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Research article

Interaction between 5-HT$_{1B}$ receptors and nitric oxide in zebrafish responses to novelty

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HIGHLIGHTS

- Serotonin and nitric oxide interact at different levels to control behavior.
- 5-HT$_{1B}$ antagonists decrease reactivity to novelty in zebrafish.
- The 5-HT$_{1B}$ inverse agonist SSB224,289 decreased bottom-dwelling and erratic swimming in zebrafish.
- The nitric oxide synthase inhibitor L-NAME blocked the effect of SSB224,289 on bottom-dwelling.

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ABSTRACT

Nitric oxide (NO) and serotonin (5-HT) interact at the molecular and systems levels to control behavioral variables, including agression, fear, and reactions to novelty. In zebrafish, the 5-HT$_{1B}$ receptor has been implicated in anxiety and reactions to novelty, while the 5-HT$_{1A}$ receptor is associated with anxiety-like behavior; this role of the 5-HT$_{1A}$ receptor is mediated by NO. This work investigated whether NO also participates in the mediation of novelty responses by the 5-HT$_{1B}$ receptor. The 5-HT$_{1B}$ receptor inverse agonist SB 224,289 decreased bottom-dwelling and erratic swimming in zebrafish; the effects on bottom-dwelling, but not on erratic swimming, were blocked by pre-treatment with the nitric oxide synthase inhibitor L-NAME. These effects underline a novel mechanism by which 5-HT controls zebrafish reactivity to novel environments, with implications for the study of neotic reactions, exploratory behavior, and anxiety-like states.

1. Introduction

An interaction between serotonin (5-HT) and the nitrergic system has been observed in the brain at the molecular [1,2], systems [3,4], and behavioral [5–9] levels. This complex interaction involves both nitric oxide (NO) mediation of 5-HT release/uptake [9–12] as well as the 5-HTergic mediation of NO activity [1,13,14]. In this sense, chronic treatment with 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) agonists has been shown to decrease anxiety-like behavior in a NO-dependent way [6], and activation of the 5-HT$_{1A}$R decreases NMDA receptor-mediated increases in NO production in cortical slices [13]. While the role of the 5-HT$_{1A}$R in anxiety-like behavior is relatively well-established, the participation of 5-HT$_{1B}$ receptors (5-HT$_{1B}$R) is emergent; 5-HT$_{1B}$R knockout mice show increased reactivity to novelty and decreased anxiety-like behavior [15], while zebrafish treated with 5-HT$_{1B}$R antagonists show decreased reactivity to novelty [16,17]. Elsewhere, we have shown that the anxiolytic-like effect of 5-HT$_{1A}$R antagonists in zebrafish is blocked by pre-treatment with the nitric oxide synthase (NOS) inhibitor l-N^{G}-nitroarginine methyl ester (l-NAME) [18]; in the present work, we analyzed whether the behavioral effects of the 5-HT$_{1B}$R inverse agonist SB 224,289 in zebrafish are also mediated by NOS.

2. Materials and methods

47 adult zebrafish from the *lof* phenotype were acquired in a local aquarium shop and kept in collective tanks (40 L, 10 animals/2 L) for at least two weeks before experiments begun. Water

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Table 1
SB224,289 decreases erratic swimming, and this effect is not blocked by L-NAME. Values refer either to mean ± S.E.M. or median ± interquartile range (IQR). *, $p < 0.05$ vs. Vehicle.

	Vehicle ($n = 9$)	SB 2.5 mg/kg ($n = 8$)	SB 5.0 mg/kg ($n = 9$)	SB 2.5 mg/kg + NAME ($n = 12$)	SB 5 mg/kg + NAME ($n = 12$)
Squares crossed (median ± IQR, N)	141 ± 89	61.5 ± 75.75	175 ± 50	125 ± 75	190 ± 85.5
Erratic swimming (median ± IQR, N)	7 ± 4	1 ± 1*	0 ± 3*	1 ± 1	3 ± 1.5
Freezing (mean ± S.E.M., s)	23.59 ± 8.25	24.85 ± 15.84	15.80 ± 7.07	14.01 ± 3.52	31.54 ± 9.27

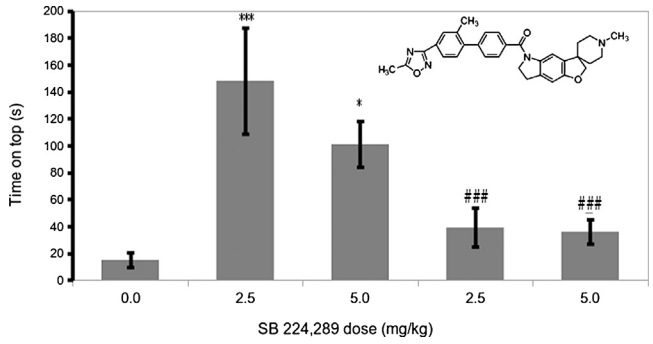

Fig. 1. The effects of SB224,289 on bottom-dwelling are blocked by pre-treatment with L-NAME. Bars refer to mean ± S.E.M. ***, $p < 0.001$ vs. Vehicle; *, $p < 0.05$ vs. Vehicle; ###, $p < 0.001$ vs. 2.5 mg/kg.

conditions, housing, and feeding conditions were standardised as per recommendations for zebrafish [19]. Animals were then swiftly and individually removed from the housing tank, cold-anesthetized (17 °C < T < 12 °C) and injected intraperitoneally [20] with either vehicle (DMSO 0.5%) or SB 224,289 (2.5 or 5.0 mg/kg). Another cohort of animals was pre-treated with l-NAME (1 mg/kg) before SB 224,289. 30 min after injection, animals were subjected to the novel tank test [21]; behavioral variables were defined as per the Zebrafish Behavior Catalog [22]. Data were analyzed using ANOVAs or Kruskal–Wallis tests whenever appropriate, with either Tukey or Bonferroni post-tests whenever p-values < 0.05. Raw data can be downloaded at figshare (doi:10.6084/m9.figshare.1272823).

3. Results

SB 224,289 dose-dependently decreased bottom-dwelling ($F_{4,41} = 6.814$, $p = 0.0003$; Fig. 1) and erratic swimming ($H_{df=4} = 20.08$, $p = 0.0005$; Table 1), without effects on freezing ($F_{4,46} = 0.6668$, NS; Table 1) or locomotion ($H_{df=4} = 8.55$, NS; Table 1). Pre-treatment with l-NAME blocked the effects of SB 224,289 on bottom-dwelling, but not on erratic swimming (Fig. 1, Table 1).

4. Discussion

The reduction in bottom-dwelling, the main response of adult zebrafish to a novel environment [23,24], as well as in erratic swimming, are indicative of reduced anxiety, stress or fear. The results from the present experiment provide a novel mechanism by which serotonin controls behavioral responses to novelty, suggesting an important interaction between the 5-HT_{1B} receptor and the nitrergic system. The specific mechanism by which NOS inhibition blocks the neotic effects of 5-HT_{1B}R antagonists in zebrafish is unclear; this interaction could be due to presynaptic effects of NO on 5-HT release or uptake [9–12] or to a postsynaptic effect, with 5-HT_{1B} receptor activation increasing NOS activity [25]. Futher experiments are needed to clarify the issue.

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