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Brain Research 955 (2002) 264–267

BRAIN RESEARCH

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Short communication

5-HT_{1A} receptor antagonist administration decreases cell proliferation in the dentate gyrus

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Accepted 12 July 2002

Abstract

This study investigated the action of 5-HT_{1A} receptor antagonists on cell proliferation in the dentate gyrus of adult rats. Three antagonists (NAN-190, *p*-MPPI and WAY-100635) all produced a statistically significant ~30% reduction in the number of BrdU-immunoreactive cells in the dentate gyrus. This suggests that 5-HT_{1A} receptor activity is important during naturally occurring cell proliferation in the dentate gyrus, and perhaps neurogenesis, and is one of the many factors involved in its regulation.

Theme: Neurotransmitters, modulators, transporters and receptors

Topic: Serotonin receptors

Keywords: Neurogenesis; 5-HT_{1A} receptor; Dentate gyrus; Hippocampus; Serotonin; Granule cell

The birth of new neurons in the brain—neurogenesis—occurs in the dentate gyrus, and other brain areas, well into adulthood in a number of mammalian species, including humans [9,19,12]. Some of the factors that regulate this process have been elucidated [18], but much remains to be determined. Serotonin (5-HT) has long been known to exert trophic effects in the central nervous system during development [32,34,22], and adulthood [24]. Some of these studies suggest that the trophic effects of 5-HT are carried out via activation of brain 5-HT_{1A} receptors [3,33]. In the present context, the dentate gyrus receives serotonergic innervation [1,15], and is enriched with 5-HT_{1A} receptors [2,16], making it a likely target for the trophic effects of 5-HT. 5-HT has also been shown to have mitogenic properties in nonneuronal systems [13,28]. Therefore, we decided to explore whether serotonin could affect the proliferation of granule cell precursors in the dentate gyrus. In our initial work on this, we found that pharmacological manipulations that increased brain 5-HT levels (fenfluramine), and those which activated 5-HT_{1A} receptors (8-OH-DPAT), also increased granule cell genesis in the dentate gyrus [21]. This has been extended by other research showing that the chronic administration of a selective serotonin reuptake inhibitor (e.g. fluoxetine) resulted in the enhancement of granule cell genesis [23], and that brain 5-HT depletion resulted in the production of fewer granule neurons [5]. In this study, using three different drugs, we specifically examine whether specific blockade of the 5-HT_{1A} receptor can affect cell proliferation in the dentate gyrus.

Adult male Sprague–Dawley rats (200–225 g) were purchased from Charles River Laboratories and were then housed in the Animal Colony at Princeton University. Rats were maintained on a 12:12 h light–dark cycle and provided with unlimited access to food and water. All animal experimentation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The 5-HT_{1A} receptor antagonists chosen for this study were NAN-190 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide; Research Biochemicals International (RBI), Natick, MA, USA), *p*-MPPI {4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide; RBI, Natick, MA, USA} and WAY-100635 (*N*-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyc-

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0006-8993/02/$ – see front matter
PII: S0006-8993(02)03477-7

lohexanecarboxamide trihydrochloride; RBI) [10,30,14]. Rats were given intraperitoneal (i.p.) injections of the 5-HT_{1A} receptor antagonists: NAN-190 (2.5 mg/kg; $n=4$); *p*-MPPI; 10 mg/kg; $n=3$); WAY-100635; 5 mg/kg; $n=4$), or saline vehicle ($n=4$). All three 5-HT_{1A} receptor antagonists were made in 0.9% saline. Thirty minutes following injection, all of the animals were injected with 200 mg/kg (i.p.) 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO, USA), a marker of DNA synthesis which labels proliferating cells and their progeny [25,26]. Two hours after BrdU injection, rats were deeply anesthetized with 100 mg/kg (i.p.) Nembutal and were transcardially perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed from the skulls, postfixed overnight, and processed for BrdU immunohistochemistry as described below. The 2 h survival time was chosen because it is more than sufficient for the uptake of BrdU [25,26], but not for the completion of cell division.

BrdU immunocytochemistry was performed according to a previously reported protocol [17]. Sections were mounted onto Vectabond-coated slides (Vector Labs., Burlingame, CA, USA) and dried under an air stream for several hours. The slides were then incubated with 0.1% trypsin in Tris buffer (pH 7.4) for 10 min, rinsed twice in phosphate buffer saline (PBS, pH 7.4), incubated 30 min in 2 M HCl, rinsed three times in PBS (pH 6.0), incubated for 20 min in 3% normal horse serum in PBS (pH 7.4), and incubated in anti-BrdU (mouse monoclonal, 1:250; Novocastra Labs., UK) in PBS with 0.5% Tween-20 overnight at 4 °C. On the following day, tissue was rinsed several times in PBS and then processed with the avidin–biotin–horseradish peroxidase method (Vectastain Elite ABC, Vector Labs.). Sections were incubated in secondary biotinylated antisera with normal serum in PBS for 90 min, incubated in avidin–biotinylated peroxidase substrate in PBS for 90 min, with PBS rinses in between, and then reacted in 3,3′-diaminobenzidine and H_2O_2 for 2–10 min. Specificity of antibody labeling was confirmed by treatment of control sections the same as described above, but without primary antisera. All slide-mounted sections were lightly counterstained for Nissl, dehydrated, cleared, and coverslipped under Permount. In all analyses conducted, slides were coded before the analysis, and the code was not broken until the analysis was completed. For counting BrdU-labeled cells, a modified version of the stereological optical disector method [31] with IMAGE PRO software (Media Cybernetics, Bethesda, MD, USA) was used on BrdU immunoperoxidase-stained sections. For every 12th section of the dentate gyrus, numbers of BrdU-labeled cells were determined at ×400 and ×1000 using an Olympus BX-60 light microscope. BrdU-labeled cells were counted throughout the entire bilateral extent of the dentate gyrus for each 1:12 series. Data were expressed as estimates of the total number of BrdU-labeled cells for each case, and then averaged for each treatment condition. The initial statistical comparisons were performed by a one-way ANOVA, followed by a posthoc Tukey HSD, and values were represented in terms of mean±S.E.M. for each experimental condition.

5-HT_{1A} antagonist treatment produced a statistically significant ~30% reduction (omnibus $F_{3,11}=7.709$; $P=0.0048$) in the number of BrdU-immunoreactive cells in the dentate gyrus after treatment with WAY-100,635 ($n=4$; 1662±95; $P<0.05$), *p*-MPPI ($n=3$; 1960±177; $P<0.05$) and NAN-190 ($n=4$; 1872±223; $P<0.05$), compared to saline vehicle-treated control rats ($n=4$; 2652±114; Fig. 1). The majority of BrdU-immunoreactive cells were found in the subgranular zone and innermost portion of the granule cell layer (Fig. 2). Some BrdU-immunoreactive cells were also found in the hilus.

The results of this study show that 5-HT_{1A} receptor antagonist treatment decreases the rate of cell proliferation in the dentate gyrus. Since all three 5-HT_{1A} receptor antagonists produced approximately the same magnitude of decrease in cell proliferation, it seems clear that this result was not a nonspecific drug effect. Given that the number of granule neurons added to the cell layer in 1 month is estimated to be about 6% of the total population [7], a decrease—such as the 30% that we observed—in dividing progenitors could have a substantial impact on the functional properties of the dentate gyrus. Since the rats in this study were perfused 2 h after BrdU administration, we can only speculate that this reduction in cell proliferation would result in fewer numbers of granule neurons over time, even though >70% of proliferating cells that survive in the dentate gyrus become neurons [9]. Previous work suggests that 5-HT depletion decreases cell proliferation and the neurogenesis in the dentate gyrus [5], and that selective serotonin reuptake inhibitors increase cell proliferation and neurogenesis [23,20].

From these previous reports, taken together with the results from this study, we suggest that the 5-HT_{1A} receptor is an important site for 5-HT's regulatory influence on neurogenesis in the dentate gyrus. Anatomically, the dentate gyrus is enriched with 5-HT_{1A} receptors [16,2], and receives serotonergic afferents from the raphe nuclei [1,27]. These results do not address whether the 5-HT_{1A} receptor is found on precursor cells, or on some other cell type in the dentate gyrus. However, it does not seem likely that the observed reduction in cell proliferation is via the blockade of 5-HT_{1A} receptors in the raphe nuclei, since local and systemic administration of 5-HT_{1A} receptor antagonists increases the firing rate of serotonergic cells, and this increase would be expected to stimulate the release of 5-HT at target sites, and in turn, at postsynaptic 5-HT_{1A} receptors. Finally, the aforementioned work from Daszuta's laboratory has shown that reductions of 5-HT lead to decreases, not increases, in cell proliferation in the dentate gyrus [5]. Future studies could resolve this issue by locally administering 5-HT_{1A} antagonists into the dentate gyrus.

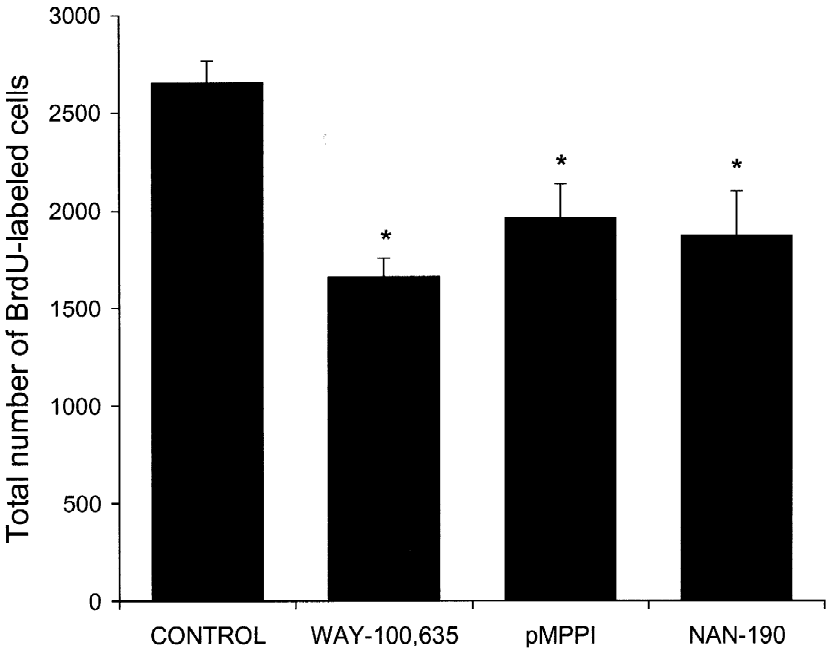

Fig. 1. Stereological estimates for the total number of BrdU-labeled cells in the dentate gyrus following 5-HT_{1A} receptor antagonist administration. Bars represent mean+S.E.M. The asterisk indicates a significant difference from Controls ($P<0.05$).

That short-term 5-HT_{1A} receptor antagonist treatment resulted in a roughly 30% decrease in cell proliferation implies that other factors are also involved in regulating the turnover and rate of addition of granule neurons in the dentate gyrus. Previous work has shown that there may be multiple factors in the regulation of hippocampal neurogenesis, such as adrenal and gonadal steroids [6,29,4], excitatory glutamatergic input [8], and opiates [11]. Therefore, it does not seem likely that long-term treatment with 5-HT_{1A} receptor antagonists would completely abolish neurogenesis in this region. Studies are currently under way to determine whether cell proliferation and neuro-

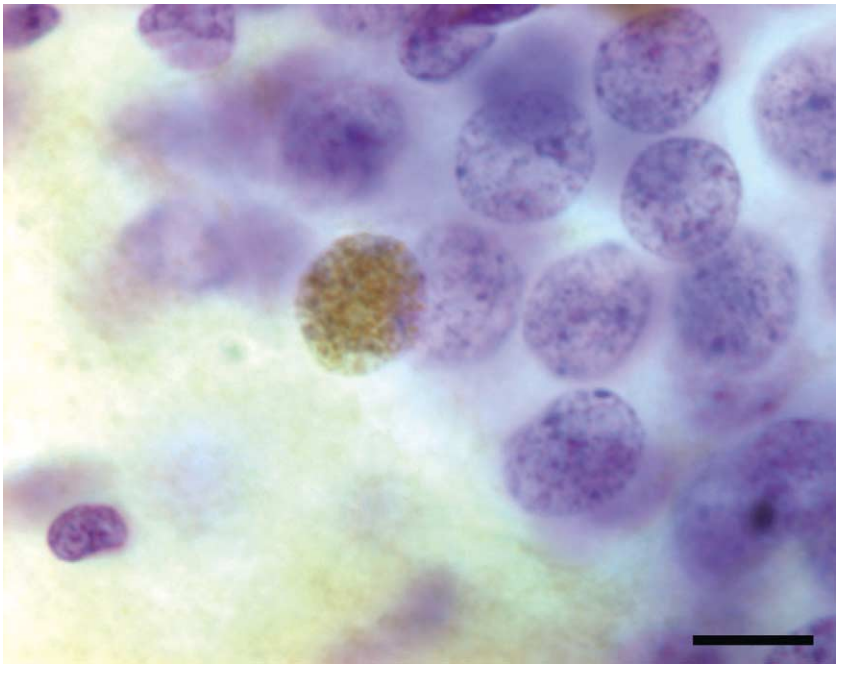

Fig. 2. Photomicrograph of BrdU-immunoreactive cell in the subgranular zone of the dentate gyrus, counterstained with cresyl violet. There was an ~30% reduction in the number of BrdU-immunoreactive cells following pharmacological treatment with three different 5-HT_{1A} receptor antagonists. Scale bar=10 μm.

genesis are affected by the physiological changes in serotonergic tone, such as that which is known to occur across the sleep–wake and light–dark cycles.

Acknowledgements

We thank Patima Tanapat for expert technical assistance, and also Casimir Fornal and Elizabeth Gould for providing conceptual and technical expertise.

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