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Short communication

Activation of the type 2 adrenal steroid receptor can rescue granule cells from death during development

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Abstract

To determine whether activation of the type 2 adrenal steroid receptor affects granule cell death in the developing dentate gyrus, we treated rat pups with the type 2 receptor agonist RU28362 and examined degenerating cells using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) and Nissl staining. RU28362 administration decreased the numbers of degenerating granule cells suggesting that type 2 receptor activation can rescue granule cells from degeneration. © 1997 Elsevier Science B.V.

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Granule neurons of the dentate gyrus are produced primarily during the postnatal period in the rat with maximal proliferation of granule cell precursors occurring on postnatal day 6 (PD 6) [1,12]. Unlike the majority of brain regions that have distinct periods of cell proliferation and cell death, the granule cell population undergoes massive cell death during the time of peak cell production [5,12]. This cell death, characterized by an increase in the number of pyknotic or degenerating cells, substantially affects the formation of the granule cell population by resulting in a net decrease in the number of granule neurons [5].

The survival of granule neurons in the dentate gyrus appears to be dependent on the levels of circulating adrenal steroids. Experimental increases in the levels of the glucocorticoid corticosterone or the mineralocorticoid aldosterone during the first postnatal week decrease the number of degenerating cells in the granule cell layer of rat pups [6]. Conversely, removing adrenal steroids by adrenalectomy (ADX) after the period of naturally occurring cell death increases the number of degenerating cells in the granule cell layer [4]. Granule cells continue to require adrenal steroids throughout life as ADX in adulthood results in massive degeneration of the granule cell layer [2,13,14].

During the first postnatal week when naturally occurring granule cell death is maximal, the levels of adrenal steroids are very low [11]. However, low levels of corticosteroid binding globulin (CBG) during this time appear to result in levels of adrenal steroid receptor activation that are comparable to that observed in the adult [15]. Our observation that administration of the mineralocorticoid aldosterone (5 mg/kg) during the first postnatal week can prevent granule cell death [6] suggested that activation of type 1, or mineralocorticoid, receptors mediates this effect. However, because type 1 receptors are probably fully occupied during the first postnatal week, their involvement in an effect mediated by increased adrenal steroids is unlikely. Although type 1 receptors have a much higher affinity for aldosterone than type 2 receptors, a high dose of aldosterone can activate type 2 receptors as well [10]. The possibility that type 2 receptor activation can prevent granule cell death has not been directly tested. In order to determine whether this is the case, we examined the numbers of degenerating cells using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL), a marker of degenerating cells, and Nissl staining in the dentate gyrus of rat pups treated with the specific type 2 receptor agonist RU28362 during the time of naturally occurring cell death.

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, PD 2, the rat pups were pooled and 5 males and 5 females were

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randomly distributed to each dam. On PD 5, rat pups received a single subcutaneous injection of either the synthetic steroid RU28362 (5 mg/kg) in sesame oil or sesame oil alone. This dose of RU28362 is sufficient to activate type 2 receptors in the adult rat [7]. Twenty-four hours after the injection, the male rat pups were either decapitated for TUNEL staining or transcardially perfused for Nissl staining. The rats were treated on PD 5 and killed on PD 6 because a significant decrease in the number of granule neurons can be detected between these 2 days [5].

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

For Nissl staining alone, brains from perfused animals were dissected from the skulls and postfixed overnight in a solution of 4.0% paraformaldehyde in 0.1 M PB. The brains were cryoprotected in 30% sucrose in PBS, frozen on dry ice and cut on a cryostat. Brain sections (18 μm thick) were thaw-mounted onto gelatinized slides. The slides were stained for Nissl using Cresyl violet and coverslipped under Permount.

The slides were coded prior to quantitative analysis and the code was not broken until the analysis was complete. Neuroanatomically matched sections from the middle dentate gyrus, where the hippocampus is oriented horizontally beneath the corpus callosum and the suprapyramidal and infrapyramidal blades are joined at the crest, were selected for light microscopic analysis. For each selected section, the number of TUNEL-stained cells or Nissl-stained pyknotic cells was counted in the suprapyramidal and infrapyramidal blades of the granule cell layer. Pyknotic cells were characterized by condensed, darkly stained spherical chromatin and pale or absent cytoplasm. A minimum of four sections for each stain were analyzed from five animals per treatment group. The cross-sectional areas of the suprapyramidal and infrapyramidal blades were then determined with a Zeiss interactive digitizing analysis system (ZIDAS) and the data were expressed as densities (number of pyknotic cells/mm^2). The data were subjected to unpaired two-tailed Student's *t*-tests.

Light microscopic examination of TUNEL-stained and Nissl-stained sections revealed degenerating cells in the granule cell layer of all brains examined. In general, cells with pyknotic morphology were TUNEL stained (Fig. 1). Some TUNEL-stained cells, however, did not appear to have the characteristics of pyknotic cells (Fig. 1). Thus, the TUNEL-stained cell counts were typically higher than those of pyknotic cells in tissue stained for Nissl alone. Quantitative analysis revealed a significant decrease in the number of TUNEL-stained cells and pyknotic cells in the granule cell layer following RU28362 treatment. This effect was observed in both the suprapyramidal and infrapyramidal blades of the granule cell layer (Figs. 2 and 3).

The results of this study suggest that activation of type 2 receptors with the agonist RU28362 prevents degeneration of granule neurons during the period of naturally occurring cell death in the neonatal dentate gyrus. In our previous study on the developing dentate gyrus, the mineralocorticoid aldosterone was able to suppress cell death [6]. Although this was interpreted at the time as suggesting that type 1 receptors are involved in suppressing cell death, it should be noted that although adrenal steroid levels are low during the first postnatal week, low levels of CBG probably result in fully occupied type 1 receptors at this time [15]. Type 2 receptors, however, are not fully occu-

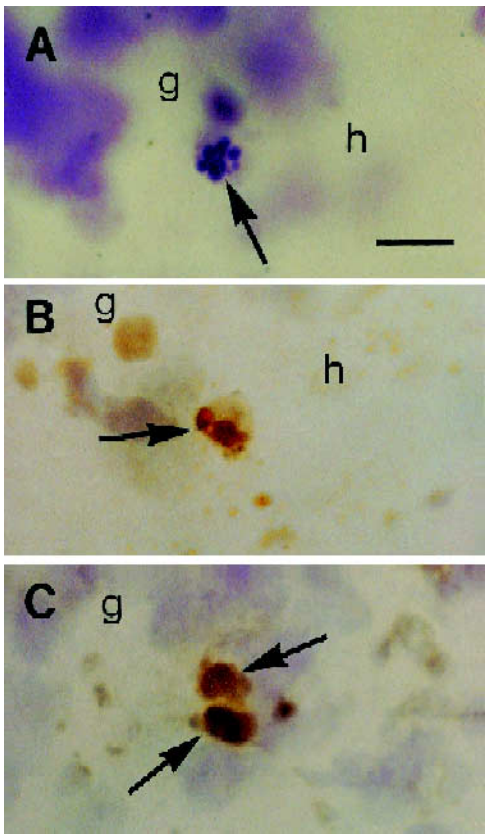

Fig. 1. Representative examples of a Nissl-stained degenerating cell (arrow, A) and TUNEL-stained degenerating cells (arrows, B, C). The TUNEL-stained cell in B has the morphological characteristics of a pyknotic cell whereas the cell in C does not. Scale bar in A represents 20 μm and applies to all frames.

pied at the time of naturally occurring cell death [15], making this receptor type available to participate in adrenal steroid-induced enhancement of granule cell survival. The observation that high doses of aldosterone can occupy type 2 receptors and have agonistic properties [10] is consistent with this possibility.

In the adult rat, ADX-induced degeneration of granule cells can be prevented with aldosterone at a dose that activated only type 1 receptors [16]. Activation of type 2 receptors with RU28362 only partially protects against granule cell death following ADX in adulthood [16], indicating that type 1 receptors play a critical role in granule cell survival in adulthood. During development, it is possible that type 1 receptor activation promotes the survival of mature, functionally connected granule cells, while type 2 receptor activation can rescue granule cells that would die naturally. The results of the present study suggest that two populations of granule cells, with different responses to activation of specific adrenal steroid receptors, may exist in the dentate gyrus.

Because type 2 receptors are only partially occupied during development [15], they are available to respond to stress-induced rises in glucorticoid levels. Although the rat pup has a diminished adrenal steroid stress response during the first two postnatal weeks [11], increases in the level of corticosterone can be detected following stressful experiences [11,15]. Stress-induced increases in corticosterone would activate unoccupied type 2 receptors and thus enhance the survival of granule neurons in the dentate gyrus, presenting a potential mechanism whereby stress could alter the development of the dentate gyrus.

Both type 1 and type 2 receptors are present in the dentate gyrus as early as PD 2 [8,9]. Our previous study has shown that the majority of degenerating cells in the dentate gyrus during the first postnatal week express type 2, but not type 1, receptors [3]. However, it is unlikely that type 2 receptors located in granule cells are directly responsible for enhanced granule cell survival following adrenal steroid treatment. The results of a recent study have shown that adrenal steroids enhance granule cell survival indirectly via NMDA receptor-mediated excitatory input (Gould et al., unpublished observations) suggesting that adrenal steroid receptors located in an afferent population may be the initial mediators of this effect.

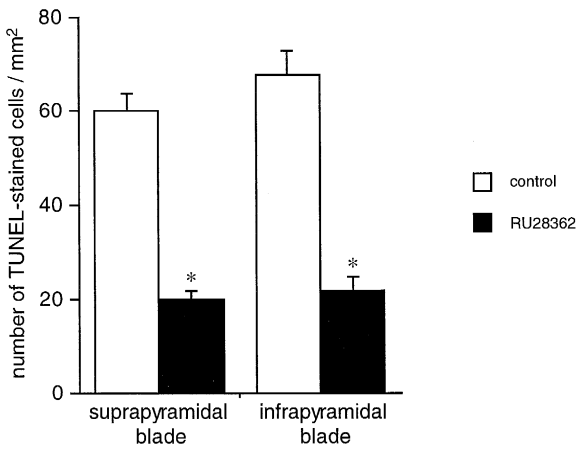

Fig. 2. The mean density of TUNEL-stained cells in the dentate gyrus of rats treated with RU28362 (solid bars) or the vehicle (open bars). Bars represent mean + S.E.M., each obtained from five animals. Treatment with RU28362 resulted in a significant decrease in the density of TUNEL-stained cells in both the suprapyramidal and infrapyramidal blades of the granule cell layer. Asterisks represent significant difference from control, $P < 0.05$, unpaired Student's *t*-tests.

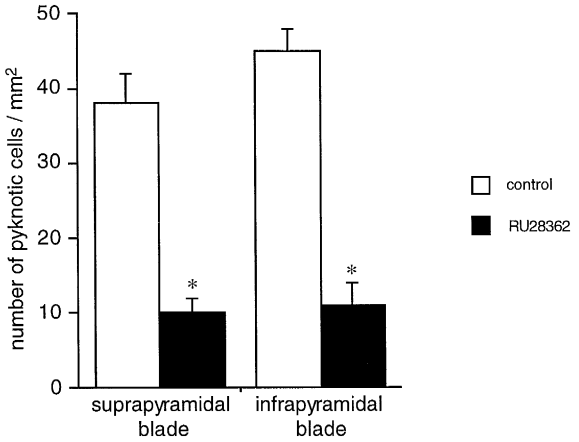

Fig. 3. The mean density of Nissl-stained pyknotic cells in the dentate gyrus of rats treated with RU28362 (solid bars) or the vehicle (open bars). Bars represent mean + S.E.M., each obtained from five animals. Treatment with RU28362 resulted in a significant decrease in the density of pyknotic cells in both the suprapyramidal and infrapyramidal blades of the granule cell layer. Asterisks represent significant difference from control, $P < 0.05$, unpaired Student's *t*-tests.

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