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Research report

Regional specific modulation of neuronal activation associated with nitric oxide synthase inhibitors in an animal model of antidepressant activity

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HIGHLIGHTS

- NOS inhibitors reduce immobility time and increase FST-induced c-FOS immunoreactivity in the lateral septum.
- NOS inhibitors reduce FST-induced c-FOS immunoreactivity in the dorsal dentate gyrus, ventral CA1 and dorsal raphe nucleus.
- Treatment with pCPA and restraint stress provokes a reduction in delta FosB in the lateral septum.
- pCPA and restraint stress provoke an increase in delta FosB in the infra-limbic cortex and nucleus accumbens.
- Regional changes in the expression of delta FosB relate to increased immobility in the FST.

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ABSTRACT

Objective: The regional specific modulation of neuronal activation following drug administration is of interest to determine brain areas involved in the behavioural effects of experimental test compounds. In the current investigation the effects of the L-arginine related NOS inhibitor N^{ω}-L-nitroarginine (L-NA) and the structurally unrelated selective neuronal NOS inhibitor 1-(2-Trifluoro-methyl-phenyl) imidazole (TRIM) were assessed in the rat for changes in regional c-FOS immunoreactivity, a marker of neuronal activation, upon exposure to the forced swimming test (FST). Behaviour and regional FOS and FosB/ΔFosB expression was assessed in naive animals and in animals exposed to stress with central serotonin-depletion which exhibit a stress related phenotype in the FST.
Methods: Male Sprague-Dawley rats (n = 5– 6 per group) were treated with the irreversible tryptophan hydroxylase inhibitor, DL-4-*p*-chlorophenylalanine (*p*CPA, 150 mg/kg, i.p.), to achieve central serotonin-depletion followed by repeated exposures to restraint stress and were then subjected to the FST. 24, 5 and 1 h prior to the test, animals were treated with either L-NA (10 mg/kg, i.p.), TRIM (50 mg/kg, i.p.) or saline vehicle (1 mg/kg i.p).
Results: pCPA treatment coupled with restraint stress increased immobility in the FST compared to naïve controls. Both NOS inhibitors decreased immobility time in 5-HT depleted and stressed animals only in keeping with their antidepressant-like properties. Brain regions analyzed for c-FOS immunoreactivity included the pre-limbic cortex, lateral septum (LS), nucleus accumbens, paraventricular hypothalamic nucleus (PVN), central amygdala, hippocampus (dorsal dentate gyrus and ventral CA1), and the dorsal raphe nucleus (DRN). Exposure to the FST increased c-FOS immunoreactivity in the LS, PVN, dentate gyrus, vCA1 and the DRN when compared to non-FST exposed controls. FST-induced c-FOS immunoreactivity was further increased in the LS following treatment with L-NA or TRIM when compared to vehicle-treated FST controls. By contrast, FST-induced c-FOS immunoreactivity was reduced in dorsal dentate gyrus, vCA1 and the DRN following treatment with L-NA or TRIM when compared to vehicle-treated FST controls. There was no difference observed in FST-induced expression of c-FOS between naïve animals and animals exposed to *p*CPA and restraint stress. This combination however provoked an increase in FosB/ΔFosB immunoreactivity in the infra-limbic cortex and nucleus accumbens with a concomitant reduction in the lateral septum, suggesting alterations to long-term, adaptive neuronal activation.

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Conclusion: This study identified a pattern of enhanced and reduced FST-related c-FOS immunoreactivity indicative of a NO-regulated network where inhibition of NO leads to activation of the septum with concomitant inhibition of the hippocampus, and the DRN. No link between FST-induced regional expression of c-FOS and increased immobility in the FST was observed in animals exposed to *p*CPA and stress. However, the 5-HT depletion regime combined with restraint stress provoked regional changes in the expression of ΔFosB which may relate to increased immobility in the FST.

1. Introduction

Due to its utility as a marker of neuronal activation, c-FOS is commonly used to delineate the neuronal circuitry that is modulated following treatment with psychoactive compounds [27]. Nitric oxide synthase (NOS) inhibitors have been shown to regulate regional neuronal activation depending upon the stimulus (i.e. type of stress) that animals are exposed to. Amir and colleagues reported that the NOS inhibitors, L-nitro-arginine-methyl ester (L-NAME; non-selective for all NOS isoforms) and 7-nitroindazole (7-NI; selective nNOS inhibitor), were capable of attenuating stress-induced c-FOS immunoreactivity within the paraventricular nucleus of the hypothalamus following a 60 min immobilisation stress in rats [1]. Such results suggest a role for nitric oxide (NO) in the regulation of stress-induced neuronal activation. Further corroborating a role for NO in stress-induced neuronal activation is that deletion of the nNOS gene in mice resulted in a decrease in immobility time in the forced swimming test (FST) along with a corresponding increase in c-FOS immunoreactivity within several brain regions such as the infralimbic cortex, dentate gyrus, periventricular nucleus of the hypothalamus and medial amygdala [21]. These studies have been useful in identifying brain regions whose activation is responsive to regulation by NOS inhibitors. Silva and colleagues reported on the regional modulation of neuronal activation associated with the antidepressant effects of 7-NI in the FST. 7-NI lowered immobility time in rats exposed to the FST while also diminishing FST-induced neuronal activation of several subcortical limbic brain regions in a similar manner to the conventional antidepressants fluoxetine and venlafaxine [23]. Such results suggest that nNOS inhibitors regulate stress-responsive regions to produce antidepressant-related responses.

Following repeated exposure to a particular stressor, the level of c-FOS protein expression begins to desensitize to the chronic nature of the stress and declines [13] and ΔFosB begins to increase in certain brain regions [18,15]. Chronic (10 day) social defeat stress has been shown to increase ΔFosB immunoreactivity within the core and shell of the nucleus accumbens of mice, with lower levels of the immediate early gene (IEG) within this brain region suggestive of an increased susceptibility to depressive-like behaviour [29]. Accumulation of ΔFosB within neurons of the nucleus accumbens following chronic stress is suggested to facilitate molecular adaptions that give rise to an active coping strategy. Treatment with the antidepressant fluoxetine over weeks increases ΔFosB immunoreactivity within the nucleus accumbens coinciding with an antidepressant-like response in social avoidance tests. Interestingly, social isolation of animals reduces the expression of this IEG within the nucleus accumbens while also increasing vulnerability towards chronic social stress [30]. This suggests that the expression of this IEG is sensitive to changes in an animal's behavioural state.

Several studies have previously documented that NOS inhibitors produce antidepressant-like properties (i.e. reduce immobility in the FST) in naive rats and mice [12,28,8,7,9,23]. Yet, the effects in animal models of depression, where animals have been subject to manipulations to provoke a depression-like phenotype, are assessed to a lesser extent. Previous work from our laboratory has shown that when central 5-HT depletion is accompanied with restraint stress the combined manipulation produces a sustained depressive-like increase in immobility in the FST in rats [10]. Consequently, a combination of 5-HT depletion and restraint stress was used in the current study to provoke depressive-related behaviour (i.e. an increase in immobility time) in the FST, to assess the effect of and the response to NOS inhibitors. In the present investigation, the antidepressant-related effects of the NOS inhibitors N^{ω}-Nitro-L-Arginine (L-NA) and 1-(2-trifluoro-methyl-phenyl) imidazole (TRIM) were determined in this model. In addition, the patterns of neuronal activation of specific brain regions were assessed in tandem by means of c-FOS immunohistochemistry. The effect of 5-HT depletion and restraint stress upon prolonged (chronic) neuronal activation was also assessed through immunohistochemical mapping of FosB/ΔFosB.

2. Methods

2.1. Animals and drug treatments

Male Sprague-Dawley (Harlan Olac, Bicester, UK) rats weighing 280–330 g at the beginning of the experiments were singly housed in standard medium sized polypropylene cages (41 × 24 cm) after acclimatisation to the animal housing facility. The animals were maintained at a constant temperature (22 ± 2 °C) and exposed to standard lighting conditions (12 h light/12 h dark cycle; lights on from 08:00 am–20:00 pm). Food and water were available *ad libitum* except when the animals were subjected to restraint stress or to behavioural tests. To induce central 5-HT depletion, the tryptophan hydroxylase inhibitor DL-4-*p*-chloro-phenylalanine (*p*CPA) was dissolved in saline solution (75 mg/mL) and was administered i.p. in an injection volume of 2 mL/kg to deliver a dose of 150 mg/kg. The NOS inhibitor L-nitro-arginine (L-NA) was dissolved in saline and sonicated to produce an injectable suspension (5 mg/mL) and was administered i.p. in an injection volume of 2 mL/kg to deliver a dose of 10 mg/kg. The selective neuronal NOS inhibitor 1-(2-trifluoro-methyl-phenyl) imidazole (TRIM) was dissolved by sonication in saline solution (50 mg/mL) and was administered at 1 mL/kg i.p. to deliver a dose of 50 mg/kg. Research involving animals in TCD is governed by directive 2010/63/EU on the protection of animals used for scientific purposes in accordance with the requirements of the S.I No 543 of 2012 and reviewed and approved by the animal research ethics committee in TCD and the Health Products Regulatory Authority (HPRA) of Ireland.

2.2. pCPA treatment and restraint stress

A combination of *p*CPA and restraint stress was applied to rats in this study as this combination induces an increase in immobility time in the FST against which it is possible to assess the antidepressant-related activity of the NOS inhibitors tested. Animals were treated with *p*CPA (150 mg/kg, i.p.) or saline once a day for 3 consecutive days to achieve cortical 5-HT depletion. Previous work from our lab has demonstrated that this dosing regime of *p*CPA produces up to a 70% depletion of cortical 5-HT [10]. 48 h fol-