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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

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Abstract

This study investigated the effects of acute and chronic (one daily i.p. injection for 14 days) treatments with the non-selective irreversible monoamine-oxidase (MAO) inhibitor phenelzine (10 and 30 mg/kg) on defensive behaviors of Swiss mice in the mouse defense test battery (MDTB) which has been designed for screening anxiolytic and anti-panic drugs. In the MDTB, subjects were confronted with a natural threat (a rat) and situations associated with this threat. MAO-A and MAO-B activities and levels of brain monoamines (serotonin (5-HT), dopamine (DA) and norepinephrine (NE)) and their deaminated metabolites were subsequently measured. Behavioral results showed that acute administration of phenelzine did not specifically modify defensive behaviors. By contrast, after chronic treatment, phenelzine produced a significant reduction in avoidance distance when the rat was approaching, an effect which is consistent with an anti-panic-like action. In addition, phenelzine displayed weak anxiolytic-like effects as it increased risk assessment responses when mice were constrained in one part of the apparatus facing the rat which remained at a constant distance. No other specific drug effect was observed. These behavioral changes were associated with a dramatic increase in 5-HT levels, in particular after chronic treatment, while levels of DA and NE increased only slightly. Importantly, no significant differences in DA and NE levels between acute and chronic regimens were observed. Levels of deaminated metabolites of monoamines were markedly decreased. Measurements of MAO activity revealed substantial reductions in both type A and B forms with a full inhibition of both forms being observed only after chronic treatment with phenelzine. These results suggest that the effects of phenelzine may be due mainly to its effects on the 5-HT system and presumably related to the full inhibition of MAO-A and/or MAO-B.

Keywords: Phenelzine; Anxiety; Panic; Defense behaviors; Monoamines; Monoamine oxidase; Swiss mice

1. Introduction

The use of monoamine oxidase inhibitors (MAOIs) for patients with depressive disorders is well established (for recent reviews, see Laux et al., 1995; Thase et al., 1995). In addition, West and Dally (1959) were the first to report that MAOIs may also be effective in the treatment of panic disorder (PD) with agoraphobia. Since then, the efficacy of MAOIs in PD has been confirmed by numerous studies and also established in the treatment of other anxiety disorders such as social phobia, obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (for reviews, see Liebowitz et al., 1990; Buller, 1995; Priest et al., 1995). Although there is evidence of a direct relationship between the plasma concentration of MAO and the antidepressant response of MAOIs (Leonard, 1993), a precise mechanism underlying the anxiolytic effects of MAOIs has not been defined. However, the norepinephrine (NE) and the serotonin (5-HT) systems have been hypothesized by many investigators to be central in the pathogenesis of some anxiety disorders, such as OCD (Zohar and Insel, 1987) and PD (Kahn and Van Praag, 1988), thereby suggesting that the therapeutic response of MAOIs may be associated with changes in monoaminergic regulation. In particular, postsynaptic 5-HT receptor hypersensitivity and NE hyperactivity, specifically

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in the locus coeruleus, have been hypothesized to underly the central biochemical dysfunction of PD (Asnis and Van Praag, 1995; Kahn et al., 1995).

Despite the clinical efficacy of MAOIs in some anxiety disorders, these drugs do not usually show anxiolytic-like effects in experimental models of anxiety in the animal laboratory. Paslawski et al., (1996) showed that the MAOI phenelzine, given acutely, produced anxiolytic-like effects in the elevated plus-maze test in rats, but most reports show that acute and/or chronic treatment with MAOIs is without effect in anxiety models (Johnston and File, 1988; Beardslee et al., 1990; Golda and Petr, 1990; Lecci et al., 1990; de Angelis, 1997). In addition, two studies reported that the MAOIs befloxatone and moclobemide exhibited anxiolytic-like effects in the elevated plus-maze in rats and the light/dark choice test in mice, respectively (Caille et al., 1996; de Angelis, 1997).The reasons for this inconsistency in drug effects are not yet clear. It has been suggested that classical animal models of anxiety are insensitive to the action of these compounds (Beardslee et al., 1990; Griebel et al., 1997b). Most of these tests have been validated pharmacologically by benzodiazepines (BZs), which represent the first-choice treatment in generalized anxiety disorders (GAD), and it is not clear whether these models are useful when testing compounds effective in other anxiety disorders.

Recently, several novel test procedures have been described as models of PD (Fontana and Commissaris, 1988; Fontana et al., 1989; Graeff, 1991; Hendrie and Neill, 1991; Martin, 1993; Jenck et al., 1995; Molewijk et al., 1995; Griebel et al., 1996). For example, it was demonstrated that flight reactions of Swiss mice confronted with a rat may serve as an experimental model for the screening of anti-panic compounds as it meets criteria for face validity and predictive validity, normally applied to such models (Griebel et al., 1996). This test is based on the work of Blanchard et al. (1993) on antipredator defense in rats. These authors designed two test batteries measuring defensive behaviors to present, approaching predators (i.e. a cat) and reactions to potential threat. The recently developed mouse defense test battery (MDTB) combines many of the features of these tests into a single procedure, eliciting and measuring reactions to both present (i.e. a rat) and anticipated threat (Griebel et al., 1995b). Pharmacological studies demonstrated that avoidance responses elicited by the presentation of a rat are specifically reduced by compounds used in the clinical management of panic such as imipramine, fluoxetine, moclobemide and the BZs alprazolam and clonazepam (Griebel et al., 1995a,c, 1997a,b,c). Other BZs such as chlordiazepoxide, diazepam and clorazepate generally failed to affect flight responses. However, these compounds affected other defense responses such as risk assessment activities, defensive threat/attack reactions and escape attempts, thereby suggesting that these defense responses may be particularly sensitive to anti-GAD agents. On the basis of these findings it was suggested that the MDTB may be useful for the screening of both anti-panic and anti-GAD drugs (Griebel et al., 1995b, 1996).

In the present study, the MDTB was used to examine the anti-panic- and anxiolytic-like properties of the MAOI phenelzine after acute and repeated administration, and to investigate neurochemical changes which may underly the behavioral effects of this MAOI (i.e. MAO-A and MAO-B activities, endogenous NE, DA and 5-HT levels). Phenelzine was chosen because its anti-panic potential was found to be superior to other drug treatments (Ashok Raj and Sheehan, 1995). Two forms of MAO have been described, distinguished by their substrate specificity and by their selective inhibition by various MAOIs. MAO-A preferentially deaminates 5-HT, NE and epinephrine, whereas MAO-B primarily deaminates phenylethylamine. Both forms of MAO metabolize dopamine (DA) and tyramine (Youdim and Finberg, 1985). A large number of studies have shown that MAOIs increase levels of MAO substrates (NE, DA, 5-HT) in the rat brain (Waldmeier and Baumann, 1983; Da Prada et al., 1989; Kumagae et al., 1991; Curet et al., 1996).

2. Materials and methods

All procedures described here are in compliance with French legislation governing research with animals.

2.1. Animals

Subjects were naive male Swiss mice aged 9 weeks at the time of testing (28–33 g), and male Long Evans rats (400–500 g). They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed singly in standard cages (mice: $30 \times 20 \times 14$ cm; rats: $44 \times 30 \times 20$ cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (22–23°C; relative humidity: 40–65%) and kept on a 12-h light/dark cycle with light onset at 06:00 h.

2.2. Drugs

Phenelzine (synthesized by the chemistry department, Synthélabo Recherche) was prepared as suspension in physiological saline containing one or two drops of Tween 80. Mice were randomly assigned to treatment with phenelzine (10 or 30 mg/kg; $n = 8–10$), or saline ($n = 30$) for 14 days, administered intraperitoneally once daily. At 24 h after the last injection mice from the saline group were divided into three treatment groups and were injected with either saline ($n = 10$) or

phenelzine (10 or 30 mg/kg; $n = 10$). Animals treated chronically with phenelzine were injected with the same dose of phenelzine. The last injection was performed on day 15, 30 min before testing was carried out. All doses are expressed as the base and were chosen on the basis of previous results with phenelzine (Caille et al., 1996).

2.3. Behavioral effects of phenelzine in the mouse defense test battery

2.3.1. Apparatus

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall ($2.0 \times 0.30 \times 0.06$). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with the experimenter. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. The room illumination was provided by one red neon tube fixed on the ceiling and two desk lamps with red bulbs placed respectively on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lux. Experiments were performed under red light between 09:30 and 15:00 h.

2.4. Procedure

2.4.1. Effects on spontaneous locomotor activity: the pre-test (1–3 min)

Subjects were placed into the runway for a 3-min familiarization period during which line crossings and wall rears were recorded.

2.4.2. Effects on flight responses: the rat avoidance test (4–6 min)

Immediately after the 3-min familiarization period, a hand-held dead rat (killed by CO_2 inhalation) was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated five times. Mean avoidance distance (cm) was calculated for each subject.

2.4.3. Effects on risk assessment: the chase (7–8 min) and the straight alley (9–11 min) tests

The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. During the chase, the number of stops (pause in movement) was recorded. After the chase was completed, the runway was converted to a straight alley by closing a door at one end. For 30 s, the hand-held rat remained at a constant distance of 40 cm from the subject and the number of approaches/withdrawals (subject must move more than 0.2 m forward from the closed door, then return to it) were recorded. Both responses are described as risk assessment activities (Griebel et al., 1995b).

2.5. Effects on defensive threat/attack responses: the forced contact test (12–13 min)

Finally, the experimenter brought the rat up to contact the subject. For each such contact, bites and vocalizations by the subjects were noted. This was repeated three times. The results were expressed as mean number of bites and mean number of vocalizations.

2.6. Effects on contextual defense: the post-test (14–16 min)

Immediately after the forced contact test, the rat was removed and the door was opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session. See Griebel et al., 1997c for additional details on this test battery.

2.7. Determination of MAO-A and MAO-B activities

The animals were decapitated 60 min after the last administration of phenelzine or saline and the brains were dissected out and rapidly frozen. The samples were kept at -80°C until assayed. The brains were homogenised in 20 vol of ice-cold 0.1 M sodium phosphate buffer (pH 7.4). Aliquots (0.1 ml) of crude membrane suspensions were incubated with [^{14}C]5-HT (final concentration 125 μM) for 5 min and with [^{14}C]PEA (final concentration 8 μM) for 1 min in a total volume of 0.5 ml at 37°C and in one experiment with [^{14}C]TYR (final concentration 100 μM) as non specific substrate for MAO, for 4 min. The reaction was stopped with 200 μl of 4 M HCl and deaminated metabolites were extracted by vigourous shaking for 10 min with 7 ml of toluene/ethyl acetate (v/v). Following extraction, the aqueous phase was frozen with liquid nitrogen and the organic layer was poured into a scintillation vial to which 10 ml of toluene containing 0.4% (w/v) 2,5-diphenyloxazol was subsequently added. After 5 min of agitation, radioactivity was measured in a scintillation spectrometer (LS-1801, Beckmann, Irvine, CA). Blank values were obtained by adding HCl prior to the substrate.

2.8. Assay of brain levels of NE, DA, 5-HT and their related deaminated metabolites

NE, 5-HT, DA, HVA, DOPAC and 5-HIAA were measured by high pressure liquid chromatography (HPLC) with electrochemical detection. Frozen tissues were sonicated in 800 μl of 0.05 M $HClO_4$ containing 0.5 mM of EDTA, 2 mM sodium metabisulfite and 3,4-DHBA (final concentration 1 ng/50 μl) as the internal standard. After centrifugation, 50 μl of the supernatant were injected onto the liquid chromatographic column using a refrigerated (4°C) autoinjector Wisp 512 (Waters, Milford, MA). Separation was achieved at room temperature. The HPLC system consisted of a pump and a stainless steel separation column (0.46 × 7 cm) packed with an Ultrasphere XL ODS C18, 3 μm particle size (Beckman, Fullertone, CA). The mobile phase contained 0.1 M NaH_2PO_4, 1 mM EDTA, 2.5 mM octane sulfonic acid, 7% CH_3CN, pH 3.4. The flow rate was 0.9 ml/min. Electrochemical detection was carried out by means of an amperometric detector (Waters 460) with a glassy carbon working electrode and an Ag/AgCl reference electrode. The detector potential was set at +0.8 V versus the reference electrode. Concentrations of each compound were calculated using a computing integrator (Maxima, Waters) with reference calibration curves obtained after injection of standards.

2.9. Statistical analysis

Data were analysed by a one-way analysis of variance (ANOVA) (line crossings, wall rearings, avoidance distance, stops, escape attempts, monoamines levels and MAO-A/B activities) or the nonparametric Kruskal–Wallis test for some infrequently occurring or highly variable behaviors in the MDTB (approaches/withdrawals, bites and vocalizations). Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test (behavioral data), Newman–Keuls (neurochemical data) or the nonparametric Siegal and Castellan test.

3. Results

3.1. Effects on spontaneous locomotor activity: the pre-test

Table 1 shows that phenelzine significantly decreased the number of line crossings at 30 mg/kg given acutely or chronically ($F_{4,41} = 5.4$, $P < 0.01$). With respect to wall rearings, although ANOVA revealed a significant main effect ($F_{4,41} = 3.4$, $P < 0.05$), post-hoc analysis failed to show significant differences between control and drugged mice.

Table 1
Locomotor activity in the runway cage before the confrontation with the rat

Treatments	Line crossings	Wall rearings
Chronic saline	118.3 ± 10.1	5.8 ± 1.5
Acute phenelzine (10 mg/kg)	109.1 ± 8.0	8.5 ± 1.6
Acute phenelzine (30 mg/kg)	78.5 ± 10.2*	5.1 ± 1.1
Chronic phenelzine (10 mg/kg)	107.6 ± 15.1	3.3 ± 0.9
Chronic phenelzine (30 mg/kg)	59.3 ± 8.4*	2.4 ± 0.8

Phenelzine or saline was administered, i.p., once a day for 2 weeks (chronic). The last injection was given 30 min before the beginning of the test (acute).
Data represent the mean ± S.E.M.
* $P < 0.05$ (Dunnett's *t*-test).

3.2. Effects on flight responses: the rat avoidance test

Fig. 1 shows that the drug significantly modified the avoidance distance ($F_{4,36} = 17.7$, $P < 0.001$). Post-hoc analysis indicated that phenelzine significantly reduced avoidance distance after a single injection at 30 mg/kg and after repeated administration of both doses.

3.3. Effects on risk assessment: (a) chase test

Fig. 2 shows that none of the drug treatments significantly affected the number of stops during the chase ($F_{4,41} = 1.3$); (b) straight alley test: statistical analysis revealed that phenelzine given repeatedly (10 and 30 mg/kg) significantly increased the number of approaches/withdrawal responses ($K = 12.04$, $P < 0.05$).

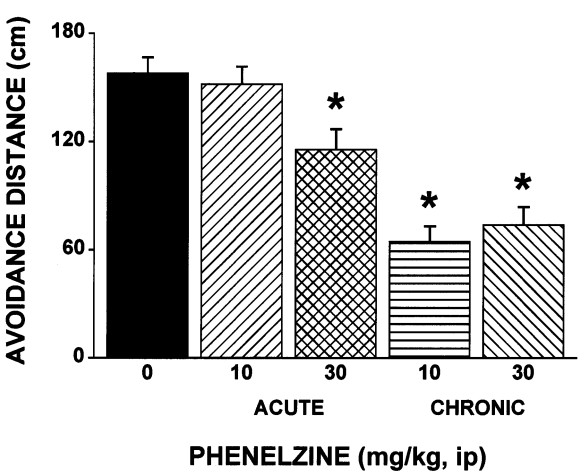

Fig. 1. Effects of acute and chronic (one daily injection for 14 days) treatments with phenelzine on a flight measure in the mouse defense test battery. Data represent the mean ± S.E.M. * $P < 0.05$ (Dunnett's *t*-test).

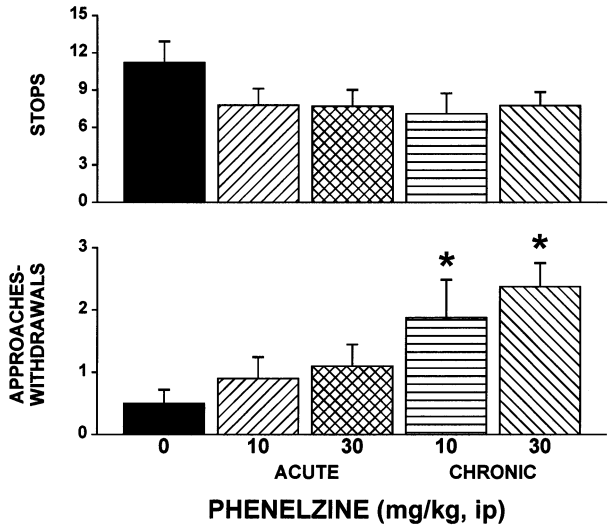

Fig. 2. Effects of acute and chronic treatments with phenelzine on two risk assessment measures in the mouse defense test battery. Data represent the mean ± S.E.M. * $P < 0.05$ (Siegal and Castellan test).

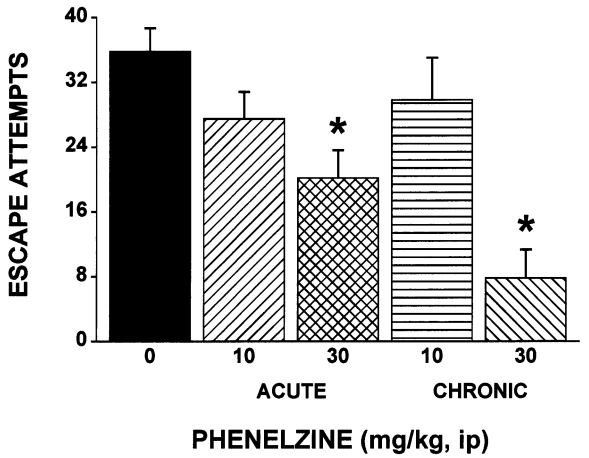

Fig. 3. Effects of acute and chronic treatments with phenelzine on escape attempts following the removal of the rat from the runway apparatus. Data represent the mean ± S.E.M. * $P < 0.05$ (Dunnett's t-test).

3.4. Effects on defensive threat/attack responses: the forced contact test

Table 2 shows that neither acute nor chronic treatment of phenelzine significantly modified bitings to the rat ($K = 2.7$) or vocalizations ($K = 5.52$).

3.5. Effects on contextual defense: the post-test

Fig. 3 shows that phenelzine significantly reduced the number of escape attempts following the removal of the rat from the runway cage ($F_{4,41} = 8.3$, $P < 0.001$) at 30 mg/kg regardless of treatment regimen, although effects were more pronounced after chronic administration

3.6. Effects on MAO-A and MAO-B activities in the mouse brain

As shown in Fig. 4, acute and chronic administration of phenelzine markedly decreased MAO-A ($F_{4,41} = 576$, $P < 0.001$) and MAO-B ($F_{4,41} = 871$, $P < 0.001$) activities in the mouse brain. While repeated administration of phenelzine decreased MAO-A and MAO-B by 98% regardless of dose, acute administration induced a dose-related inhibition of both forms of the enzyme. At 10 mg/kg of acute phenelzine, MAO-A and MAO-B activities were decreased by 93 and 74%, respectively, whereas the higher dose inhibited both forms by 98 and 90%, respectively. When compared to the acute groups, chronic phenelzine produced a significantly larger decrease in MAO-B.

Table 2
Defensive threat and attack responses of mice confronted with a rat

Treatments	Vocalizations	Bitings
Chronic saline	2.9 ± 0.1	2.5 ± 0.2
Acute phenelzine (10 mg/kg)	2.5 ± 0.3	2.2 ± 0.3
Acute phenelzine (30 mg/kg)	2.7 ± 0.3	1.7 ± 0.4
Chronic phenelzine (10 mg/kg)	3.0 ± 0.0	2.0 ± 0.5
Chronic phenelzine (30 mg/kg)	3.0 ± 0.0	1.9 ± 0.5

Phenelzine or saline was administered, i.p., once a day for 2 weeks (chronic). The last injection was given 30 min before the beginning of the test (acute).
Data represent the mean ± S.E.M.

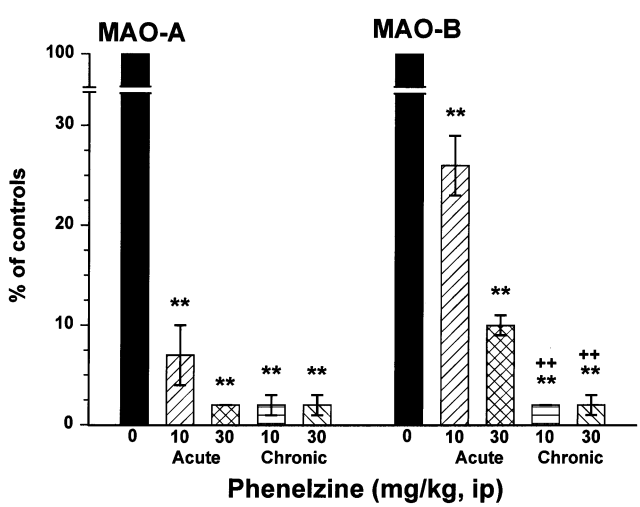

Fig. 4. Effects of acute and chronic (one daily injection for 14 days) treatments with phenelzine on MAO-A and MAO-B activities in the mouse brain. The animals were sacrificed 60 min after the last injection of saline or phenelzine. Data represent the mean ± S.E.M. ($n = 8$–10 animals per group). Control activities (nmol/min per mg of tissue) were for MAO-A, 0.0804 ± 0.0008 and for MAO-B, 0.087 ± 0.0017. ** $P < 0.001$ (vs vehicle control), $^{++}$ $P < 0.001$ (vs acute, Newman–Keuls test).

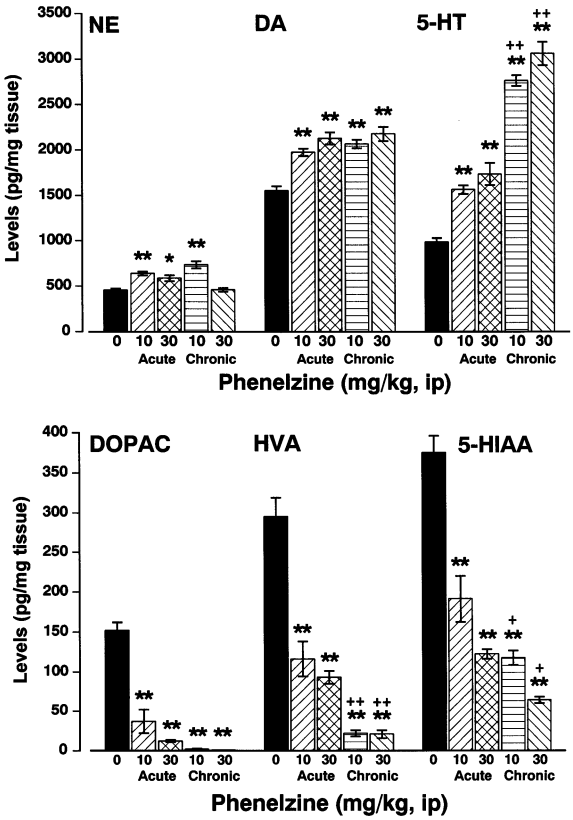

Fig. 5. Effects of acute and chronic (one daily injection for 14 days) treatments with phenelzine on the levels of NE, DA, 5-HT, DOPAC, HVA and 5-HIAA in the mouse brain. The animals were sacrificed 60 min after the last injection of saline or phenelzine. Data represent the mean ± S.E.M. ($n = 8–10$ animals per group).* $P < 0.01$, ** $P < 0.001$ (vs vehicle control), $^{+}$ $P < 0.05$, $^{++}$ $P < 0.001$ (vs acute, Newman–Keuls test).

3.7. Effects on the levels of monoamines and their metabolites in mouse brain

Fig. 5 shows that tissue levels of NE were significantly increased in both acute groups and in animals treated chronically with 10 mg/kg of phenelzine ($F_{4,41} = 18.92$, $P < 0.0001$). DA ($F_{4,41} = 19.97$, $P < 0.0001$) and 5-HT ($F_{4,41} = 175.46$, $P < 0.0001$) levels were significantly increased in all drug-treated animals. HVA ($F_{4,41} = 49.17$, $P < 0.0001$), DOPAC ($F_{4,41} = 55.06$, $P < 0.0001$) and 5-HIAA (($F_{4,41} = 46.33$, $P < 0.0001$) levels were significantly decreased in all groups. Increases in NE levels were very similar after acute (10 and 30 mg/kg, +40 and +29%, respectively) and chronic administration (10 mg/kg, +62%) of phenelzine. Similarly, DA levels were increased by 27–40% regardless of dose and treatment regimen. In contrast, chronic treatment (10 and 30 mg/kg) with phenelzine produced a significant greater increase in 5-HT levels (+180 and +210%, respectively) than did acute administration (59% with 10 mg/kg and 76% with 30 mg/kg). Similarly, decreases in HVA and 5-HIAA levels were significantly greater after chronic than after acute administration.

4. Discussion

This study investigated the effects of acute and chronic treatments with the MAOI phenelzine on behavioral and neurochemical changes in Swiss mice subjected to the MDTB. Behavioral results indicated that chronic phenelzine produced a profile which may be consistent with an anti-panic-like action. These effects are associated with changes in MAO-A and MAO-B activities and monoamines levels.

4.1. Behavioral effects

The present data are generally consistent with previous studies in this laboratory using the MDTB (Griebel et al., 1996). Thus, in reaction to an approaching hand-held, dead rat, control mice showed active flight responses. When the control mice ran to escape the chasing rat, they frequently displayed risk assessment, consisting of an abrupt movement arrest. This was in contrast to the inescapable straight alley test, where control animals showed less active risk assessment responses (approaches/withdrawals). Finally, defensive threat and attack to the rat almost invariably occurred upon forced contact.

Previous findings from the MDTB have shown that BZs (e.g. chlordiazepoxide, diazepam, clorazepate) (Griebel et al., 1995b, 1996) as well as the atypical anxiolytics buspirone and gepirone (Griebel et al., 1995d, 1998) decreased escape attempts following the removal of the rat, while the clinically effective anti-panic compounds imipramine, fluoxetine and moclobemide failed to produce such effects (Griebel et al., 1995a, 1997b). In the present study, both acute and chronic phenelzine at 30 mg/kg produced a significant reduction in escape attempts, although the lower dose was inactive. However, these effects may have been contaminated by behavioral impairment as indicated by the decrease in line crossings observed before the exposure to the rat.

The observation that PD patients usually report an urgent desire to flee (DSM-IV, 1994), has led several authors to suggest that panic symptoms are due to pathological, spontaneous activation of neuronal mechanisms underlying flight reactions (Deakin and Graeff, 1991; Graeff, 1991). In accordance with this suggestion, data from the MDTB clearly demonstrated that anti-panic agents specifically decrease animals' flight responses. Thus, the clinically effective anti- panic agents imipramine, fluoxetine, alprazolam, clonazepam and moclobemide reduced avoidance distance when the rat was first placed in the runway cage (Griebel et al.,

1995a,c, 1996, 1997b). Furthermore, the anti-GAD agents chlordiazepoxide, diazepam, buspirone and gepirone generally failed to affect this response in a selective manner (i.e. at non sedative doses) (Griebel et al., 1995b,d, 1996, 1998) Together, these findings led to the suggestion that avoidance reactions elicited by exposure to a natural threat may serve as an effective experimental model of panic (Griebel et al., 1996). The present finding with phenelzine is in agreement with this idea as chronic treatment with the drug clearly reduced the mouse-stimulus distance at which flight occurred.

Extensive pharmacological investigations with the rat and the mouse defense test batteries have shown that BZs mainly affected risk assessment responses (Blanchard et al., 1993; Griebel et al., 1995b). In the present study, none of the treatments significantly affected risk assessment activities during the chase test. By contrast, in the straight alley situation, risk assessment was increased following repeated administration. However, the failure of phenelzine to reduce risk assessment during the chase indicates only partial efficacy in affecting these behaviors, and therefore suggests a weaker anxiety-reducing potential compared to classical anxiolytics. Overall, this behavioral profile on risk assessment closely resembles that recently obtained in the MDTB after chronic treatment with the MAOI befloxatone (Griebel et al., 1997b). It is noteworthy that the significant increase of an active behavior (i.e. approaches followed by withdrawal responses) after chronic phenelzine at 30 mg/kg in the straight alley situation contrasts with the decrease in pre-test line crossings. This indicates that responses to highly threatening stimuli (i.e. an approaching rat) may involve central mechanisms that can override the hypolocomotor effect seen during the pre-test where there is no discrete threat stimulus and levels of defensiveness are undoubtedly lower.

Previous studies in rats and mice with traditional (e.g. BZs) and atypical (5-HT_{1A} receptor ligands) anti-anxiety agents (Blanchard et al., 1988, 1989; Griebel et al., 1995b,d, 1996, 1997a), showed that these drugs specifically attenuated vocalizations and bitings upon forced contact with a natural threat stimulus (i.e. a human or a rat), indicating that defensive threat/attack is sensitive to a range of anxiolytic drugs. In the present study, neither acute nor chronic treatment with phenelzine modified these responses, thereby confirming recent data from the MDTB with MAOIs which provided no evidence that such drugs may be involved in the modulation of these defense reactions (Griebel et al., 1997b).

In summary, although the behavioral profile displayed by phenelzine is consistent with an anxiolytic-like effect, the finding of an action upon a limited number of defense responses suggests a weaker anxiolytic-like potential compared to that of classical anxiolytics.

4.2. Neurochemical effects

In agreement with previous reports, our results showed that acute and repeated administration of phenelzine decreased MAO-A and MAO-B activities (Dyck et al., 1988; McKenna et al., 1992). However, the greater inhibition of MAO-A than of MAO-B activity after acute treatment of 10 mg/kg of phenelzine (-93 vs -74%) suggests that this drug inhibits preferential MAO-A activity. In contrast, chronic treatment with phenelzine induced a maximal and non-selective inhibition of both forms of MAO.

Phenelzine produced significant decreases of DOPAC, HVA and 5-HIAA levels and increases in DA, NE and 5-HT levels, effects which are consistent with those previously reported with phenelzine (McKim et al., 1983; Baker et al., 1984; Dyck et al., 1988; McKenna et al., 1992) and other MAOIs (Waldmeier and Baumann, 1983; Da Prada et al., 1989; Kumagae et al., 1991; Curet et al., 1996). After acute administration, phenelzine decreased DOPAC, HVA and 5-HIAA levels in a dose-dependent manner, an effect which is correlated with MAO-A and MAO-B inhibition. In contrast, increases of NE, DA and 5-HT were similar at 10 and 30 mg/kg. Following chronic administration, decreases of DOPAC and HVA were maximal and similar with both dosages. The decrease of 5-HIAA levels was slightly more pronounced after 30 mg/kg. Interestingly, while both acute and repeated administration of phenelzine induced similar increases in NE and DA levels, the increase in 5-HT was more marked after repeated administration. This latter effect is related with a maximal inhibition of MAO-A and MAO-B activities ($>98\%$ for both forms), whereas after acute administration, MAO-A and MAO-B were not completely inhibited (-93 and -74% with 10 mg/kg, and -98 and -90% with 30 mg/kg, respectively). Taken together these data suggest that when MAO-A is fully inhibited by phenelzine (30 mg/kg), 5-HT can be deaminated by the remaining 10% MAO-B activity. The greatest effect of phenelzine on 5-HT level was obtained only after complete ($>98\%$) inhibition of both forms of MAO. The larger increase of 5-HT as compared to NE and DA may be explained by the fact that the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase, is less sensitive to the end product-inhibition than tyrosine hydroxylase, the rate-limiting enzyme for the synthesis of NE and DA (Millard and Gal, 1971).

5. Conclusion

Taken together, the present results show that complete inhibition of MAO-A/B ($>98\%$) activities which gave rise to a dramatic rise in 5-HT levels was associated with the effects of phenelzine on defense. This

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suggests that the effects of phenelzine may primarily involve MAO- A/B activities and 5-HT. The absence of clear differences of NE and DA levels between acute and chronic treatments also suggests that these monoamines may have had minimal influence in the behavioral effects of phenelzine in the MDTB. These findings are in agreement with clinical evidence suggesting that the 5-HT system may be primarily involved in the pathogenesis of PD (Kahn and Van Praag, 1988; Westenberg and Den Boer, 1993a,b; Den Boer and Westenberg, 1995; Kahn et al., 1995). However, the involvement of other central mechanisms in the behavioral effects of the drug cannot be totally ruled out. Paslawski et al. (1996) recently demonstrated that the anxiolytic-like effects of a single injection of 15 mg/kg phenelzine in the elevated plus-maze test in rats was associated with a 2-fold increase in whole brain levels of GABA. It is therefore possible that other mechanisms are involved in the anxiety-reducing action of the drug, but the importance of each of these mechanisms in the therapeutic action of phenelzine remains to be determined.

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