Biochemical Pharmacology, Vol. 59, pp. 1253–1263, 2000.
© 2000 Elsevier Science Inc. All rights reserved.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00244-6

Time-Dependent Changes in Brain Monoamine Oxidase Activity and in Brain Levels of Monoamines and Amino Acids Following Acute Administration of the Antidepressant/Antipanic Drug Phenelzine

Marise B. Parent,†‡ Marie K. Habib* and Glen B. Baker*†§*

Departments of *Psychology and †Psychiatry (§Neurochemical Research Unit), University of Alberta, Edmonton, Alberta, Canada T6G 2E9

ABSTRACT. Phenelzine (PLZ) is a non-selective monoamine oxidase (MAO) inhibitor commonly used to treat depression and panic disorder. Acute administration of PLZ produces several neurochemical changes, including an increase in brain levels of the catecholamines norepinephrine (NE) and dopamine (DA), of 5-hydroxytryptamine (5-HT), and of the amino acids alanine and γ-aminobutyric acid (GABA). The goal of the present series of experiments was to characterize the time course of these PLZ-induced changes. Male Sprague–Dawley rats were sacrificed 6, 24, 48, 96, 168, or 336 hr after acute PLZ administration (15 or 30 mg/kg, i.p., based on free base weight). Whole brain levels of monoamines and amino acids were determined using HPLC, and MAO A and B activities were determined using a radiochemical procedure. The results indicated that PLZ changed amino acid levels 6 and 24 hr after injection, but not 48 hr later. In contrast, the effects of PLZ on MAO activity and monoamines were longer-lasting. For example, PLZ-induced increases in dopamine and 5-HT were observed 1 week after injection, and PLZ-induced inhibition of MAO activity persisted for 2 weeks. Thus, in addition to demonstrating that the effects of PLZ on MAO activity and monoamines were long-lasting, these results indicate that the effects of PLZ on MAO activity and on brain levels of monoamines and amino acids are temporally dissociated. These findings regarding the long-term effects of PLZ on neurochemistry will have considerable critical implications for the design and interpretation of behavioral studies of the acute effects of PLZ. BIOCHEM PHARMACOL **59**;10:1253–1263, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. phenelzine; antidepressants; monoamine oxidase; amino acids; monoamines; panic disorder

PLZ‖ is a potent, long-term inhibitor of MAO. In humans, it is commonly used in the treatment of psychiatric disorders, such as panic disorder and depression, particularly depression associated with anxiety, panic, and phobia [1–3]. In rodents, PLZ has antipanic and anxiolytic effects in the mouse defense test battery and the elevated plus maze [4, 5]. 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 [6].

As expected based on its MAO-inhibiting properties, PLZ increases brain levels of the monoamines DA, NE, and 5-HT [7–11]. Interestingly, PLZ also produces large increases in rat brain levels of the amino acids GABA and alanine and decreases levels of the amino acid glutamine; these changes have been reported in hypothalamus, frontal cortex, and whole brain [10, 12–18]. The exact mechanisms through which PLZ increases alanine and GABA levels are not understood completely, although the effects appear to involve, at least in part, inhibition of the enzymes that break down GABA and alanine, GABA-T and ALA-T, respectively [12–18]. PLZ is a remarkable drug in that in addition to being an MAO inhibitor, it is also a substrate for MAO [19–22]. PLZ-induced increases in GABA, alanine, and glutamine are blocked by prior treatment with other MAO inhibitors, a finding that suggests that a PLZ metabolite produced by the action of MAO on PLZ mediates the effect of PLZ on levels of these amino acids [13, 17, 18].

PLZ is an irreversible MAO inhibitor, inhibiting both MAO-A and MAO-B as soon as 1 hr after administration [4, 10, 15]. The degree of inhibition remains virtually unchanged 24 hr later [15]; however, the extent to which MAO remains inhibited by PLZ beyond 24 hr apparently has not been established. Similarly, information indicating

‡ Corresponding author: Dr. Marise B. Parent, University of Alberta, Department of Psychology, P-220 Biological Sciences, Edmonton, Alberta, Canada T6G 2E9. Tel. (780) 492-5268; FAX (780) 492-1768; E-mail: mparent@ualberta.ca

‖ *Abbreviations*: PLZ, phenelzine (2-phenylethylhydrazine); MAO, monoamine oxidase; DA, dopamine; NE, norepinephrine; 5-HT, serotonin (5-hydroxytryptamine); GABA, γ-aminobutyric acid; GABA-T, GABA transaminase; ALA-T, alanine transaminase; HPLC-EC, HPLC with electrochemical detection; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindole-3-acetic acid; and NMDA, *N*-methyl-D-aspartate.

Received 13 July 1999; accepted 8 October 1999.

whether PLZ affects monoamines and amino acids beyond 24 hr after a single injection is also not available. Data regarding the duration of the effects of PLZ on neurochemistry are crucial for experiments examining the effects of acute administration of PLZ on behavior. For example, we recently examined the effects of acute administration of PLZ on learning and memory in rats [6]. In this study, shock avoidance was assessed hours and days after PLZ administration, and the effects of PLZ on water maze performance were examined in the same rats 4–10 days later. Information regarding the long-term effects of PLZ on neurochemistry would have allowed us to know if the neurochemical effects of PLZ persisted when PLZ was re-administered and when behavior was re-tested. Consequently, the goal of the present experiment was to determine the duration of the neurochemical changes produced by acute PLZ administration. The profile of PLZ-induced changes in brain MAO inhibition and in brain levels of monoamines and amino acids was examined in different rats 6 hr, 1 day, 2 days, 4 days, 1 week, and 2 weeks following administration of a single dose of PLZ.

MATERIALS AND METHODS

All animal procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Subjects

Male Sprague–Dawley rats (250–300 g; Ellerslie) were used. The rats were housed two per cage in a temperature-controlled (19–21°) animal room with a 12-hr light/dark cycle. The rats were given free access to food (Purina Rat Chow) and tap water and were kept in the animal room under these conditions for at least 4 days prior to the injections. Also, the rats were handled for 3 min 1–3 days prior to the injections.

Drug Administration and Procedure

PLZ sulfate (Sigma) was dissolved in distilled water and injected i.p. The doses of PLZ (15 or 30 mg/kg) are expressed as their free bases and were selected based on their demonstrated efficacy in increasing GABA and alanine and inhibiting MAO activities in the brain [14, 15, 17]. Rats were injected randomly with either PLZ or vehicle and decapitated either 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Their brains were removed, cut in half, frozen solid immediately in isopentane on solid carbon dioxide, transferred to plastic vials, and stored at −80° until the biochemical analyses were performed. The 6-hr period was selected because previous results have shown that PLZ-induced increases in NE, DA, 5-HT, GABA, and alanine would be pronounced at this time [10, 14, 15, 23].

Biochemical Analyses

For the biochemical analyses, frozen brain halves were allowed to thaw partially and were randomly assigned to be homogenized in 5 vol. of ice-cold isotonic potassium chloride; aliquots were used for MAO assays. The other half of each brain was homogenized in 5 vol. of ice-cold 0.1 N perchloric acid containing 10 mg EDTA/100 mL and 0.05 mM ascorbic acid. The homogenates were centrifuged at 12,000 *g* for 15 min at 4°, and aliquots were used for analysis of monoamines, acid metabolites, and amino acids.

Brain MAO activity was analyzed *ex vivo* by a modification of the radiochemical method of Lyles and Callingham [24]. Briefly, the homogenates were incubated with either [^{14}C]5-HT or [^{14}C]β-phenylethylamine (DuPont NEN Research Products) as the substrates for MAO-A and MAO-B, respectively. The corresponding radioactive aldehyde and acid products were extracted with a mixture of ethyl acetate and toluene. The radioactivities (dpm) were measured in a Beckman LS 6000SC scintillation counter after the addition of scintillation fluid (Ready Safe; Beckman Instruments, Inc.).

HPLC-EC was used to determine brain levels of NE, DA, 5-HT, DOPAC, and 5-HIAA. The HPLC-EC method and apparatus used are described in detail in Ref. 25.

Amino acid levels (alanine, asparagine, GABA, glutamate, glutamine, serine, and taurine) were determined using HPLC with fluorescence detection following derivatization with *o*-phthaldialdehyde. For more details see Ref. 26.

Statistical Analysis

The monoamine and amino acid data were analyzed with a two-way ANOVA with drug treatment (0, 15, or 30 mg/kg of PLZ) and drug-sacrifice interval (6 hr, 1 day, 2 days, 4 days, 1 week, or 2 weeks) as the two factors, followed by *post hoc* comparisons where appropriate. For significant interactions, *post hoc* comparisons were made between drugged rats and rats injected with vehicle and sacrificed at the same time. This resulted in 12 comparisons, and the α level ($P < 0.05$) was reduced accordingly ($P < 0.004$). The MAO data were also analyzed by two-way ANOVA with two levels of the drug treatment factor (15 and 30 mg/kg of PLZ). Independent *t*-tests were used to compare MAO activities in drug- and vehicle-injected rats 2 weeks after injection. A portion of the GABA, NE, DA, and 5-HT data for rats in the 6- and 48-hr groups has been reported previously in Ref. 6.

RESULTS

MAO Activity

Both doses of PLZ inhibited MAO-A and MAO-B activity (MAO-A: $F_{1,67} = 23.23$, $P < 0.001$; MAO-B: $F_{1,67} = 36.17$, $P < 0.001$; see Fig. 1). Six hours after PLZ was injected, MAO-A activity was inhibited 95 and 97% by

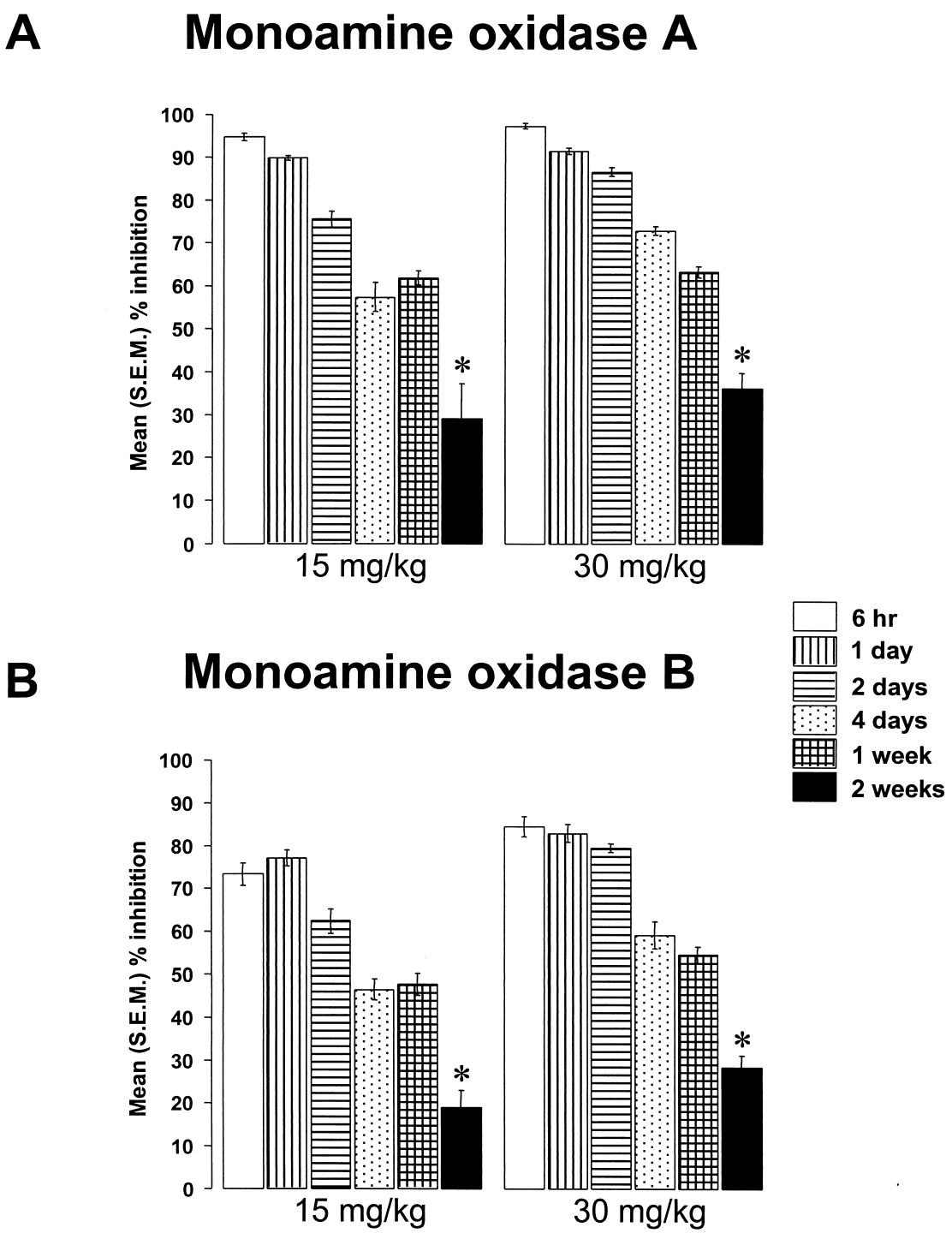

FIG. 1. Effect of acute phenelzine (15 or 30 mg/kg, i.p., based on free base weight) on brain (A) MAO-A and (B) MAO-B activities 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Results are expressed as percent inhibition compared with vehicle controls injected and sacrificed at the same time (N = 4–11 per group). Key: (*) $P < 0.05$ vs same dose − 6 hr and vehicle − 2 weeks). Combined control (vehicle) values (pmol · min^{-1} · mg^{-1} tissue) across different time points and runs were 73 ± 3 and 53 ± 2 for MAO-A and -B, respectively (means ± SEM, N = 55).

PLZ at the 15 and 30 mg/kg doses, and MAO-B activity was inhibited 73 and 84%. Averaged across all time points, the 30 mg/kg dose of PLZ produced greater inhibition of MAO-A and -B than did the 15 mg/kg dose ($P < 0.001$). PLZ-induced inhibition of MAO-A and MAO-B activity decreased as the interval between the drug injection and time of sacrifice increased (MAO-A: $F_{5,67} = 206.7$, $P < 0.001$; MAO-B: $F_{5,67} = 103.84$, $P < 0.001$). However, MAO-A and MAO-B activity remained significantly inhibited 2 weeks after administration of either dose of PLZ

(MAO-A: vehicle vs 15 mg/kg, $t_7 = 7.71$, $P < 0.001$; vehicle vs 30 mg/kg, $t_8 = 7.56$, $P < 0.01$; MAO-B: vehicle vs 15 mg/kg, $t_8 = 3.18$, $P < 0.05$; vehicle vs 30 mg/kg, $t_8 = 5.87$, $P < 0.001$).

Monoamines

PLZ increased NE, DA, and 5-HT levels (NE: $F_{2,117} = 81.223$, $P < 0.001$; DA: $F_{2,117} = 56.07$, $P < 0.001$; 5-HT: $F_{2,117} = 120.01$, $P < 0.001$; see Fig. 2). Compared with control levels, NE, DA, and 5-HT levels were higher in rats given either dose of PLZ ($P < 0.001$). The effects of PLZ were dose dependent. NE, DA, and 5-HT levels were higher in rats given 30 mg/kg of PLZ than in rats given 15 mg/kg of PLZ ($P < 0.05$). The effects of PLZ were also time dependent (NE: $F_{10,117} = 3.72$, $P < 0.001$; DA: $F_{10,117} = 3.73$, $P < 0.001$; 5-HT: $F_{10,117} = 11.006$, $P < 0.001$). Compared with rats injected with vehicle and sacrificed at the same time, NE levels were elevated significantly in rats sacrificed 6 hr, 1 day, or 2 days after either dose of PLZ was administered ($P < 0.001$). NE levels were also increased significantly 4 days after the 30 mg/kg dose of PLZ was injected ($P < 0.001$), but were not significantly different from control levels 1 or 2 weeks after injection of either dose. DA levels were increased significantly 6 hr and 1 day after the lower 15 mg/kg dose of PLZ was administered ($P < 0.001$), but were not significantly different from control levels at any subsequent time point. Following administration of the higher dose of PLZ (30 mg/kg), DA levels were elevated significantly 6 hr, 1 day, 4 days, and 1 week later ($P < 0.01$), but were not elevated significantly 2 days or 2 weeks after injection. The 15 mg/kg dose of PLZ significantly increased 5-HT levels 6 hr and 1 day after injection ($P < 0.001$), but did not produce any significant increases at subsequent time points. The higher dose of PLZ significantly increased 5-HT levels 6 hr, 1 day, 2 days, 4 days, and 1 week after injection ($P < 0.01$), but not 2 weeks later.

PLZ decreased DOPAC and 5-HIAA levels (DOPAC: $F_{2,117} = 172.754$, $P < 0.001$; 5-HIAA: $F_{2,117} = 42.885$, $P < 0.001$; see Fig. 3). Compared with levels observed in rats injected with vehicle, both doses of PLZ significantly decreased DOPAC and 5-HIAA levels ($P < 0.001$). However, the effects of PLZ on DOPAC and 5-HIAA levels did not differ as a function of dose ($P > 0.05$). The PLZ-induced decreases in DOPAC and 5-HIAA levels were time dependent (DOPAC: $F_{10,117} = 7.384$, $P < 0.001$; 5-HIAA: $F_{10,117} = 4.38$, $P < 0.001$). DOPAC levels were decreased significantly 6 hr, 1 day, 2 days, 4 days, and 1 week following injection of either dose of PLZ ($P < 0.001$), but were not different from control levels 2 weeks later. 5-HIAA levels were decreased 6 hr, 1 day, and 2 days after injection of either dose of PLZ ($P < 0.01$), but were not significantly different from control levels at subsequent time points.

Amino Acids

PLZ significantly elevated alanine, asparagine, and GABA levels in a time-dependent manner (alanine: $F_{10,117} = 35.448$, $P < 0.001$; asparagine: $F_{10,117} = 3.169$, $P < 0.01$; GABA: $F_{10,116} = 38.22$, $P < 0.001$; see Fig. 4). Alanine and GABA levels were increased significantly 6 hr after injection of either dose of PLZ ($P < 0.001$) and remained significantly increased 24 hr after injection of the 30 mg/kg dose ($P < 0.001$). Asparagine levels were increased 6 hr after injection of either dose of PLZ ($P < 0.001$), but were not elevated significantly at any subsequent time point.

PLZ administration also decreased glutamine, glycine, and serine levels significantly in a time-dependent manner (glutamine: $F_{10,117} = 5.546$, $P < 0.001$; glycine: $F_{10,117} = 2.548$, $P < 0.01$; serine: $F_{10,116} = 2.882$, $P < 0.01$; see Fig. 5). Both doses of PLZ decreased glutamine levels significantly 6 hr after injection ($P < 0.01$); however, there was no significant effect of PLZ on glutamine at the later time points. Glycine levels were depressed significantly 6 hr after the higher dose of PLZ (30 mg/kg) was administered ($P < 0.001$) and 24 hr after injection of either dose of PLZ ($P < 0.001$). Glycine levels were not different from control levels at subsequent time points. Both doses of PLZ decreased serine levels 6 hr and 1 day after injection ($P < 0.01$); however, there was no significant effect of PLZ on serine at any other time.

PLZ did not affect glutamate (dose: $F_{2,117} = 2.165$, $P > 0.05$; time: $F_{5,117} = 1.452$, $P > 0.05$; dose x time: $F_{10,117} = 0.788$, $P > 0.05$) or taurine levels (dose: $F_{2,117} = 1.192$, $P > 0.05$; time: $F_{5,117} = 1.094$, $P > 0.05$; dose x time: $F_{10,117} = 1.062$, $P > 0.05$; data not shown).

DISCUSSION

In agreement with previous results, the present findings indicate that acute administration of the antidepressant/antipanic drug PLZ affects brain MAO activity and brain levels of monoamines, their acid metabolites, and amino acids. To our knowledge, the present report is the first to assess these multiple effects of PLZ simultaneously and over such a lengthy (2-week) period. Such a period was chosen for the present study, not only because it covers the period of time in which we have conducted behavioral studies [6], but also because it represents the minimum period generally required for clinical improvement to become apparent with many antidepressants. In addition, it corresponds to the period of time required in patients for full recovery of MAO activity after withdrawal from PLZ. The results demonstrate that a single dose of PLZ significantly inhibited brain MAO-A and -B activity, increased brain levels of alanine, asparagine, GABA, NE, DA, and 5-HT, and decreased brain levels of glutamine, glycine, serine, and the amine metabolites DOPAC and 5-HIAA.

Importantly, the findings show that the effects of PLZ on MAO activity, monoamines, and amino acids were temporally dissociated. PLZ-induced changes in MAO activity

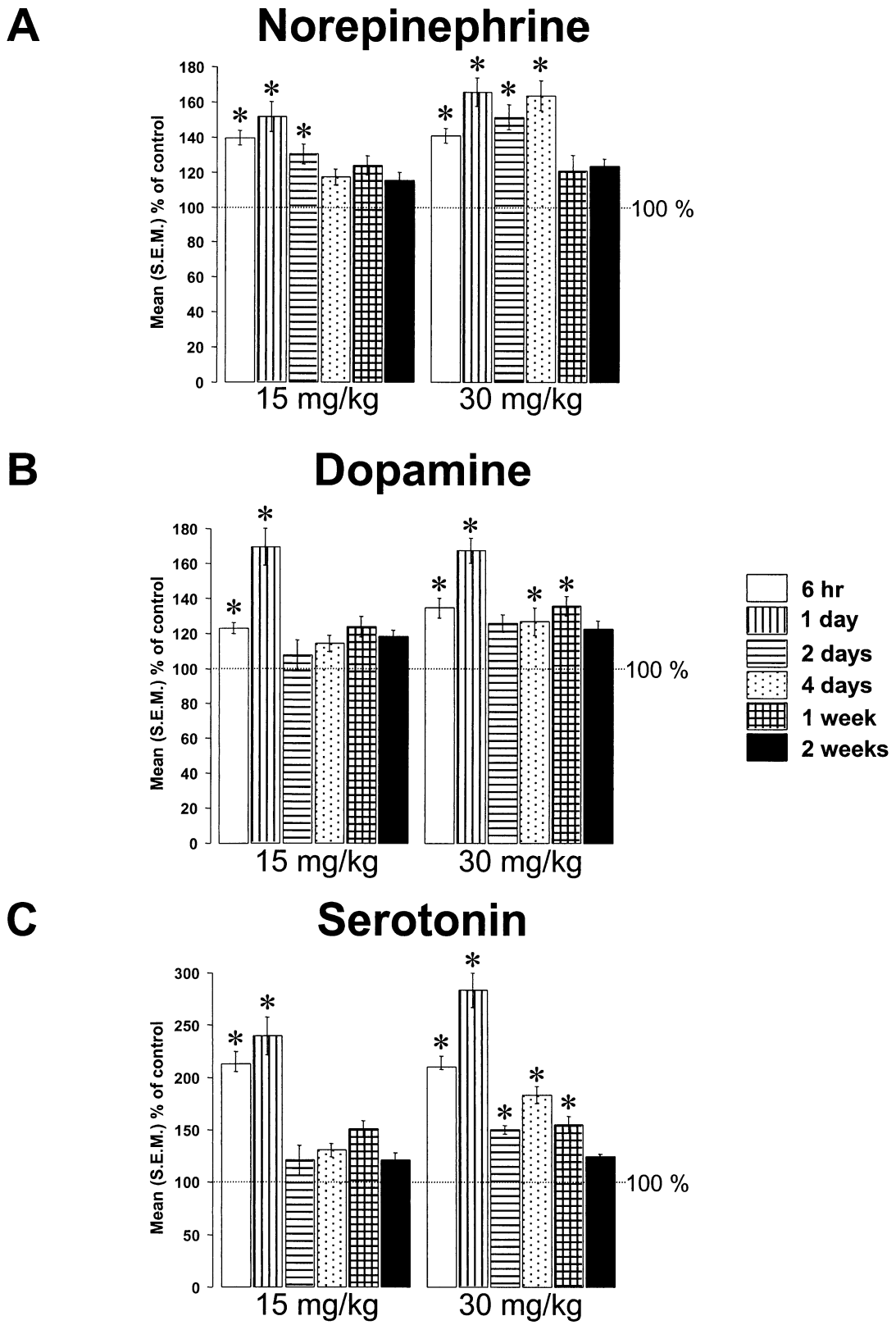

FIG. 2. Effect of acute phenelzine (15 or 30 mg/kg, i.p., based on free base weight) on whole brain levels of (A) NE, (B) DA, and (C) 5-HT (serotonin) 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Results are expressed as percent of vehicle controls injected and sacrificed at the same time (N = 5–11 per group). Key: (*) $P < 0.01$ vs vehicle − same time. Combined control (vehicle) values across different time points and runs were 273 ± 9 ng/g for NE, 618 ± 20 ng/g for DA, and 318 ± 13 ng/g for 5-HT (means ± SEM, N = 55). The 48-hr data and a portion of the 6-hr data were reported previously in Ref. 6.

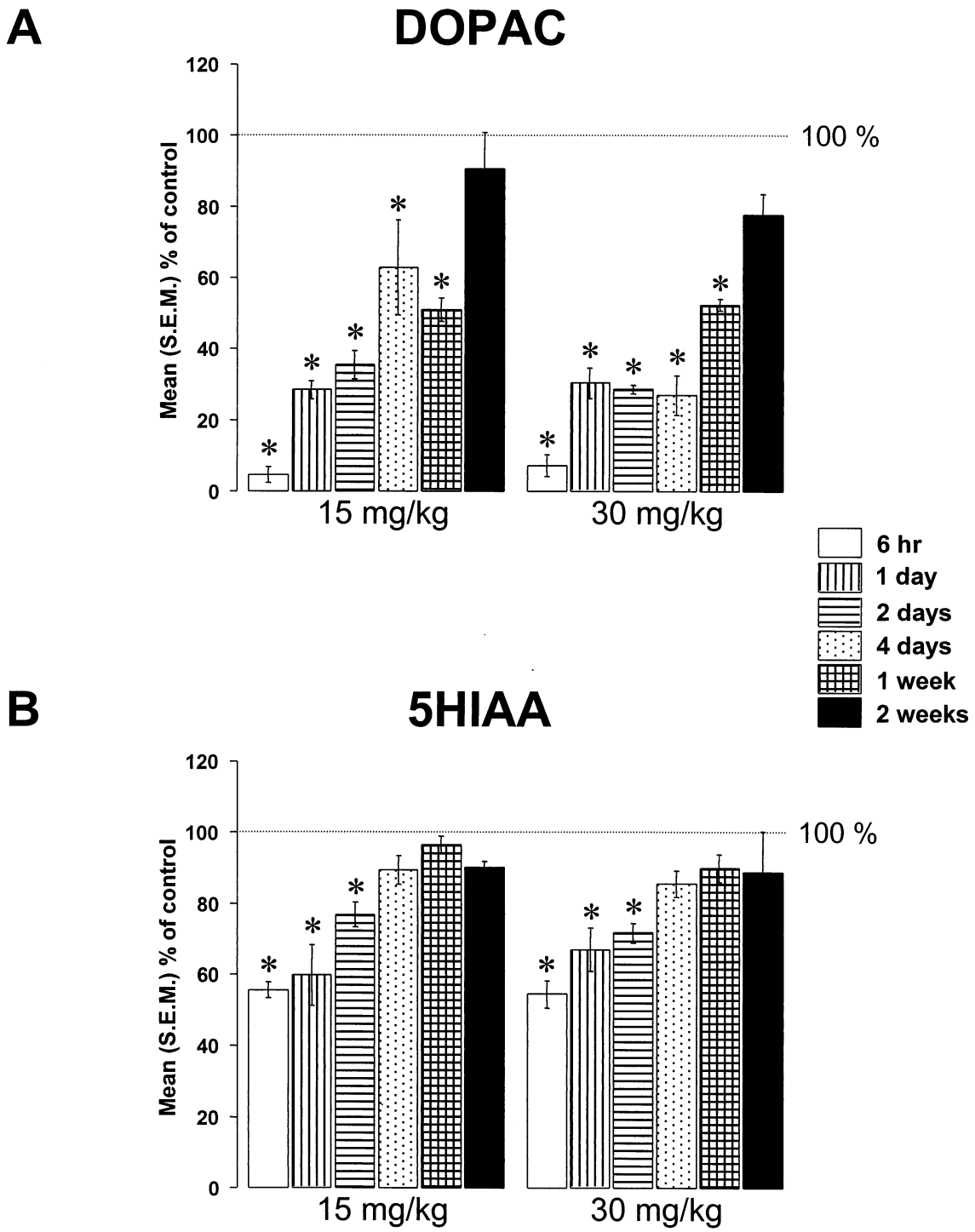

FIG. 3. Effect of acute phenelzine (15 or 30 mg/kg, i.p., based on free base weight) on whole brain levels of (A) DOPAC and (B) 5-HIAA 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Results are expressed as percent of vehicle controls injected and sacrificed at the same time (N = 5–11 per group). Key: (*) $P < 0.01$ vs vehicle − same time. Combined control (vehicle) values across different time points and runs were 119 ± 7 ng/g for DOPAC and 317 ± 16 ng/g for 5-HIAA (means ± SEM, N = 55).

and in monoamine levels were more prolonged than the effects of PLZ on amino acids. Specifically, the changes in the amino acids asparagine, alanine, GABA, and glutamine that were observed 6 hr and 1 day after one injection of PLZ were no longer evident 48 hr later. In contrast, MAO-A and -B activities remained significantly inhibited 2 weeks after PLZ was injected, and DA and 5-HT levels were elevated for at least 1 week. Also, NE levels were elevated significantly for at least 4 days. This temporal dissociation between the effects of PLZ on amino acids and monoamines is likely related to

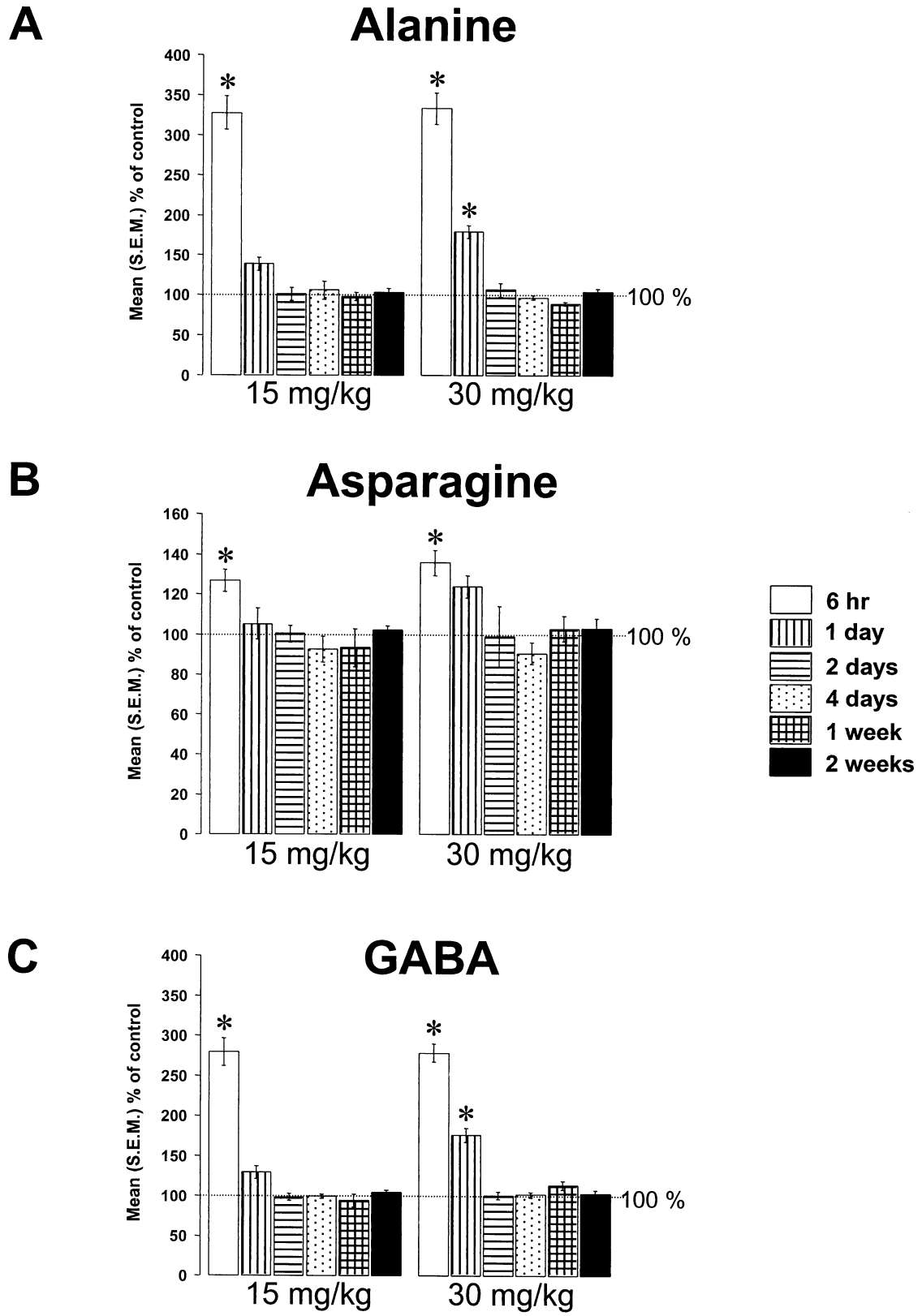

FIG. 4. Effect of acute phenelzine (15 or 30 mg/kg, i.p., based on free base weight) on whole brain levels of (A) alanine, (B) asparagine, and (C) GABA 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Results are expressed as percent of vehicle controls injected and sacrificed at the same time (N = 5–11 per group). Key: (*) $P < 0.001$ vs vehicle − same time. Combined control (vehicle) values across different time points and runs were 62 ± 2 μg/g for alanine, 33 ± 6 μg/g for asparagine, and 317 ± 13 μg/g for GABA (means ± SEM, N = 54–55). The 48-hr GABA data and a portion of the 6-hr GABA data were reported previously in Ref. 6.

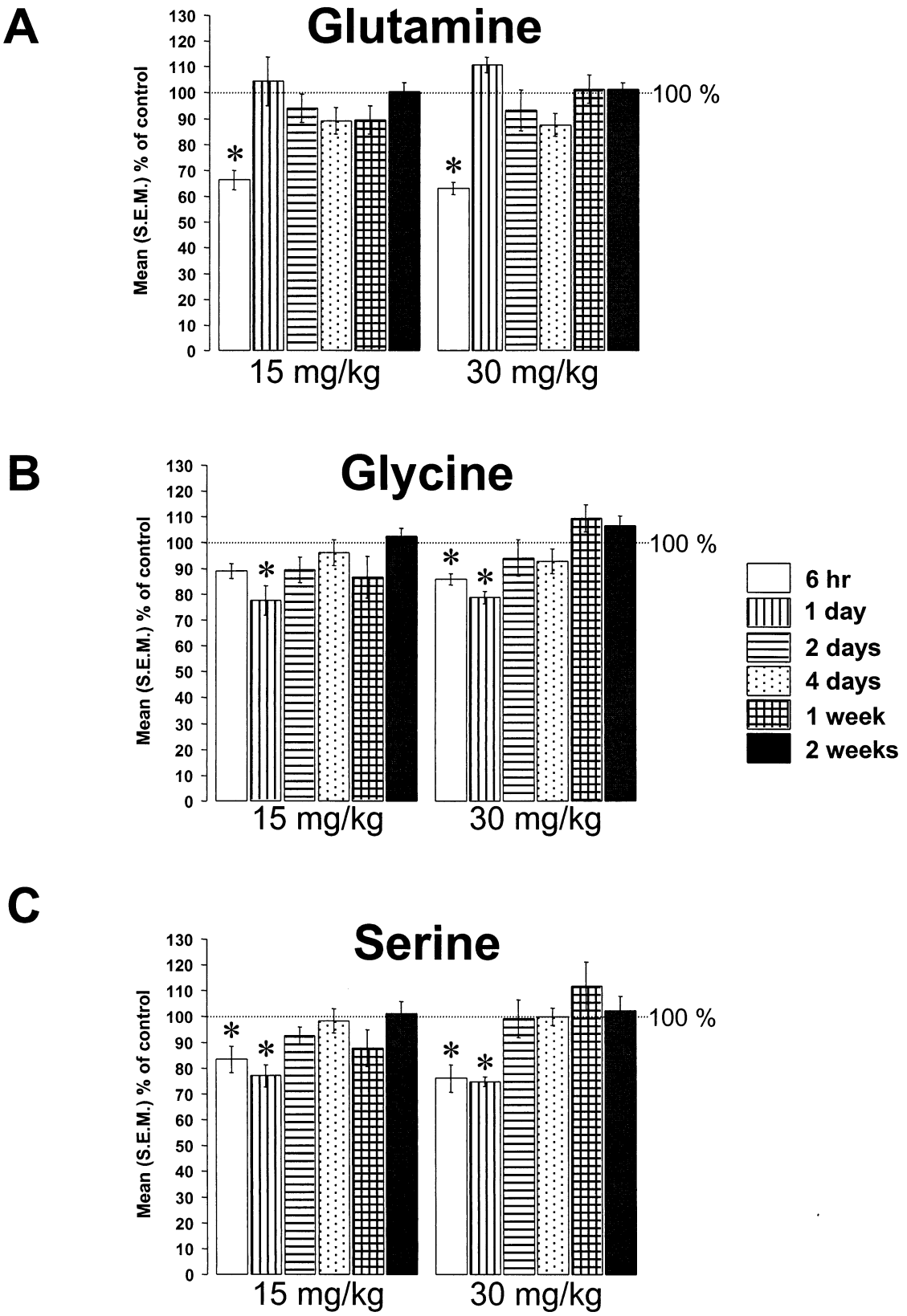

FIG. 5. Effect of acute phenelzine (15 or 30 mg/kg, i.p., based on free base weight) on whole brain levels of (A) glutamine, (B) glycine, and (C) serine 6 hr, 24 hr (1 day), 48 hr (2 days), 96 hr (4 days), 168 hr (1 week), or 336 hr (2 weeks) after injection. Results are expressed as percent of vehicle controls injected and sacrificed at the same time (N = 5–11 per group). Key: (*) $P < 0.01$ vs vehicle − same time. Combined control (vehicle) values across different time points and runs were 689 ± 19 μg/g for glutamine, 123 ± 3 μg/g for glycine, and 97 ± 2 μg/g for serine (means ± SEM, N = 55).

differences in the degree to which PLZ administration inhibits the enzymes that degrade monoamines and amino acids. PLZ inhibits MAO activity to a greater extent than it does GABA-T and ALA-T activity [10, 13, 16].

The present findings add to the growing body of evidence indicating that PLZ produces dramatic increases in whole brain levels of GABA [5, 10, 15, 17, 18]. Combined with previous findings [15], the present findings indicate that PLZ elevates whole brain levels of GABA for at least 24 hr, but less than 48 hr after injection. These PLZ-induced increases in GABA may play an important role in the therapeutic effects of PLZ in panic disorder [27]. In rodents, the anxiolytic effects of PLZ in the plus maze are not mimicked by the *N*-acetylated metabolite of PLZ, N^2-acetylphenelzine [5]. These findings implicate PLZ-induced increases in GABA in the anxiolytic effects of PLZ because N^2-acetylphenelzine inhibits MAO and increases brain levels of biogenic amines, but does not increase brain GABA levels [10, 28].

The present findings also corroborate previous results indicating that PLZ produces large increases in whole brain levels of alanine [10, 14]. The implications of these PLZ-induced increases in alanine are unclear because there is limited information available on the role of alanine in the central nervous system. Alanine is related metabolically to lactate, a substance that can produce panic attacks [28]. If PLZ-induced increases in alanine result in decreases in levels of lactate, then alanine may participate in the antipanic effects of PLZ [14]. Alanine levels are increased by the convulsant pentamethylenetetrazole [29], hypocapnia [30], hypoxia [31], seizures [32], and ischemia [33]. Evidence suggests that alanine may serve as a source of glutamate during recovery from ischemia and hypoxia [34]. Alanine can activate NMDA receptors in a manner analogous to glycine, although it is less potent than glycine [35]. Also, alanine may contribute to the decreases in glutamine that are observed following PLZ administration ([17] and present findings), because alanine can inhibit glutamine synthetase [36]. This decrease in glutamine could, in turn, be related to the increases in GABA or asparagine that are observed following PLZ administration. Glutamine is an important GABA precursor [37, 38], and glutamine also contributes to the biosynthesis of asparagine [39].

In addition to the previously reported changes in GABA, glutamine, and alanine, the present findings are also the first to show that acute administration of PLZ elevates whole brain levels of asparagine, although the implications of these increases for physiological function are unclear. Asparagine apparently does not act as a neurotransmitter, but it can contribute to the biosynthesis of the excitatory neurotransmitter aspartate [39]. Asparagine is also critically involved in protein synthesis. Decreases in brain temperature lead to decreases in asparagine levels, possibly reflecting a decrease in protein synthesis [40]. Asparagine plays a critical role in post-translational modification of proteins to form glycoproteins, and it is through this *N*-glycosylation that asparagine residues are involved in functional control of neural cell adhesion molecules [41, 42]. Finally, asparagine residues are also presumed to be involved in controlling calcium permeation and magnesium channel blockade in NMDA receptors [43].

The findings are also the first, to our knowledge, to show that PLZ decreases whole brain levels of the amino acid serine. The consequences of these decreases are not apparent, although serine is metabolized in the body to glycine [44, 45]. High levels of serine are associated with seizures [46] and Huntington's disease [47]. The brain contains surprisingly high concentrations of D-serine, which is derived from glial cells and may serve as an intrinsic modulator of the NMDA receptor glycine site [48–51]. Through the supply of L-serine, astroglial cells contribute to cerebral development and neuronal survival [52, 53]. Given that the HPLC assay used in the present experiment does not separate the D and L enantiomers of the amino acids, it is not clear whether the observed decrease reflects a decrease in L-serine, D-serine, or both.

The findings confirm previous results indicating that a threshold value of MAO inhibition (>85%) appears to be required to produce changes in brain levels of catecholamines [54], whereas 5-HT concentrations can be elevated at levels of inhibition of MAO as low as 65% [55]. We observed that NE, 5-HT, 5-HIAA, and DOPAC returned to baseline levels at the later time points, even though MAO activity remained significantly inhibited at these times. Our findings are also in agreement with previous results indicating that PLZ is a potent nonspecific inhibitor of both forms of MAO, that PLZ is slightly more potent at inhibiting MAO-A than MAO-B, and that PLZ inhibits MAO activity in a dose-dependent manner [4, 5, 7, 9, 10, 15, 56]. The present results extend these previous findings by demonstrating that PLZ-induced inhibition of MAO is long-lasting. Although MAOs remained inhibited 2 weeks after a single injection of PLZ, the degree of inhibition was lower than that observed at earlier time points.

The present findings support the contention that the MAO-inhibiting effects of PLZ are independent of its effects on amino acids, such as GABA [13, 18]. Although MAO levels were inhibited by greater than 80% 2 days following administration of the higher dose of PLZ, GABA and alanine levels were at baseline at this point. This interpretation is compatible with previous results indicating that the dose-dependent effects of PLZ on MAO and GABA are dissociated. Specifically, low doses of PLZ (2.5 mg/kg) that inhibit MAO activity (MAO-A, ~83%; MAO-B, ~58%) do not affect GABA levels [15].

In conclusion, the results demonstrated, for the first time to our knowledge, that acute administration of the antipanic/antidepressant drug PLZ produces changes in MAO activity and monoamines that persist for as long as 2 weeks. In contrast, the effects of PLZ on brain levels of amino acids, including GABA and alanine, were relatively short-lived (less than 48 hr). These findings regarding the long-term effects of PLZ on neurochemistry will have

considerable critical implications for the design and interpretation of behavioral studies of the acute effects of PLZ.

We are grateful for the invaluable technical assistance provided by Gail Rauw and Rick Strel and the statistical advice provided by Dr. Connie Varnhagen. This research was supported by grants from NSERC (OGP0194532 and EGP0195857) to M. B. P. and from the Medical Research Council of Canada (MT-12235) and the George and Dorothy Davey Endowment Fund to G. B. B.

References

1. Ashok RB and Sheehan DV, Somatic treatment strategies in panic disorder. In: *Panic Disorder: Clinical, Biological, and Treatment Aspects* (Eds. Asnis GM and van Praag HM), pp. 279–313. Wiley, New York, 1995.
2. Ballenger JC, Pharmacotherapy of the panic disorders. *J Clin Psychiatry* **47**(Suppl): 27–32, 1986.
3. Johnson MR, Lydiard RB and Ballenger JC, MAOIs in panic disorder and agoraphobia. In: *Clinical Advances in Monoamine Oxidase Inhibitor Therapies* (Ed. Kennedy SH), pp. 205–224. American Psychiatric Press, Washington, DC, 1994.
4. Griebel G, Curet O, Perrault G and Sanger DJ, Behavioral effects of phenelzine in an experimental model for screening anxiolytic and anti-panic drugs: Correlation with changes in monoamine-oxidase activity and monoamine levels. *Neuropharmacology* **37**: 927–935, 1998.
5. Paslawski T, Treit D, Baker GB, George M and Coutts RT, The antidepressant drug phenelzine produces antianxiety effects in the plus-maze and increases in rat brain GABA. *Psychopharmacology (Berl)* **127**: 19–24, 1996.
6. Parent MB, Habib MK and Baker GB, Task-dependent effects of the antidepressant/antipanic drug phenelzine on memory. *Psychopharmacology (Berl)* **142**: 280–288, 1999.
7. Baker GB, LeGatt DF, Coutts RT and Dewhurst WG, Rat brain concentrations of 5-hydroxytryptamine following acute and chronic administration of MAO-inhibiting antidepressants. *Prog Neuropsychopharmacol Biol Psychiatry* **8**: 653–656, 1984.
8. Baker GB, Greenshaw AJ and Coutts RT, Chronic administration of monoamine oxidase inhibitors: Implications for interactions between trace amines and catecholamines. In: *Progress in Catecholamine Research, Part A: Basic Aspects and Peripheral Mechanisms* (Eds. Dahlstrom A, Belmaker RH and Sandler M), pp. 569–572. Alan R. Liss, New York, 1988.
9. Blier P, de Montigny C and Azzaro AJ, Modification of serotonergic and noradrenergic neurotransmissions by repeated administration of monoamine oxidase inhibitors. Electrophysiological studies in rat central nervous system. *J Pharmacol Exp Ther* **237**: 987–994, 1986.
10. McKenna KF, Baker GB and Coutts RT, N^2-Acetylphenelzine: Effects on rat brain GABA, alanine, and biogenic amines. *Naunyn Schmiedebergs Arch Pharmacol* **343**: 478–482, 1991.
11. McKim RH, Calverley DG, Dewhurst WG and Baker GB, Regional concentrations of cerebral amines: Effects of tranylcypromine and phenelzine. *Prog Neuropsychopharmacol Biol Psychiatry* **7**: 783–786, 1983.
12. Perry TL and Hansen S, Sustained drug-induced elevation of brain GABA in the rat. *J Neurochem* **21**: 1167–1175, 1973.
13. Popov N and Matthies H, Some effects of monoamine oxidase inhibitors on the metabolism of γ-aminobutyric acid in rat brain. *J Neurochem* **16**: 899–907, 1969.
14. Wong JTP, Baker GB, Coutts RT and Dewhurst WG, Long-lasting elevation of alanine in brain produced by the antidepressant phenelzine. *Brain Res Bull* **25**: 179–181, 1990.
15. Baker GB, Wong JTF, Yeung JM and Coutts RT, Effects of the antidepressant phenelzine on brain levels of γ-aminobutyric acid (GABA). *J Affect Disord* **21**: 207–211, 1991.
16. McManus DJ, Baker GB, Martin IL, Greenshaw AJ and McKenna KF, Effects of the antidepressant/antipanic drug phenelzine on GABA concentrations and GABA-T activity in the rat brain. *Biochem Pharmacol* **43**: 2486–2489, 1992.
17. Paslawski TM, Sloley BD and Baker GB, Effects of the MAO inhibitor phenelzine on glutamine and GABA concentrations in rat brain. *Prog Brain Res* **106**: 181–186, 1995.
18. Todd KG and Baker GB, GABA-elevating effects of the antidepressant/antipanic drug phenelzine in brain: Effects of pretreatment with tranylcypromine, (-)-deprenyl and clorgyline. *J Affect Disord* **35**: 125–129, 1995.
19. Clineschmidt BV and Horita A, The monoamine oxidase catalyzed degradation of phenelzine-1-^{14}C, an irreversible inhibitor of monoamine oxidase—I. Studies *in vitro*. *Biochem Pharmacol* **18**: 1011–1020, 1969.
20. Clineschmidt BV and Horita A, The monoamine oxidase catalyzed degradation of phenelzine-1-^{14}C, an irreversible inhibitor of monoamine oxidase—II. Studies *in vivo*. *Biochem Pharmacol* **18**: 1021–1028, 1969.
21. Tipton KF and Spire IPC, Oxidation of 2-phenylethylhydrazine by monoamine oxidase. *Biochem Pharmacol* **21**: 268–270, 1972.
22. Yu PH and Tipton KF, Deuterium isotope effect of phenelzine on the inhibition of rat liver mitochondrial monoamine oxidase activity. *Biochem Pharmacol* **38**: 4245–4251, 1989.
23. Dewar K, Dyck LE, Durden DA and Boulton AA, Involvement of brain trace amines in the behavioural effects of phenelzine. *Neurochem Res* **13**: 113–119, 1988.
24. Lyles GA and Callingham BA, *In vitro* and *in vivo* inhibition by benserazide of clorgyline-resistant amine oxidases in rat cardiovascular tissues. *Biochem Pharmacol* **31**: 1417–1424, 1982.
25. Baker GB, Coutts RT and Rao TS, Neuropharmacological and neurochemical properties of N-(2-cyanoethyl)-2-phenylethylamine, a prodrug of 2-phenylethylamine. *Br J Pharmacol* **92**: 243–255, 1987.
26. Sloley BD, Kah O, Trudeau VL, Dulka JG and Peter RE, Amino acid neurotransmitters and dopamine in brain and pituitary of the goldfish: Involvement in the regulation of gonadotropin secretion. *J Neurochem* **58:** 2254–2262, 1992.
27. Breslow MF, Fankhauser MP, Potter RL, Meredith KE, Misiaszek J and Hope DG Jr, Role of γ-aminobutyric acid in antipanic drug efficacy. *Am J Psychiatry* **146**: 353–356, 1989.
28. Shear MK, Pathophysiology of panic: A review of pharmacologic provocative tests and naturalistic monitoring data. *J Clin Psychiatry* **47**(Suppl): 18–26, 1986.
29. Clarke DD, Lajtha AL and Maker HS, Intermediary metabolism. In, *Basic Neurochemistry* (Eds. Siegel GJ, Agranoff BW, Albers RW and Molinoff PB), 4th Edn, pp. 541–551. Raven Press, New York, 1989.
30. Norberg K, Changes in the cerebral metabolism induced by hyperventilation at different blood glucose levels. *J Neurochem* **26**: 353–359, 1976.
31. Norberg K and Siesjö BK, Cerebral metabolism in hypoxic hypoxia. II. Citric acid cycle intermediates and associated amino acids. *Brain Res* **86**: 45–54, 1975.
32. Chapman AG, Meldrum BS and Siesjö BK, Cerebral metabolic changes during prolonged epileptic seizures in rats. *J Neurochem* **28**: 1025–1035, 1977.
33. Folbergrová J, Ljunggren B, Norberg K and Siesjö BK, Influence of complete ischemia on glycolytic metabolites, citric acid cycle intermediates, and associated amino acids in the rat cerebral cortex. *Brain Res* **80**: 265–279, 1974.
34. Erecinska M, Nelson D, Nissim I, Daikhin Y and Yudkoff M,

Cerebral alanine transport and alanine aminotransferase reaction: Alanine as a source of neuronal glutamate. *J Neurochem* **62**: 1953–1964, 1994.
35. Thomson AM, Glycine modulation of the NMDA receptor-channel complex. *Trends Neurosci* **12**: 349–353, 1989.
36. Stryer L, *Biochemistry,* 2nd Edn, p. 502. W.H. Freeman, San Francisco, 1981.
37. Paulsen RE, Odden E and Fonnum F, Importance of glutamine for γ-aminobutyric acid synthesis in rat neostriatum *in vivo*. *J Neurochem* **51**: 1294–1299, 1988.
38. Peng L, Hertz L, Huang R, Sonnewald U, Petersen SB, Westergaard N, Larsson OM and Schousboe A, Utilization of glutamine and of TCA cycle constituents as precursors of transmitter glutamate and GABA. *Dev Neurosci* **15**: 367–377, 1993.
39. McGilvery RW and Goldstein GW, *Biochemistry: A Functional Approach,* 3rd Edn. W.B. Saunders, Philadelphia, 1983.
40. Conradi NG, Nystrom B, Hamberger A and Sourander P, Changes in brain temperature and free amino acids in normal and protein deprived suckling rats exposed to room temperature. *Neurochem Res* **13**: 657–661, 1988.
41. Milev P, Meyer-Puttlitz B, Margolis RK and Margolis RU, Complex-type asparagine-linked oligosaccharides on phosphacan and protein-tyrosine phosphatase-ζ/β mediate their binding to neural cell adhesion molecules and tenascin. *J Biol Chem* **270**: 24650–24653, 1995.
42. Sorkin BC, Hoffman S, Edelman GM and Cunningham BA, Sulfation and phosphorylation of the neural cell adhesion molecule. *Science* **225**: 1476–1478, 1984.
43. Masu M, Nakajima Y, Moriyoshi K, Ishii T, Akazawa C and Nakanashi S, Molecular characterization of NMDA and metabotropic glutamate receptors. *Ann NY Acad Sci* **707**: 153–164, 1993.
44. Rodwell VW, Biosynthesis of the nutritionally nonessential amino acids. In: *Harper's Biochemistry* (Eds. Murray RK, Granner DK, Mayes PA and Rodwell VW), pp. 293–298. Appleton & Lange, Norwalk, CT, 1996.
45. Yudkoff M, Diseases of amino acid metabolism. In: *Basic Neurochemistry: Molecular, Cellular, and Medical Aspects* (Eds. Siegel GJ Agranoff BW, Albers RW, Fisher SK and Uhler MD), 6th Edn, pp. 887–915. Lippincott-Raven Publishers, Philadelphia, 1999.
46. von Essen C, Rydenhag B, Nystrom B, Mozzi R, van Gelder N and Hamberger A, High levels of glycine and serine as a cause of the seizure symptoms of cavernous angiomas? *J Neurochem* **67**: 260–264, 1996.
47. Bonilla E, Prasad AL and Arrieta A, Huntington's disease: Studies on brain free amino acids. *Life Sci* **42**: 1153–1158, 1988.
48. Chouinard ML, Gaitan D and Wood PL, Presence of the *N*-methyl-D-aspartate-associated glycine receptor agonist, D-serine, in human temporal cortex: Comparison of normal, Parkinson, and Alzheimer tissues. *J Neurochem* **61**: 1561–1564, 1993.
49. Hashimoto A, Oka T and Nishikawa T, Extracellular concentration of endogenous free D-serine in the rat brain as revealed by *in vivo* microdialysis. *Neuroscience* **66**: 635–643, 1995.
50. Kumashiro S, Hashimoto A and Nishikawa T, Free D-serine in post-mortem brains and spinal cords of individuals with and without neuropsychiatric diseases. *Brain Res* **681**: 117–125, 1995.
51. Nagata Y, Horiike K and Maeda T, Distribution of free D-serine in vertebrate brains. *Brain Res* **634**: 291–295, 1994.
52. Mitoma J, Furuya S and Hirabayashi Y, A novel metabolic communication between neurons and astrocytes: Non-essential amino acid L-serine released from astrocytes is essential for developing hippocampal neurons. *Neurosci Res* **30**: 195–199, 1998.
53. Mitoma J, Kasama T, Furuya S and Hirabayashi Y, Occurrence of an unusual phospholipid, phosphatidyl-L-threonine, in cultured hippocampal neurons. Exogenous L-serine is required for the synthesis of neuronal phosphatidyl-L-serine and sphingolipids. *J Biol Chem* **273**: 19363–19366, 1998.
54. Gey KF and Pletscher A, Activity of monoamine oxidase in relation to the 5-hydroxytryptamine and norepinephrine content of the rat brain. *J Neurochem* **6**: 239–243, 1961.
55. Hampson DR, Baker GB and Coutts RT, Neurochemical changes in rat brain amines after short- and long-term inhibition of monoamine oxidase by a low dose of tranylcypromine. *Biol Psychiatry* **23**: 227–236, 1988.
56. Dyck LE, Juorio AV, Durden DA and Boulton AA, Effect of chronic deuterated and non-deuterated phenelzine on rat brain monoamines and monoamine oxidase. *Naunyn Schmiedebergs Arch Pharmacol* **337**: 279–283, 1988.