M Animal Care Products). Following surgery, the rats were kept in a warm temperature-controlled environment until recovery from anesthesia. Doxapram hydrochloride (Dopram-V; 7.0 mg/kg, i.p.; Ayerst Laboratories) was administered to those rats that experienced respiratory problems during recovery. Two days following surgery, the patency of each cannula was checked, and betadine was applied to the surgical wound (10% Povidone-iodine; Purdue Frederick).

2.3. Drugs

PLZ (phenelzine sulfate; Sigma Chemicals) was dissolved in distilled water and administered i.p. (15 or 30 mg/kg, free base weight). The doses of PLZ were selected based on their demonstrated efficacy in increasing GABA levels in the brain [3,12,13,16].

PEH was synthesized at the University of Alberta (E. Knaus, Faculty of Pharmacy and Pharmaceutical Sciences),

dissolved in pure sunflower oil (Smart Choice brand), and administered i.p. (29.6 mg/kg, free base weight). This dose is equivalent, on a molar basis, to the 30 mg/kg dose of PLZ.

2.4. In vivo microdialysis

Each rat was allowed to recover from surgery for at least 4 days and handled for 3 min on two separate occasions before microdialysis procedures were initiated. Between 7:00 and 9:00 a.m. on the day of microdialysis, the rat was placed in a round Plexiglas bowl (BAS) that contained a mixture of clean bedding and bedding from the rat's cage. After 5 min, the rat was attached to the tether. Following a 1-hr habituation period, a microdialysis probe (2 mm membrane for nucleus accumbens; 4 mm membrane for caudate-putamen; BAS) was inserted into the guide cannula. The probe was perfused at the rate of 1 μL/min with artificial cerebrospinal fluid (aCSF; NaCl, 145.0 mM; KCl, 3.0 mM; $CaCl_2$, 1.5 mM; $MgCl_2$, 1.0 mM; NaH_2PO_4, 2.0 mM; Na_2HPO_4, 2.0 mM; dextrose, 2.0 mM; pH 7.3; filtered and degassed). After a 2-hr stabilization period, three 25-min samples were collected, and then vehicle, PLZ, or PEH was administered (i.p.). Following the injection, eleven 25-min samples were collected. The samples were kept on dry ice during the experiment and then transferred to a $-80°$ freezer until the biochemical assays were performed.

2.5. In vitro GABA recovery

To estimate the recovery of GABA across the dialysis membrane, the probe was removed after the eleventh sample was collected, placed into a 5 μM GABA solution (Sigma), perfused with aCSF at a rate of 1 μL/min for 25 min at room temperature, and then the amount of GABA present in the sample was measured later.

2.6. Histology

After the completion of the microdialysis experiments, the rats were euthanized with an overdose of pentobarbital (100 mg/kg) and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and stored in a 10% formalin solution until they were sectioned into slices (40 μm) collected consecutively through the extent of the guide cannula tract. The sections were mounted onto glass slides and stained with thionin, and the cannula location for each rat was verified by an observer who was unaware of the rat's treatment or results.

2.7. Neurochemical analyses

GABA levels were determined using HPLC with fluorescence detection following derivatization with *o*-phthaldialdehyde [41]. Briefly, 5-μL portions of the samples were reacted with fluoraldehyde reagent (Pierce Chemicals) in the injection loop of an Alliance 2690XE solvent management system (Waters) before injection onto a Waters μBondapak C18 precolumn connected to a Waters Spherisorb ODSD2 C18 column (4.6 $\times$ 250 mm, 5 μm) maintained at a constant temperature of 30°. A Waters Alliance 2690XE pump and sample management system were used to mix two mobile phases to form a gradient. The system was coupled to a Shimadzu RF10 fluorescence detector (excitation and emission wavelengths of 260 and 455 nm, respectively).

2.8. Statistical analyses

The dialysis data were expressed as mean percent of baseline. As is typical with time series data, the variance increased as a function of time [42]. Consequently, the post-baseline data were expressed as the log (base 10) of the percent change of baseline and analyzed using a two-way mixed model ANOVA, with one between factor (dose) and one within factor (time). Post hoc comparisons were made using Fisher's adjusted least significant differences tests (LSD). An alpha level of 0.05 was used as the criterion of statistical significance.

3. Results

3.1. In vitro recovery

The recovery of GABA across the microdialysis membrane was 31 $\pm$ 1.66% (mean $\pm$ SEM).

3.2. Effects of PLZ on extracellular GABA levels in the caudate-putamen

The results of the histological analyses indicated that 19 rats had cannulae located in the region of the caudate-putamen (Fig. 2A). The administration of PLZ increased extracellular GABA levels in the caudate-putamen in a dose- and time-dependent manner, $F(20,160) = 5.53$; $P < 0.0005$ (Fig. 2B). The LSD post hoc tests indicated that there were no significant differences in extracellular GABA levels at any time following the vehicle injection ($P > 0.05$ for all comparisons). In comparison with rats given vehicle alone, extracellular GABA levels in rats treated with 15 or 30 mg/kg of PLZ were significantly higher ($P < 0.05$ for all comparisons). The 30 mg/kg dose of PLZ produced significantly larger increases in extracellular GABA levels than did the 15 mg/kg dose ($P < 0.05$ for all comparisons). Also, the magnitude of the PLZ-induced elevation in extracellular GABA levels increased significantly over time. That is, for both doses of PLZ, extracellular GABA levels were higher at several later post-injection sampling periods relative to earlier ones ($P < 0.05$ for all comparisons).

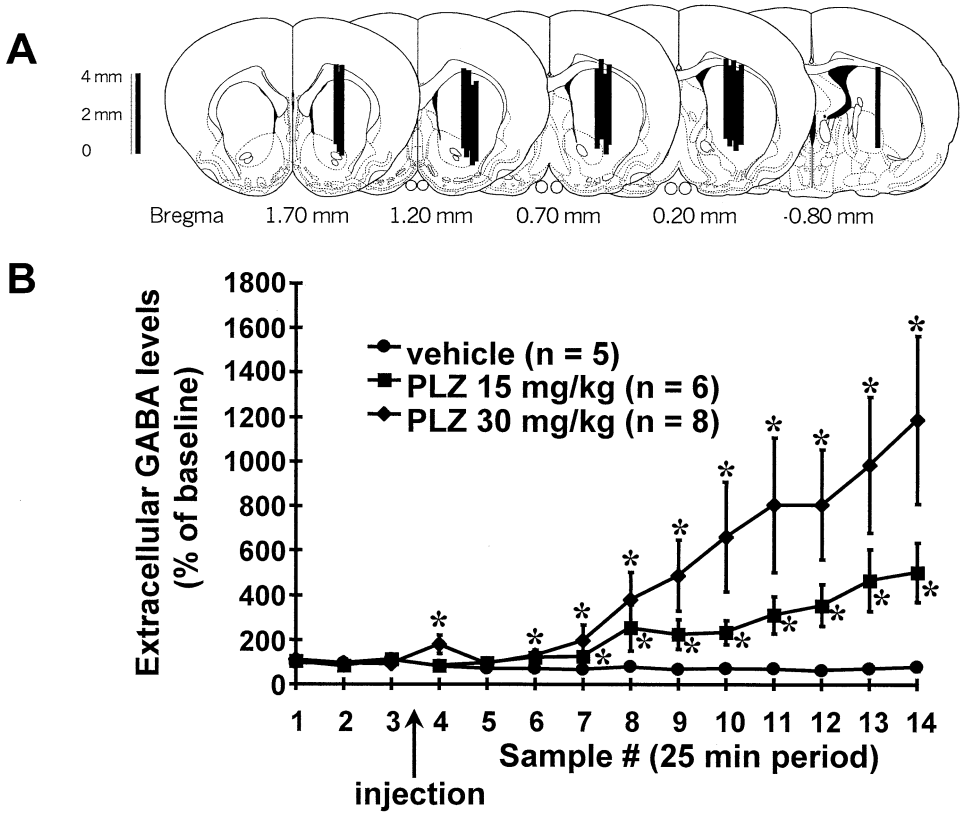

Fig. 2. (A) Approximate distribution of microdialysis probes in the caudate-putamen. Atlas plates were adapted from Ref. 40. The anteroposterior planes are in millimeters, relative to bregma. (B) Effects of i.p. administration of vehicle or PLZ (15 or 30 mg/kg, free base weight) on extracellular GABA levels in the caudate-putamen. Values are means ± SEM. Baseline values for all three groups combined = 1.624 ± 0.151 pmol/25 μL, not corrected for probe recovery. Key: (*) $P < 0.05$ vs vehicle—same sample period.

3.3. Effects of PLZ on extracellular GABA levels in the nucleus accumbens

The results of the histological analyses indicated that 15 rats had cannulae located in the region of the nucleus accumbens (Fig. 3A). The administration of PLZ affected extracellular GABA levels in the nucleus accumbens in a time-dependent manner, $F(20,120) = 4.22$; $P < 0.01$ (Fig. 3B). The LSD post hoc tests indicated that there were no differences in extracellular GABA levels at any time following the vehicle injection ($P > 0.05$ for all comparisons). Compared with rats given vehicle, rats given 15 or 30 mg/kg of PLZ had significantly higher extracellular GABA levels. However, the effects of PLZ were not dose-dependent. There were no significant differences between the increases in extracellular GABA levels produced by either the 15 or 30 mg/kg dose of PLZ ($P > 0.05$ for all comparisons). The magnitude of the PLZ-induced elevation in extracellular GABA levels increased significantly over time. That is, for both doses of PLZ, extracellular GABA levels were higher at several later post-injection sampling intervals relative to earlier ones ($P < 0.05$ for all comparisons).

3.4. Effects of PEH on extracellular GABA levels in the caudate-putamen

The results of the histological analyses indicated that 6 rats had cannulae located in the region of the caudate-putamen (Fig. 4A). All of the rats in this experiment participated in two microdialysis sessions. In one session they were given vehicle and in the other they were given PEH. At least 5 days separated the two sessions, and the order of the treatments was counterbalanced.

The administration of PEH increased extracellular GABA levels in the caudate-putamen in a time-dependent manner, $F(10,110) = 7.75$; $P < 0.01$ (Fig. 4B). The LSD post hoc tests indicated that there were no differences in extracellular GABA levels at any time following the vehicle injection ($P > 0.05$ for all comparisons). In comparison with rats given vehicle, rats given PEH had significantly higher extracellular GABA levels ($P < 0.05$ for all comparisons). Also, the magnitude of the PEH-induced elevation in extracellular GABA levels significantly increased over time. Extracellular GABA levels were higher at several later post-injection sampling periods relative to earlier ones ($P < 0.05$ for all comparisons).

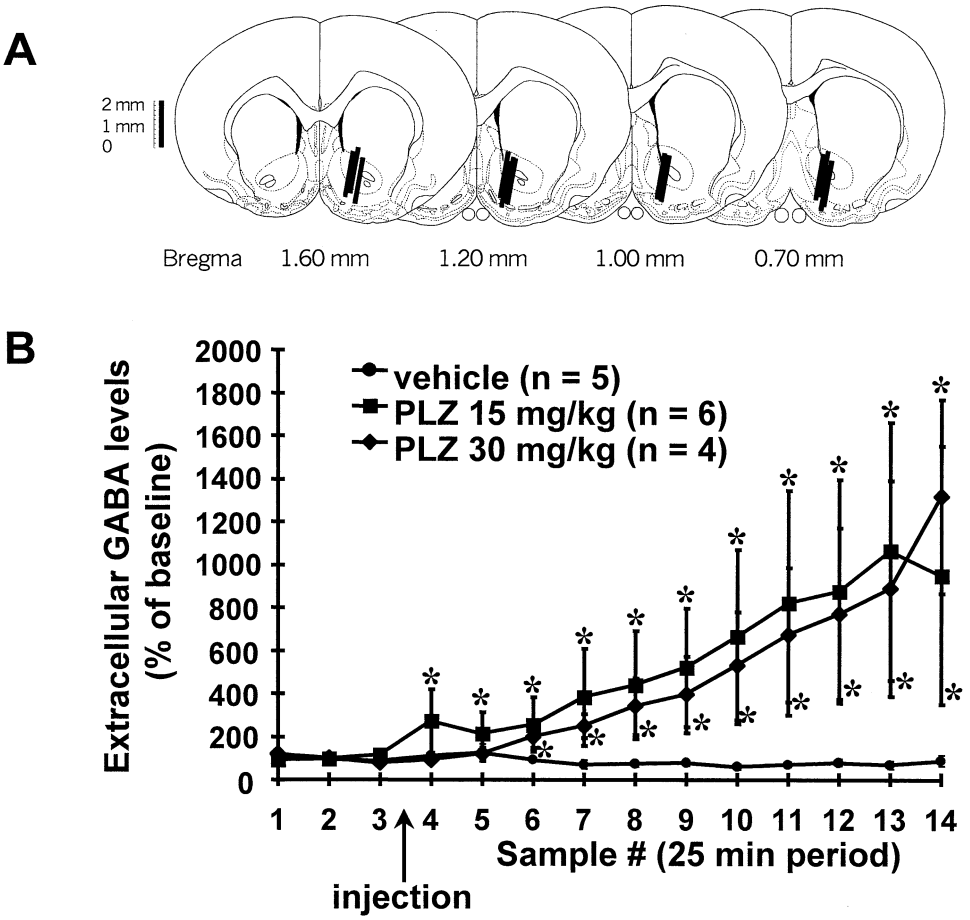

Fig. 3. (A) Approximate distribution of microdialysis probes in the nucleus accumbens. Atlas plates were adapted from Ref. 40. The anteroposterior planes are in millimeters, relative to bregma. (B) Effects of intraperitoneal administration of vehicle or PLZ (15 or 30 mg/kg, free base weight) on extracellular GABA levels in the nucleus accumbens. Values are means ± SEM. Baseline values for all three groups combined = 1.027 ± 0.132 pmol/25 μL, not corrected for probe recovery. Key: (*) $P < 0.05$ vs vehicle—same sample period.

4. Discussion

The present findings demonstrated that acute administration of the antidepressant/antipanic drug PLZ increases extracellular GABA levels in the caudate-putamen and nucleus accumbens. Also, the results showed that PEH, a putative metabolite of PLZ that does not inhibit MAO, mimics the effects of PLZ, and produces large increases in GABA levels in the caudate-putamen. The present findings add to the growing body of evidence indicating that PLZ dramatically increases GABA levels in the brain [2,3,9,12, 16,21]. However, in comparison with assays in homogenates of brain tissue, measures of GABA levels in dialysates provide a more dynamic measure of activity in GABAergic neurons, because dialysis provides a continuous measure of the net activity of release and clearance mechanisms in the extracellular space. To our knowledge, the present findings are the first to show that PLZ- and PEH-induced increases in brain GABA occur, at least in part, at the extracellular level and, moreover, that they occur in a conscious, freely-moving rat. The present results demonstrate that the effects of PLZ on extracellular GABA levels are observed in both the nucleus accumbens and caudate-putamen. We are confident that we were able to dissociate the sampling of these two closely situated areas of the striatum, because the effects of PLZ were not identical in both regions. Specifically, the 15 mg/kg dose of PLZ produced a larger increase in extracellular GABA levels in the nucleus accumbens than it did in the caudate-putamen. Also, others have shown that different populations of GABA neurons exist in the dorsal and ventral striatum [30], and that the effects of various manipulations on GABA levels in dialysates collected from the caudate-putamen and nucleus accumbens are dissociable [43–46].

The present results also demonstrated that PLZ rapidly increases extracellular GABA levels. In both brain regions, PLZ-induced increases in extracellular GABA levels were observed in the first 25-min dialysis sample collected following PLZ administration. Increases in whole brain GABA levels have been reported to occur as soon as 1 hr following one injection of PLZ [2,12]. We recently demonstrated that one injection of PLZ (30 mg/kg) increases whole brain GABA levels 6 and 24 hr after injection [3].

The present findings suggest that PEH does not com-

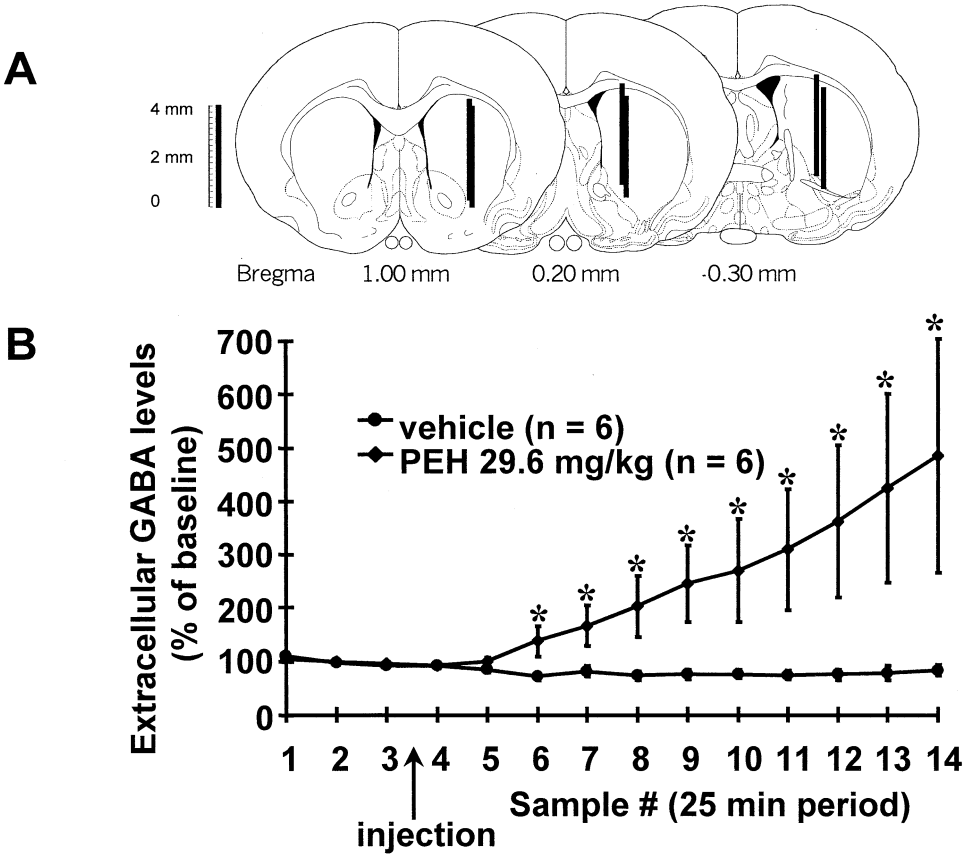

Fig. 4. (A) Approximate distribution of microdialysis probes in the caudate-putamen of rats given sunflower oil or PEH. Atlas plates were adapted from Ref. 40. The anteroposterior planes are in millimeters, relative to bregma. (B) Effects of intraperitoneal administration of vehicle or PEH (29.6 mg/kg, free base weight) on extracellular GABA levels in the caudate-putamen. Values are means ± SEM. Baseline values for both groups combined = 1.744 ± 0.163 pmol/25 μL, not corrected for probe recovery. Key: (*) $P < 0.05$ vs vehicle—same sampling period.

pletely mimic the effects of PLZ. For example, PLZ produced larger increases in extracellular GABA levels than did PEH. Also, the effects of PLZ on extracellular GABA levels were observed sooner than were those of PEH. Nonetheless, the fact that PEH does increase GABA levels may provide insights into the mechanisms underlying the effects of PLZ. The free hydrazine component in PLZ appears to be essential to the GABA-elevating properties of PLZ [47,48]. Hydrazine itself elevates GABA and inhibits GABA-T [49]. PEH maintains the hydrazine portion present in the parent molecule and elevates GABA ([23], present findings). PLZ and N^2-acetylPLZ differ by substitution on the hydrazine moiety [2], and N^2-acetylPLZ does not increase brain GABA levels [2,9]. Similarly, the MAO-inhibiting antidepressants iproniazid and nialamide, which have substitutions on the hydrazine group, do not inhibit GABA-T or increase GABA levels in the brain [47]. Tranylcypromine, a commonly used MAO inhibitor that does not have a hydrazine group, also does not produce an elevation of GABA levels after acute or chronic administration [15,21,50].

The present data do not allow us to draw conclusions regarding the source of the increase in extracellular GABA levels that is observed following PLZ administration. Increases in GABA may be derived from impulse-dependent vesicular stores, from a metabolic pool that can be glial or neuronal, and possibly from reversal of GABA re-uptake [51]. The findings of previous *in vivo* microdialysis studies indicate that evoked increases in extracellular GABA levels measured from the caudate-putamen and nucleus accumbens of conscious rats are prevented, in part, by manipulations that prevent neural impulses and calcium-dependent processes [52–55]. In a very recent study on rat hippocampal neurons in primary cell culture, it was concluded that inhibition of GABA-T induces spontaneous and enhances depolarization-evoked GABA efflux through reversal of the GABA transporter system [56]. It is likely that a portion of the PLZ-induced increases in GABA is also derived from glial sources and that perfusing the microdialysis probe with the reversible glial metabolic inhibitor fluorocitrate [57] would also partially prevent the effects of PLZ. Regardless of whether future findings reveal that the source of PLZ-induced increases in GABA are glial or neuronal, the results will be physiologically relevant because they will help determine how PLZ augments extracellular GABA levels.

In summary, the present findings demonstrated that the antipanic/antidepressant drug PLZ produces large increases in extracellular levels of GABA in the caudate-putamen and nucleus accumbens of conscious rats. In addition, we have

shown that PEH, a putative metabolite of PLZ, also increases extracellular levels of GABA in the caudate-putamen. Future studies will be directed at determining whether these increases participate in the therapeutic effects of PLZ. We have recently obtained evidence indicating that PLZ also affects memory storage in a task-dependent manner. Specifically, PLZ enhances shock avoidance on a memory test, but impairs spatial water maze retention performance [13]. Given the extensive evidence indicating that the GABAergic neurotransmitter system is involved in memory processes [58–60], it is also possible that changes in striatal GABA participate in the effects of PLZ on memory.

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