

Short communication

Adrenal steroids suppress granule cell death in the developing dentate gyrus through an NMDA receptor-dependent mechanism

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Abstract

Treatment with the NMDA receptor antagonist MK-801 prevented the adrenal steroid-induced suppression of cell death, determined by both morphological identification of pyknotic cells and TUNEL staining, in the dentate gyrus in rat pups. This finding suggests that adrenal steroids naturally promote granule cell survival via NMDA receptor activation. © 1997 Elsevier Science B.V.

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Cell death is an important developmental phenomenon that contributes to the determination of the final number of neurons within a given neural region [1]. The majority of mammalian brain regions undergo cell death, typically detected as an increase in the number of pyknotic cells and a decrease in the number of healthy cells, several days after a discrete period of cell proliferation [1]. In contrast, the granule cell population of the rat dentate gyrus undergoes a period of massive cell death during the time of maximal neurogenesis, the first postnatal week [6,11]. During this period of coinciding neuron production and elimination in the dentate gyrus, a net decrease in the number of granule neurons can be detected suggesting that granule cell death outweighs granule cell production [6].

Previous studies have indicated that adrenal steroids regulate granule cell survival throughout the life of the rat. Treatment of rat pups with adrenal steroids during the first postnatal week, when basal and stress levels of adrenal steroids are low [10] and the rate of granule cell death is high [6], significantly enhances granule cell survival [7]. In addition, removal of circulating adrenal steroids at times ranging from the end of the second postnatal week throughout adulthood, when adrenal steroid levels are relatively high [10] and the rate of granule cell death is low [5,6], induces apoptotic cell death in the granule cell

population [5,7,12–14]. However, the observation that many degenerating cells in the developing dentate gyrus do not express adrenal steroid receptors [4] suggests that these hormones do not influence cell survival directly.

Blockade of the NMDA receptor subtype of the glutamate receptor during the first postnatal week dramatically increases granule cell death throughout the dentate gyrus [3]. This observation suggests that NMDA receptor activation normally enhances granule cell survival and presents the possibility that adrenal steroids suppress cell death through NMDA receptors. In order to test this possibility, we examined the density of degenerating cells, using Nissl staining and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) in the dentate gyrus granule cell layer of rat pups treated with either an NMDA receptor antagonist, corticosterone or both.

Timed pregnant (15 day) Sprague–Dawley rats (Charles River) were housed individually and provided with unlimited access to food and water. On the day after birth, postnatal day (P) 2, the rat pups were pooled, and 5 males and 5 females were randomly distributed to each dam. On P2, P3, P4, and P5, rat pups received one of the following daily treatments: (1) injection of MK-801 (0.1 mg/kg in saline, i.p.) followed 1 h later by injection of corticosterone (cort) (5.0 mg/kg in sesame oil, s.c.); (2) injection of MK-801 followed 1 h later by injection of sesame oil; (3) injection of saline followed 1 h later by injection of cort; or (4) injection of saline followed 1 h later by injection of sesame oil. Twenty-four hours after the last cort or oil injection, the rat pups were anesthetized with

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Metofane and either transcardially perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (PB) for Nissl staining or decapitated for TUNEL staining. These treatment regimens for MK-801 and cort have previously been found to increase and decrease, respectively, the number of degenerating cells in the dentate gyrus [3,7].

The brains of perfused rats were removed and postfixed overnight in a solution of 4.0% paraformaldehyde in PB, cryoprotected in 30% sucrose in phosphate-buffered saline, and frozen on dry ice. Coronal sections (18 μm thick) were cut on a cryostat, thaw-mounted onto gelatinized slides, stained for Nissl using Cresyl violet and coverslipped under Permount.

For TUNEL staining, the brains of decapitated animals were dissected out and immediately frozen on dry ice. Coronal sections (18 μm) were cut with a cryostat, thaw-mounted onto gelatinized slides, fixed in 4.0% paraformaldehyde in 0.1 M PB (30 min), blocked in 0.3% hydrogen peroxide in methanol (20 min) and permeabilized in 0.1% Triton X-100 in 0.1% sodium citrate (5 min). The sections were then incubated in TdT and fluorescein-labeled dUTP (Boehringer Mannheim) for 1 h (37°C), anti-fluorescein conjugated with horseradish peroxidase (Boehringer Mannheim) for 1 h (37°C) and diaminobenzidine with hydrogen peroxide in PB for 10 min. The sections were then stained for Nissl using Cresyl violet and coverslipped under Permount. Control sections were treated as described above without TdT and demonstrated no non-specific staining.

The slides were coded prior to quantitative analysis, and the code was not broken until the analysis was complete. Neuroanatomically matched sections were selected from the middle dentate gyrus, where the hippocampus is oriented horizontally and the suprapyramidal and infrapyramidal blades are joined at the crest. On each selected Nissl-stained section, the number of pyknotic cells was counted in the granule cell layer (suprapyramidal and infrapyramidal blades combined). Pyknotic cells were identified by their condensed, darkly stained spherical chromatin and light or absent cytoplasm. On each selected TUNEL-stained section, the number of labeled cells was determined in the granule cell layer (suprapyramidal and infrapyramidal blades combined). The cross-sectional areas of the granule cell layer were determined with a Zeiss Interactive Digitizing Analysis System (ZIDAS) and the counts were expressed as densities (number of degenerating cells/ mm^2). The data were subjected to two-way ANOVA (cort \times MK-801) followed by Tukey HSD post-hoc comparisons.

Pyknotic cells and TUNEL-stained cells were observed in the granule cell layer of the dentate gyrus in all sections from the control group (Fig. 1). Quantitative analysis of pyknotic cell density indicated a significant interaction between cort and MK-801 treatments. Post-hoc comparisons demonstrated that cort injections resulted in significantly lower pyknotic cell densities than oil injections in

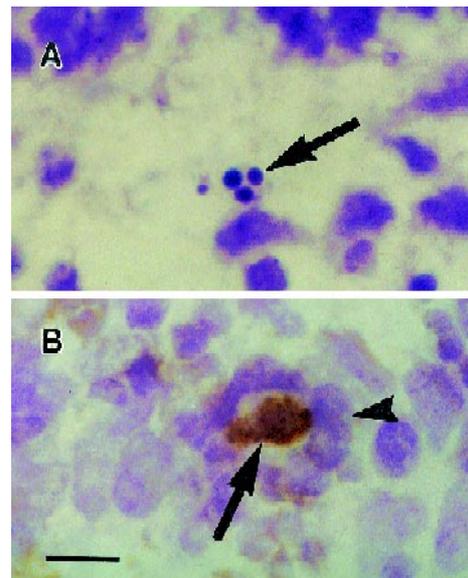


Fig. 1. Representative degenerating cells in the dentate gyrus. A: Nissl-stained degenerating cell (arrow) with pyknotic morphology. B: TUNEL-stained degenerating cell (arrow) that appears to be morphologically indistinguishable from neighboring cells (arrowhead). Scale bar in B equals 20 μm and applies to both frames.

rats not treated with MK-801. However, no significant change was found with cort treatment in animals that received MK-801 injections (Fig. 2). MK-801-treated rats had significantly higher pyknotic cell densities compared to that of saline-treated rats, both cort-injected and oil-injected (Fig. 2). The mean cross-sectional area of the granule cell layer was not significantly altered by any of the treatments, indicating that the observed differences in degenerating cell density reflect changes in the total number of dying cells.

A significant interaction between cort and MK-801 treatment was also indicated by quantitative analysis of density of TUNEL-stained cells (Fig. 3). The relationships

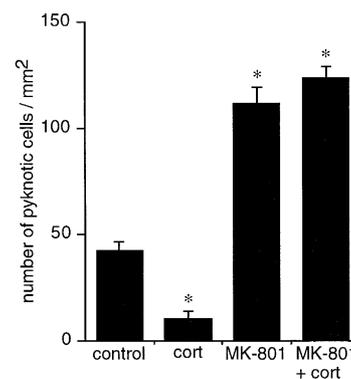


Fig. 2. Treatment with MK-801 prevents the corticosterone-induced decrease in density of pyknotic cells. Bars represent mean \pm S.E.M. each obtained from 5 animals. Asterisk represents significant difference from control ($P < 0.05$). No significant difference was observed between MK-801 and MK-801 + cort.

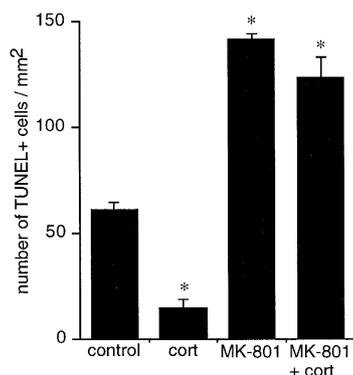


Fig. 3. Treatment with MK-801 prevents the corticosterone-induced decrease in density of TUNEL-stained cells. Bars represent mean + S.E.M. each obtained from 5 animals. Asterisk represents significant difference from control ($P < 0.05$). No significant difference was observed between MK-801 and MK-801 + cort.

between the treatment groups revealed by post-hoc comparisons of TUNEL-stained cell data were similar to those seen in the examination of Nissl-stained pyknotic cells (Fig. 3). However, the counts of TUNEL-stained cells were slightly higher than those of pyknotic cells in all groups (compare Fig. 2 with Fig. 3). Although many TUNEL-stained cells in tissue counterstained for Nissl appeared pyknotic based on their morphological characteristics, i.e., condensed, darkly stained spherical chromatin with pale or absent cytoplasm, some degenerating cells in TUNEL-stained tissue did not appear pyknotic (Fig. 1). Thus, it appears that TUNEL also stains degenerating cells shortly before they become morphologically identifiable as pyknotic cells.

The results of this study demonstrate that cort and NMDA receptor antagonists both affect developmental granule cell survival in the dentate gyrus, but that the effect of cort can be prevented by a blockade of NMDA receptor activation. It is unlikely that the failure to find an additive effect of the two treatments is due to a floor or ceiling effect, since these drugs produce opposite effects. Collectively, these findings suggest that adrenal steroids influence granule cell survival via NMDA receptors.

Several recent studies have demonstrated effects of adrenal steroids on excitatory amino acids in the hippocampus of the adult rat. First, stress-induced increases in cort have been shown to increase glutamate release in the hippocampus of adult rats [8,16]. Second, adrenal steroids have been shown to alter LTP, an NMDA receptor-mediated electrophysiological response [2], in the dentate gyrus of adult rats [9,15]. Third, adrenal steroids have been shown to alter the expression of NMDA receptor subunit mRNAs in the adult rat hippocampus [17]. The results of these studies present several different, but not mutually exclusive, mechanisms whereby adrenal steroids could influence granule cell survival in the developing dentate gyrus: (1) by increasing the release of glutamate; (2) by potentiating the responses of existing NMDA receptor

channels; or (3) by increasing the expression of NMDA receptors. The extent to which these mechanisms underlie the action of adrenal steroids on NMDA receptor-mediated cell death in the developing dentate gyrus remains to be determined.

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