IS THERE A NEGATIVE CORRELATION BETWEEN A PHENELZINE SUB-ACUTELY INDUCED ANTIDEPRESSANT-LIKE BEHAVIOUR AND HIPPOCAMPAL 5-HT?

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Abstract

Objective The aim of the current study was to evaluate the behavioural activity (immobility time in the forced swim test; FST) of the antidepressant and anti-panic drug phenelzine in relation to any neurochemical corollaries (hippocampal noradrenaline and 5-HT levels) in rats. Methods The monoamines were determined using high performance liquid chromatography with electrochemical detection). Results Acute and 7-day sub-acute phenelzine treatments evoked a significant reduction in immobility time in the forced swim test with an ED₅₀ ~ 10.0 mg/kg. The decreased immobility time after both acute and sub-acute treatment was associated with significant elevation of hippocampal noradrenaline and 5-HT levels compared to control. Notably, there was a significant negative correlation between the sub-acute phenelzine dose-related decrement in augmented hippocampal 5-HT levels in the hippocampus with respect to FST performance. Conclusion This may suggest either that phenelzine antidepressant-like activity does not correlate closely with elevated 5-HT levels in the hippocampus and may signify that other mechanisms are more critically operative or that the FST is perhaps not the best predictor of antidepressant potential.

Key words: Phenelzine; Forced swim test; Noradrenaline; Serotonin; Immobility time
Introduction
Depression is a prevailing neuropsychiatric illness currently ranked third among the global burden of disabling diseases and it is estimated that it will be number one by the year 2030 (WHO, 2004). According to the monoamine hypothesis of depression, attenuated monoaminergic (noradrenaline and serotonin) signaling underlies the pathogenesis of depression (1-2). This concept was further reinforced by the fact that clinically effective antidepressants (e.g. fluoxetine) increased monoamine levels in the brain by reuptake inhibition raising their intrasynaptic concentrations (3). Another class of antidepressants including phenelzine elevates the levels of monoamines in the brain by non-selectively inhibiting the enzyme monoamine oxidase A/B (MAO A/B) responsible for monoamine degradation (4-5).

Phenelzine has a variety of actions in addition to MAO inhibition and these may well contribute to its pharmacological and therapeutic profile. Such actions include inhibition of primary amine oxidase, effects on functional availability of glutamate, inhibition of GABA transaminase to enhance brain GABA levels, sequestration of reactive aldehydes, and effects on brain derived neurotrophic factor (BDNF) (6).

Despite difficulties in mimicking the symptoms of depression in rodents, numerous animal models of disease have been developed including the forced swim test (FST) (7). Because of its simplicity, high specificity and reliability, it is a commonly used tool to assess the potential antidepressant activity of various compounds (8).

The aim of the present study was to establish to what degree a correlation between elevated noradrenaline-serotonin (NA-5-HT) brain levels and FST dose efficacy reflected their contribution to phenelzine’s antidepressant proclivity. To this end, neurochemical determinations were performed using high performance liquid chromatography (HPLC) with electrochemical detection (9) on hippocampal tissues dissected immediately after completion of the FST in phenelzine treated rats.

Methods
Animals
Male Sprague Dawley rats (180 – 220 g) were maintained under standard environmental conditions i.e. room temperature of 25 ± 1 °C with 12:12 hour light / dark cycle. Food and water were available ad libitum. All experiments were conducted in accordance with the guidelines by the National Institute of Health (NIH publication no. 85-23, revised 1985).

Chemicals
5-Hydroxytryptamine (5HT), noradrenaline (NA), ethylenediaminetetraacetic acid (EDTA) and phenelzine were obtained from Sigma Aldrich, USA. Acetonitrile, methanol and 1-octane sulfonic acid and sodium dihydrogen phosphate were provided by Fisher chemicals, UK.

Forced swim test (FST)
A pretest session was conducted by placing subjects in a forced swim test (FST) tank (dimensions: 18 x 18 x 50 cm; filled with water at 25.0 ± 1.0 °C, up to a level of 18 cm) for 15 minutes. Any animals exhibiting nose bleeding during this period were excluded from subsequent study. Twenty-four hours later, study animals were then injected intraperitoneally (i.p.) with phenelzine (10, 20 or 30 mg/kg) once (acutely) or daily for seven consecutive days (sub-acutely). Each day, 1 h after treatment, the duration of behavioural immobility (s) was noted after placing the animals in the FST tank (5 minutes) as described by (7). Immediately after completion of FST, the hippocampus of each animal was removed after killing and dissected on ice (10), then immersed in liquid nitrogen prior to storage at -80 °C. The following day, neurochemical analysis was performed as described below.

Monoamine Neurotransmitter Analysis
The hippocampus obtained from each animal was prepared as detailed below. The samples containing monoamine neurotransmitters (NA and 5-HT) were analysed using high performance liquid chromatography with electrochemical detection (Schimadzu). A reversed phase nucleosil column (C18, 250/4.6 mm) and phosphate buffer (0.1 M sodium dihydrogen phosphate, 1 mM EDTA and 2.5 mM 1-octane sulfonic acid, pH 3.4) was used at flow rate of 0.5 ml/min.

In order to dissect the hippocampus, the skull of each rat was carefully removed by cutting through the interaural lines in an upward direction from both sides of skull. The brain was exposed and gently removed and placed on a chilled petri dish. The cortex was removed to expose the hippocampus on both halves of the brain (10). Then it was dissected and immediately frozen in liquid nitrogen prior to
storage at ~80 ºC. For monoamine analysis, the hippocampal were weighed, homogenized in perchloric acid (1 M) in a proportion of 100 mg/ml and centrifuged (15,000 rpm for 20 minutes at 4 ºC). The supernatant was collected and filtered (0.22 µm) using a syringe driven filter unit (Millex-GV, Millipore) and immediately subjected to HPLC analysis (injection volume = 25 µl). Standard curves were used to obtain the concentrations of the monoamines and their respective metabolites in the samples.

Statistical analysis
Immortality time in the FST was expressed in seconds as (mean ± S.E.M). Monoamine levels were determined in ng/g of tissue wet weight (mean ± S.E.M). Differences among means were analysed using one-way ANOVA with post hoc Duncan’s multiple range test and subsequently least significant difference (LSD). Pearsons correlation coefficient (r) was calculated for hippocampal monoamine levels (NA and 5-HT against FST with respect to dose). Asterisk(s) indicated significance levels i.e. *P<0.05, and **P<0.005 as compared to the respective control.

Results
Effect of phenelzine treatment on the immortality time of rats in the forced swim test (FST)
Acute doses of phenelzine (10, 20 and 30 mg/kg), induced significant (P<0.005) dose dependent reductions (49%, 63% and 73%, respectively) in the immortality time of rats in the FST. Sub-acute phenelzine administration of the same doses also caused significant dose-related reductions (P<0.005, 53%, 81% and 91%) in the FST (Table 1).

Effect of phenelzine treatment on the levels of monoamines in rat hippocampus
Acute phenelzine treatment induced significant increases (17% and 18%, P<0.05) in NA levels in the hippocampus at doses of 10 and 20 mg/kg respectively, whereas no significant change was seen at the highest tested dose of 30 mg/kg compared to control (Table 2 and Figure 1). In the case of hippocampal 5-HT, significant increases (49 %, P<0.005; 47 % and 26 %, P<0.05) were observed in response to acute doses of 10, 20 and 30 mg/kg respectively.
Following sub-acute phenelzine administration, hippocampal NA was significantly elevated by progressively decreasing magnitudes (55%, P<0.005; 33% and 28%, P<0.05) in response to 10, 20 and 30 mg/kg doses respectively (Table 2). The level of 5-HT was also raised by values (258%, 253% and 213%, P<0.005) which diminished with increasing dose (10, 20 and 30 mg/kg).

Evaluation of correlation coefficients between decreased FST and hippocampal monoamine levels with respect to phenelzine dose
The apparent decline of raised hippocampal NA and decreasing FST immobility time with phenelzine doses did not generate any significant Pearson correlation values either acutely (r = - 0.087, P = 0.715) or sub-acute ( r = - 0.418, P = 0.067). Likewise, there was no significant correlation between the seeming decline in enhanced 5-HT levels with regard to FST and acute dose of phenelzine (r = - 0.352, P = 0.128). However it was highly notable that there was a significant negative correlation between the sub-acute phenelzine dose-related decrement in augmented hippocampal 5-HT levels in the hippocampus with respect to FST performance (r = - 0.647, P = 0.02).

Discussion
A comparative study was performed involving an antidepressant-like behavioural activity in the forced swim test and neurochemical effects (hippocampal NA and 5-HT monoamine levels) of phenelzine in rats. The phenelzine doses examined were within the range of those selected in a previous behavioural and neurochemical study (11). The hippocampal brain region was chosen because it is related to three major hypotheses of depression i.e. monoamine, neurogenic and the hypothalamic-pituitary-adrenal (HPA) axis (12-13). The hippocampus undergoes atrophy during recurrent depressive illness (14) and it is one of the brain regions mediating the diverse symptoms of depression in addition to being a target for stress hormones (15).

Both acute and sub-acute phenelzine treatment significantly (P<0.005) decreased the immortality time (ED50 ~ 10 mg/kg, Table 1) of rats compared to the controls in the FST. This accords with an earlier report (16) and it has been attributed to elevated levels of monoamines caused by inhibition of monoamine oxidase enzyme in the brain (17). Our neurochemical data revealed that acute phenelzine treatment (10 and 20 mg/kg) induced a significant elevation of hippocampal NA levels whereas at a higher dose (30 mg/kg) there was no significant change from control (Table 2, Figure 1).
It appeared that there was a declining trend in elevated hippocampal NA with decreasing immobility time in the FST with respect to dose, but subsequent calculation of the Pearson correlation coefficient indicated that this tendency was not significant. Following sub-acute phenelzine treatment, a similar relationship between dose and NA levels was also obtained (Table 2) though once again there was no significant inverse correlation. In the case of 5-HT, acute phenelzine treatment produced an elevation of hippocampal 5-HT level which declined marginally with increasing dose but it did not yield a negative correlation with FST latency. However, sub-acute phenelzine administration did generate a significant negative correlation between these two parameters even though the highest dose (30 mg/kg) still induced a 5-HT hippocampal level that was markedly greater than the control (Table 2).

In addition, sub-acute phenelzine treatment elevated the hippocampal 5-HT content to much higher levels than those produced by acute treatment which is in accordance with an earlier report for this drug (17). The actions of sub-acuteley administered phenelzine are somewhat at variance since there is an inverse correlation between 5-HT levels in the hippocampus and activity in the FST with respect to dose. This may signify that mechanisms other than elevated hippocampal 5-HT are more critically operative and give rise to this finding. Apropos this concept, it has been shown that phenelzine increases brain GABA levels by a factor of up to four times (18) while GABA-transaminase activity is not inhibited by more than half, even at high dose levels (19).

This infers that other, as yet unidentified mechanisms may also be implicated in the GABA elevating activity of phenelzine. In this regard, phenelzine has been reported to reduce KCl-evoked glutamate release (20). It also stimulates a short-term decrease not only in brain levels of glutamine and glutamate but also the glutamine-glutamate cycling flux between neurons and glia (Yang and Shen, 2005) plus astrocytic glutamate release (21). The lack of positive correlation and the discrepancy between 5-HT levels and behaviour is reflected by the two antidepressants fluoxetine and tianeptine which possess opposing modes of action i.e. inhibition or enhancement of 5-HT reuptake respectively (16, 22). Additionally, the therapeutic efficacy of antidepressants has been ascribed partly to functional under activity of some central 5-HT systems (23). It is noteworthy that like phenelzine, tianeptine can also modulate glutamatergic hippocampal activity (24-25). Furthermore, it has been shown that fluoxetine and desipramine act differently in 5-HT depleted rodents (26). Equally, clinical studies have revealed that simultaneous disruption of indoleamine and catecholamine function did not exacerbate symptoms in unmedicated depressed subjects suggesting that monoamines regulate mood during depression via indirect mechanisms (27). The aforementioned reports endorse our data exhibiting an absence of, or even negative correlation between elevated hippocampal 5-HT and behavior in the FST. Contrary to our findings, the MAO-B inhibitor deprenyl has been reported to produce a moderate increase in 5-HT dialysates from the raphe nuclei but not the frontal cortex (28). However the experiments were performed using in vivo microdialysis which samples extracellular 5-HT and the studies were not undertaken in the hippocampus, nor was any overt stress involved. Additionally, the current findings relate to earlier reports which questioned the validity of the FST as an appropriate animal model to assess antidepressant potential (29-30). Moreover, several other hypotheses (involving the neurogenic, hypothalamic-pituitary adrenal axis, neurotrophic factors, glutamate, gamma amino butyric acid and leptin mechanisms) have been proposed to underlie the pathogenesis of depression (31-36). Hence, any antidepressant propensity of phenelzine should be viewed in the light of such theories. Moreover, phenelzine displays a multiplicity of mechanisms which in addition to an antidepressant effect inevitably contribute towards a diversity of other applications encompassing the treatment of anxiety disorders such as panic disorder and social anxiety disorder. Phenelzine also has protective actions against neuro and gliotoxicity and suggestions have been made that it should be developed for treatment of psychological and neurological disorders, particularly those involving neurodegeneration (6, 21)

In conclusion, our data clearly demonstrated that the sub-acute phenelzine treatment induced a negative correlation between the sub-acute phenelzine dose-related decrement in augmented hippocampal 5-HT levels in the hippocampus with respect to FST performance. This may suggest either that phenelzine antidepressant activity does not correlate closely with 5-HT levels in the hippocampus and/or
that the FST is perhaps not the best predictor of antidepressant potential.

References

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Table-1. Effect of acute and sub-acute phenelzine treatment on the immobility time(s) of rats in the forced swim test (FST).

<table>
<thead>
<tr>
<th>Phenelzine treatment</th>
<th>Dose (mg/kg)</th>
<th>C</th>
<th>o</th>
<th>n</th>
<th>t</th>
<th>r</th>
<th>o</th>
<th>l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>104 ± 9s*** (49% ↓)</td>
<td>75 ± 8s*** (63% ↓)</td>
<td>54 ± 8s*** (73% ↓)</td>
<td>204 ± 6s</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-acute</td>
<td>99 ± 7s*** (53% ↓)</td>
<td>40 ± 7s*** (81% ↓)</td>
<td>19 ± 8s*** (91% ↓)</td>
<td>210 ± 5s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values represent the mean immobility time(s) ± S.E.M. (n = 5) in animals treated with 10, 20 and 30 mg/kg of phenelzine. Numbers in brackets indicate the percentage change in the immobility time as compared to control: (↓)decrease and(↑) increase *** (p<0.005) as compared to the control.
Table 2. Effect of acute or sub-acute phenelzine treatment on the levels of monoamines in the rat hippocampus immediately after the forced swim test

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Hippocampal tissue wet weight</th>
<th>ng/g</th>
<th>5-HT</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA</td>
<td>655 ± 20</td>
<td>424 ± 29</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>765 ± 42*</td>
<td>632 ± 39***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>776 ± 39*</td>
<td>625 ± 43***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>668 ± 26</td>
<td>535 ± 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-acute</td>
<td>Control</td>
<td>603 ± 38</td>
<td>352 ± 36</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>935 ± 74***</td>
<td>1260 ± 127***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>804 ± 43*</td>
<td>1242 ± 167***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>774 ± 63*</td>
<td>1101 ± 81***</td>
<td></td>
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</tr>
</tbody>
</table>

Figure 1

The figure shows the percentage changes in adrenaline (AD), noradrenaline (NA), and serotonin (5-HT) levels in the rat hippocampus after acute and sub-acute phenelzine treatment. The y-axis represents the percentage change, while the x-axis represents the minutes post-treatment.