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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 ED_{50} ~ 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 ºC 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 ^{o}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 ^{o}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 hippocampi 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

Immobility 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 immobility 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 immobility 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 declination 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-acutely (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 hypothalamus-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 immobility time (ED_{50} ~ 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-acutely 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 dyalisates 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

1. Elhwuegi AS. Central monoamines and their role in major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2004;28(3):435-51.
2. Piñeyro G, Blier P. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacological reviews. 1999;51(3):533-91.
3. Reneric J-P, Lucki I. Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology. 1998;136(2):190-7.
4. aan het Rot M, Mathew SJ, Charney DS. Neurobiological mechanisms in major depressive disorder. Canadian Medical Association Journal. 2009;180(3):305-13.
5. Ansorge MS, Hen R, Gingrich JA. Neurodevelopmental origins of depressive disorders. Current opinion in pharmacology. 2007;7(1):8-17.
6. MacKenzie EM, Song M-S, Dursun SM, Tomlinson S, Todd KG, Baker GB. Pheneizine: An Old Drug That May Hold Clues to The Development of New Neuroprotective Agents. Klinik Psikofarmakoloji Bulteni. 2010;20(2).
7. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730-2.
8. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in pharmacological sciences. 2002;23(5):238-45.
9. Bergquist J, Ściubisz A, Kaczor A, Silberring J. Catecholamines and methods for their identification and quantitation in biological tissues and fluids. Journal of neuroscience methods. 2002;113(1):1-13.
10. Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain-I. Journal of neurochemistry. 1966;13(8):655-69.
11. Maki Y. [A behavioral and neurochemical study on the mechanism of the anxiolytic effect of monoamine oxidase inhibitors]. [Hokkaido igaku zasshi] The Hokkaido journal of medical science. 2001;76(3):133-42.
12. Dranovsky A, Hen R. Hippocampal neurogenesis: regulation by stress and antidepressants. Biological psychiatry. 2006;59(12):1136-43.
13. McEwen BS. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Annals of the New York Academy of Sciences. 2001;933(1):265-77.
14. McEwen BS. Stress and hippocampal plasticity. Annual review of neuroscience. 1999;22(1):105-22.
15. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nature Reviews Neuroscience. 2006;7(2):137-51.
16. Uzbay TI. Tianeptine: potential influences on neuroplasticity and novel pharmacological effects. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2008;32(4):915-24.
17. Baker GB, LeGATT DF, Coutts RT, Dewhurst WG. Rat brain concentrations of 5-hydroxytryptamine following acute and chronic administration of MAO-inhibiting antidepressants. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 1984;8(4):653-6.
18. Baker GB, Wong JT, Yeung JM, Coutts RT. Effects of the antidepressant phenelzine on brain levels of γ-aminobutyric acid (GABA). Journal of affective disorders. 1991;21(3):207-11.
19. Popov N, Matthies H. Some effects of monoamine oxidase inhibitors on the metabolism of gamma-aminobutyric acid in rat brain. J Neurochem. 1969 Jun;16(3):899-907.
20. Michael-Titus A, Bains S, Jeetle J, Whelpton R. Imipramine and phenelzine decrease glutamate overflow in the prefrontal cortex—a possible mechanism of neuroprotection in major depression? Neuroscience. 2000;100(4):681-4.
21. Song MS, Baker GB, Dursun SM, Todd KG. The antidepressant phenelzine protects neurons and astrocytes against formaldehyde-induced toxicity. Journal of neurochemistry. 2010;114(5):1405-13.
22. Mennini T, Mocaer E, Garattini S. Tianeptine, a selective enhancer of serotonin uptake in rat brain. Naunyn-Schmiedeberg's archives of pharmacology. 1987;336(5):478-82.
23. Ögren S, Fuxe K, Agnati L, Gustafssons J, Jonsson G, Holm A. Reevaluation of the indoleamine hypothesis of depression. Evidence for a reduction of functional activity of central 5-HT systems by antidepressant drugs. Journal of neural transmission. 1979;46(2):85-103.
24. Kole MH, Swan L, Fuchs E. The antidepressant tianeptine persistently modulates glutamate receptor currents of the hippocampal CA3 commissural associational synapse in chronically stressed rats. European Journal of Neuroscience. 2002;16(5):807-16.
25. Zoladz PR, Muñoz C, Diamond DM. Beneficial effects of tianeptine on hippocampus-dependent long-term memory and stress-induced alterations of brain structure and function. Pharmaceuticals. 2010;3(10):3143-66.
26. Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology. 1999;147(2):162-7.
27. Berman RM, Sanacora G, Anand A, Roach LM, Fasula MK, Finkelstein CO, et al. Monoamine depletion in unmedicated depressed subjects. Biological psychiatry. 2002;51(6):469-73.
28. Celada P, Artigas F. Monoamine oxidase inhibitors increase preferentially extracellular 5-hydroxytryptamine in the midbrain raphe nuclei. A brain microdialysis study in the awake rat. Naunyn-Schmiedeberg's archives of pharmacology. 1993;347(6):583-90.
29. Abbas G, Naqvi S, Mehmood S, Kabir N, Dar A. Forced swimming stress does not affect monoamine levels and neurodegeneration in rats. Neuroscience bulletin. 2011;27(5):319-24.
30. Borsini F, Volterra G, Meli A. Does the behavioral “despair” test measure “despair”? Physiology & behavior. 1986;38(3):385-6.
31. Barbon A, Popoli M, La Via L, Moraschi S, Vallini I, Tardito D, et al. Regulation of editing and expression of glutamate α-amino-propionic-acid (AMPA)/kainate receptors by antidepressant drugs. Biological psychiatry. 2006;59(8):713-20.
32. Cryan JF, Slattery DA. GABA< sub> B</sub> Receptors and Depression: Current Status. Advances in pharmacology. 2010;58:427-51.

33. David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, et al. Neurogenesis-dependent and-independent effects of fluoxetine in an animal model of anxiety/depression. Neuron. 2009;62(4):479-93.
34. Frost P, Bornstein S, Ehrhart-Bornstein M, O'Kirwan F, Hutson C, Heber D, et al. The prototypic antidepressant drug, imipramine, but not Hypericum perforatum (St. John's wort), reduces HPA-axis function in the rat. Hormone and metabolic research. 2003;35(10):602-6.
35. Lu X-Y. The leptin hypothesis of depression: a potential link between mood disorders and obesity? Current opinion in pharmacology. 2007;7(6):648-52.
36. Réus GZ, Stringari RB, Ribeiro KF, Ferraro AK, Vitto MF, Cesconetto P, et al. Ketamine plus imipramine treatment induces antidepressant-like behavior and increases CREB and BDNF protein levels and PKA and PKC phosphorylation in rat brain. Behavioural brain research. 2011;221(1):166-71.

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

Phenelzine treatment	**Dose (mg/kg)**			**Control**
	10	**20**	**30**	
Acute	104 ± 9s*** (49% ↓)	75 ± 8s*** (63% ↓)	54 ± 8s*** (73% ↓)	204 ± 6s
Sub-acute	99 ± 7s*** (53% ↓)	40 ± 7s*** (81% ↓)	19 ± 8s*** (91% ↓)	210 ± 5s

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

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

Figure-1