Adult hippocampal cell birth and death in relation to stress, aging and the vasculature

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Increased P27Kip1 Protein Expression in the Dentate Gyrus of Chronically Stressed Rats Indicates G1 Arrest Involvement

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Abstract

Various chronic stress paradigms decrease new cell proliferation in the hippocampal dentate gyrus, yet the exact underlying mechanism is still unclear. In the G₁ phase of the cell cycle, both stimulatory and inhibitory signals derived from the extracellular environment converge. Corticosteroids, which increase during stress and are well-known anti-mitotics, cause cells in vitro to arrest in the G₁ phase. Following 3 weeks of unpredictable stress, we therefore expected a change in protein expression of various important G₁ cell cycle regulators in the adult rat subgranular zone. Using quantitative immunocytochemistry, we show that particularly cyclin-dependent kinase inhibitor p27Kip1 expression is significantly increased. In addition, 3 weeks of recovery after stress normalized the numbers of p27Kip1-expressing cells, consistent with the recovered adult cell proliferation in these animals. P27Kip1-positive cells do not overlap with GFAP-staining and only to a limited extent with Ki-67-expressing cells. Numbers of cyclin E- and cyclin D₁-expressing cells did not change after chronic stress. These results indicate that chronic stress causes cycling cells in the adult hippocampus to arrest in G₁, thereby providing more mechanistic insight in the stress-induced decrease in cell proliferation.
Introduction

Exposure to both acute and chronic stress decreases newborn cell proliferation in the hippocampal dentate gyrus (DG) (Czeh et al., 2002; Fuchs et al., 2001; Gould et al., 1997; Gould et al., 1998; Heine et al., 2004a; Tanapat et al., 2001), a brain region where both neurogenesis and apoptosis continue to take place in adult life. Although elevated glucocorticoid (GC) levels during stress are believed to be the main regulators of the decreased new cell birth (Cameron and McKay, 1999; Gould et al., 1991; Heine et al., 2004a; Sloviter et al., 1989), the underlying mechanism is so far unclear.

Regulation of mammalian cell proliferation by extracellular signals is thought to occur primarily during the G1 phase of the cell cycle (Pardee, 1989). During this interval, both stimulatory and inhibitory signals derived from the extracellular environment converge onto the cell cycle control machinery, the engine of which is driven by the cyclic expression of cyclins and cyclin-dependent kinases (Cdks) and opposed by Cdk inhibitors. Growth factor receptor coupled tyrosine kinases e.g. activate various cytoplasmic signaling molecules, which ultimately lead to the activation of G1 phase specific regulatory proteins like cyclin D1 and E (reviewed in Sherr, 1993). These cyclins bind to Cdk-4 and Cdk-2, respectively, to form complexes, which eventually lead to the stimulation of other factors necessary for the initiation of the S phase (Kato et al., 1993; Weinberg, 1995).

In addition to cyclin and Cdk expression, Cdk inhibitors like CIP/KIP and INK4 family members are essential for G1 transition (Cheng et al., 1999; Sherr and Roberts, 1999; Polyak et al., 1994; Serrano, 1997). Overexpression of Cdk inhibitors p21Cip1 or p27Kip1, induced by e.g. deprivation of growth factors, causes cells to arrest in G1 by complex formation with G1 cyclin / Cdks and so inhibition of their kinase activity (Cheng et al., 1999; Harper et al., 1995; Lepley and Pelling, 1997; Rivard et al., 1996; Sherr and Roberts, 1999).

Many in vitro studies have demonstrated that GCs or glucocorticoid receptor (GR) activation, via modulation of specific G1 cell cycle proteins, leads to an inhibition of proliferation and induces cell cycle arrest (Baghdassarian et al., 1998; Corroyer et al., 1997; Fernandes et al., 1999; Goya et al., 1993; Greenberg et al., 2002; Jiang et al., 2002; Ramalingam et al., 1997; Rogatsky et al., 1997; Sanchez et al., 1993). Following exposure to GCs (or dexamethasone) decreased levels of cyclin D, Cdk-4, Cdk-2, E2F, reduced pRb phosphorylation (Fernandes et al., 1999; Greenberg et al., 2002; Rogatsky et al., 1997), and increased levels of the cell cycle inhibitors p21Cip1 (Greenberg et al., 2002) and p27Kip1 (Jiang et al., 2002) were observed. It is so far unknown whether similar mechanisms apply.
Part II: Stress - Cell Cycle

to the in vivo situation and whether this bears any relevance to the stress effects on adult proliferation in the DG.

The present study therefore tested the hypothesis that chronic stress inhibits progression of the cells through the G1 phase by regulating expression of G1 cell cycle proteins. We used quantitative immunocytochemistry and stereology to assess the total number of cyclin E, D1 and p27Kip1-positive cells in the hippocampal subgranular zone (SGZ) of control and chronically stressed rats, and of rats that were allowed to recover after stress. These numbers were compared to the total number of cells in cell cycle, i.e. Ki-67 positive cells.

Material & Methods

Animals

All animals were male Wistar rats (Harlan, the Netherlands) studied at 10 weeks of age. Two rats were housed together under controlled conditions (21°C room temperature, 60% humidity, lights on from 8.00-20.00h) with food and water available ad libitum. Rats were randomly assigned to the control (n = 13), chronic stress (n = 11) or acute stress group (n = 3). The local animal ethical committee of the University of Amsterdam approved of all experiments.

Stress protocols

Animals were acutely or chronically stressed according to a multiple unpredictable stress paradigm as described earlier in detail (Heine et al., 2004a). Briefly, chronically stressed rats were exposed to different stressors twice daily for 21 days, consisting of (cold) immobilization, forced (cold) swimming, crowding, isolation and vibration. A recovery group was included that was allowed to survive for an additional 3 weeks following stress exposure. The acutely stressed rats were stressed for one day, by cold immobilization (1 h at 4°C) in the morning and by forced swimming (30 min at 25°C) in the afternoon. To exclude effects of handling of the stressed rats, control rats were handled twice daily. On forehand, certain criteria were set for excluding animals from the study based on weight loss, or the possible occurrence of wounds. No animals were excluded from the study.

Brain tissue preparation

Animals were deeply anaesthetized in the morning by i.p. injection of pentobarbital sodium salt (Nembutal 1 ml/kg bodyweight; A.U.V., Cuijk, The Netherlands) and then
perfused transcardially with 0.9% physiological saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). After in situ post fixation overnight at 4°C, the brains were taken out and the two hemispheres separated by a midline cut. The left hemisphere was then equilibrated in 30% sucrose overnight, frozen and sectioned in a coronal plane at 40 μm thickness using a sliding microtome. Sections were stored at -20°C in 2% Dimethylsulphateoxide (DMSO), 20% glycerol, 0.05M Tris buffer pH 7.6 until used.

**Immunocytochemistry**

Free-floating sections were washed in 0.01M phosphate buffered saline (PBS) pH 7.4 (for cyclin D1 immunocytochemistry, wash and incubation procedures were done with 0.05M Tris buffered saline, pH 7.6) and placed in plastic jars filled with citrate buffer (0.01 M, pH 6.0) and put in a microwave (MW) oven for 10 min at 400W. For standardization purposes, two filled jars were always used, irrespective of the number of sections that rotated in the central part of a domestic MW oven (Samsung M 6235, 800 W). After 30 min cooling at room temperature, endogenous peroxidase activity was blocked by 1.5% peroxide treatment for 15 min. After several rinses in PBS, 5% Normal Goat Serum (NGS) / 0.3% Triton X-100 / 1% Bovine serum albumine (BSA) in PBS (for cyclin D1 immunocytochemistry; 4% NGS / 0.3% Triton X-100 in TBS) was applied for 1 hr in order to prevent nonspecific binding. Sections were incubated with the primary antibody mouse monoclonal anti-p27Kip1 (K25025; 1:3000; BD Transduction Laboratories) or rabbit polyclonal anti-cyclin E (sc-481; 1:1000; Santa Cruz Biotechnology, Heerhugowaard, The Netherlands) diluted in 1% NGS / 0.3% Triton X-100 / 0.1% BSA in PBS (PBS+), or with the primary antibody rabbit polyclonal anti-cyclin D1 (sc-753; 1:50; Santa Cruz Biotechnology, Heerhugowaard, The Netherlands) diluted in 3% NGS / 0.3% Triton X-100 in TBS (TBS+), for 1 hr at RT and then overnight at 4°C. Specificity of these antibodies has been demonstrated before elsewhere. For negative control, the first antibody was omitted. With intermittent rinses in PBS, sections were then incubated with biotinylated sheep anti-mouse IgG (1:200) or biotinylated sheep anti-rabbit (1:100) (Amersham Life Sciences, Den Bosch, Netherlands, 1:200) in PBS+ / TBS+ for 1.5 hrs and amplified with Avidine Biotine complex (ABC, 1:800, Vectastain Elite, Brunschwig Chemie, Amsterdam, The Netherlands) in PBS / BSA 1% for 2 hrs. The ABC signal was further amplified with biotinylated tyramide (1:500, produced and kindly provided by Dr. I. Huetinga, Netherlands Institute for Brain Research, Amsterdam, The Netherlands) and 0.01% peroxide in PBS for 30 min followed by another 1.5 hr incubation in ABC (1:1000). Color development was performed with nickel-enhanced diaminobenzidine (0.50 mg/ml DAB / 0.04% nickel
ammonium sulphate / 0.01% H₂O₂) after which sections were mounted, dried, dehydrated, passed through xylene and coverslipped with Entellan (Merck).

P27Kip1 and GFAP / Ki-67 immunofluorescence

After washing free-floating sections in 0.01M PBS pH 7.4, pretreatment and blocking was done as described above. Sections were incubated overnight with the primary antibodies mouse monoclonal α-p27Kip1 (1:500) and rabbit polyclonal α-GFAP (Chemicon International, Amsterdam, the Netherlands, 1:200) or α-Ki-67 (Novocastra, New Castle, UK, 1:100) in PBS+. The next day, sections were washed, blocked with PBS+ for 30 min, the first antibodies were subsequently detected with Alexa Fluor 546 and Alexa Fluor 488, respectively and embedded in Vectashield (Vector Laboratories). P27Kip1 / GFAP (p27Kip1 / Ki-67) double immunostained sections were evaluated using a Zeiss LSM 510 (Carl Zeiss, Jena, Germany) confocal laser-scanning device equipped with a Plan-NeoFluar 100x / 1.3-oil lens.

Quantification and stereology

For stereological quantification of the p27Kip1 (DAB immunocytochemical labeling), cyclin D₁ and cyclin E-positive cell numbers, serial sections (40 μm, every 12th section) were taken through the entire rostro-caudal extent of one hippocampus hemisphere. The numbers of newborn cells were assessed in the SGZ, in a stereological approach. The total number of immuno-positive cells per animal was estimated by multiplying the sum of all the positive cells found in the serial sections by 12.

Statistics

Statistical analysis was performed using an unpaired Student’s t-test with a two-tailed p-value. Differences are considered significant, when the two-tailed P-value < 0.05. When standard deviations were not equally distributed between the two groups, a non-parametrical Mann-Whitney-U-test was applied to the data.

Results

Three weeks of unpredictable stress significantly decreased the numbers of proliferating (Ki-67 positive) cells in the hippocampal DG (Heine et al., 2004a). To examine changes in the G₁ phase of the cell cycle, we stereologically assessed the total number of various important G1 cell cycle regulators in the SGZ. Immunocytochemistry
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revealed a large population of p27Kip1-positive cells throughout the SGZ (Fig. 1A upper panel), with lower amounts in the hilar region. Confocal analysis showed that P27Kip1-positive cells in the SGZ did not co-label with GFAP, although a close apposition to the processes of GFAP-expressing astrocytes was apparent (Fig. 1B left + lower panel, see Appendix). Furthermore, the population of p27Kip1-expressing cells hardly overlapped with the population of Ki-67-expressing cells (Fig. 1B right panel, see Appendix).

*Figure 1A:*

![p27, cyclin D1, cyclin E immunocytochemistry](image)

*Figure 1A: Typical examples of p27Kip1, cyclin D1, and cyclin E immunocytochemistry. A large population of p27Kip1-expressing cells is present particularly in the SGZ, while also expression is found in the hilar region. Cyclin D1 immunostaining visualizes less but still considerable numbers of positive cells in the SGZ, but with a great variation in individual protein expression levels. The single cyclin D1-positive cells (arrow head) often have a higher intensity compared the clusters of positive cells (arrow). Cyclin E immunocytochemistry gave a low number of positive cells in the SGZ (arrow).*

Total numbers of p27Kip1-positive cells were significantly increased after chronic stress (p = 0.01, unpaired t-test equal variances) as presented in Fig. 2A. Following an additional three weeks of recovery after chronic stress, these numbers returned to control levels and were not different anymore from the control group (p = 0.39, unpaired t-test equal variances). Compared to the chronic stress group, the recovery group was not significantly
different \( (p = 0.27, \) unpaired t-test equal variances). The increase in p27Kip1-positive cells, indicates that increased numbers of \( G_0 / G_1 \) resting cells are present in the SGZ of chronically stressed animals. Acute stress did not change the number of p27Kip1-positive cells \( \) (Control: 42296 ± 2178 (SEM); Acute Stress: 40896 ± 1878; \( p = 0.96, \) unpaired t-test equal variances).

Cyclin D1 expression is induced in mid \( G_1 \) phase, and forms the rate limiting step in the formation of the cyclin D / Cdk-4 complex. As such, it plays a significant role in controlling \( G_1 \) phase progression. Cyclin D1 immunocytochemistry revealed positive cells in the SGZ with different individual protein levels, likely due to their presence in different phases of the cell cycle (Fig. 1). The stereologically determined number of cyclin D1-positive cells are presented in Fig. 2B. Total numbers were not changed after chronic stress or following 3 more weeks of recovery \( \) (Fig. 2B).

Cyclin E expression peaks only shortly in late \( G_1 \). Indeed, cyclin E immunolabeling revealed low numbers of positive cells (Fig. 1). The total number of cyclin E-positive cells did not significantly differ between the groups (Fig. 2C).

*Figure 2:*

![Figure 2](image-url)

**Figure 2:** Quantification of the mean total numbers (± SEM) of p27Kip1- (A), cyclin D1- (B) and cyclin E-positive cells (C) in the SGZ of the 10-week old rat hippocampus, compared to the numbers of Ki-67-positive cells (A) as estimated before (Heine et al., 2004a). A) After 3 weeks of chronic stress \( (n = 11) \) the number of p27Kip1-positive cells increased significantly \( (p = 0.01, \) unpaired t-test equal variances) compared to control animals \( (n = 10) \). After an additionally 3 weeks of recovery \( (n = 7) \), the numbers returned to normal \( (p = 0.39) \), however were not yet significantly different from the stress group \( (p = 0.27) \). This trend is consistent with the decreased
number of Ki-67-positive cells in the same animals, suggesting a chronic stress-induced G₁ cell cycle arrest. B): The number of cyclin D₁-expressing cells did not change between the groups. C): There was also no significant change in the number of cyclin E-expressing cells.

We next tested whether the number of cells expressing any of the specific cell cycle markers correlated with the numbers found for the other markers within the same treatment group. Ki-67 numbers were found to correlate significantly with the cyclin D₁ cell numbers in the chronically stressed rats (R = 0.65; Fig. 3). Other correlations were not significant.

Figure 3: Within the group of chronically stressed rats, the Ki-67 numbers were found to correlate significantly with the cyclin D₁ cell numbers (R = 0.65; p = 0.03).

Discussion

To better understand the 30% reduction in adult proliferation after chronic stress (Heine et al., 2004a), we examined changes in protein expression of important G₁ cell cycle regulators in the SGZ of the rat hippocampus. We found significant increase in the number of p27Kip1-expressing cells in the SGZ after chronic, but not after acute stress. Furthermore, the recovery group showed levels that were not significantly different from the control or stress groups (Fig. 2). No changes were found in cyclin D₁- or cyclin E-expressing cell numbers. Notably, when reductions in adult proliferation are found in vivo using the S phase markers 3H-thymidine, Bromodeoxyuridine (BrdU), or the cell cycle-associated protein Ki-67, this can be caused, either by lower numbers of cycling cells in total or by a decrease in cell cycle length. Furthermore, cells in the early G₁ phase are not detected by Ki-67 immunocytochemistry, but are, on the contrary, by p27Kip1 immunocytochemistry. In agreement, our fluorescent double labeling showed that the p27Kip1-positive cells overlap to a limited extent with Ki-67 expression. The presently found increase in p27Kip1
expression, therefore, indicates that chronic stress has caused increases in the proportion of both newly formed cells in the SGZ, and of already proliferating cells, to arrest in G₁. This subsequently caused fewer cells to progress towards the S phase.

By preventing cells from progressing through the cell cycle, p27Kip₁ plays an important role in the regulation of cell proliferation (Fero et al., 1996; Kiyokawa et al., 1996; Nakayama et al., 1996). Previously, it has e.g. been shown that p27Kip₁ expression is induced by various anti-mitotic signals, such as cAMP in macrophages (Kato et al., 1994), rapamycin in T-lymphocytes (Nourse et al., 1994), serum deprivation in fibroblasts (Coats et al., 1996; Pagano et al., 1995), transforming growth factor-beta (Slingerland et al., 1994) and contact inhibition in epithelial cells (Polyak et al., 1994). P27Kip₁ overexpression inactivated G₁ cyclin / Cdk complexes and resulted in G₁ arrest (Massague and Polyak, 1995; Polyak et al., 1994; Sherr and Roberts, 1999; Toyoshima and Hunter, 1994), further underscoring its important role in control of G₁ arrest.

Glucocorticoids are well known for their anti-mitotic action in various cell types. Some studies suggest this effect occurs through G₁ arrest (Goya et al., 1993; Samuelsson et al., 1999; Sanchez et al., 1993), (Goya et al., 1993; Samuelsson et al., 1999; Sanchez et al., 1993), while decreases in growth-promoting factors might be involved as well (Fernandes et al., 1999; Greenberg et al., 2002; Rogatsky et al., 1997). Furthermore, corticosteroids caused an increase in CIP/KIP family members, i.e. the cell cycle inhibitor p21Cip₁ in fibroblasts (Greenberg et al., 2002; Ramalingam et al., 1997) and hepatoma cells (Cha et al., 1998), as well as in p27Kip₁ expression levels in different carcinoma cell lines (Rogatsky et al., 1999; Rogatsky et al., 1997; Zhu et al., 2003; Zhuang and Burnstein, 1998). GRs are thought to be involved in these corticosteroid effects, since in mouse mammary hyperplastic epithelial cell lines, the corticosterone-induced increase in p27Kip₁ protein level could be reversed by treatment with the glucocorticoid receptor blocker RU486 (Jiang et al., 2002). This suggests that GR signaling might also be involved in the observed increase in p27Kip₁ expression chronic stress, which is associated with increased corticosteroid levels (Heine et al., 2004a). The present study supports this view. If cells slow down in their progression through the G₁ phase, a coincident change in the number of cyclin D₁- and cyclin E-expressing cells might be expected as well. Especially since cyclin E is expressed after the restriction point, where cells can still complete division without growth factors (Ekholm et al., 2001; Pardee, 1974; Pardee, 1989). One explanation for the present lack of changes in cyclin D₁ and E, could be that G₁ arrest reflects an inhibition of the cyclin E / Cdk-2 and cyclin D / Cdk-4 complex activity in a stoichiometric manner (Cheng et al., 1998). P27Kip₁ overexpression could e.g. have
prolonged the time needed to synthesize these cyclins in amounts sufficient to exceed the rate-limiting threshold required for the G₁/S phase transition (Mitsuhashi et al., 2001). As such, p27Kip1 could have prolonged the presence of these cyclins. Such accumulative changes could cause comparable numbers of cyclin D₁ and E to be present when studied histologically.

Another possibility relates to the fact that cyclin E is expressed for such a short period of time (number of cyclin E-positive cells was around 6% of all the Ki-67-positive cells) that significant changes, if any, are not detected due to the small numbers. Treatments causing more robust decreases in proliferation might result in detectable changes in the cyclin E levels. With regard to cyclin D₁, some additional reasons are worth mentioning. First, cyclin D₁ level peaks during the G₁ phase, but remains low during the following cell cycle phases (Sherr, 1993). That is probably why our immunolabeling gave such a diverse, mixed group of cells with low and high expression. Our quantification only involved the total number of cyclin D₁-expressing cells present, since other approaches would be highly arbitrary, and did not distinguish between high and low expression levels. Furthermore, even if a smaller number of cells had expressed cyclin D₁, accumulation of fewer cells in G₁ histologically would have yielded relatively high numbers not different from controls, and could hence have masked an actual lower incidence. In addition, although some studies did find changes in cyclin D₁ (Goya et al., 1993; Greenberg et al., 2002), in one case even in the DG, albeit after adrenalectomy (Postigo et al., 1998), other studies generally failed to find regulation by glucocorticoids, which is in agreement with our findings (Corroyer et al., 1997; Ramalingam et al., 1997).

Next to the subventricular zone (SVZ) (Alvarez-Buylla and Garcia-Verdugo, 2002), the SGZ is a unique area where new neurons still arise in the adult brain of different species. Many quiescent cells of other tissue types show high p27Kip1 levels (Harper and Elledge, 1996; Kato et al., 1994; Sherr and Roberts, 1995), which decline upon e.g. mitogen-induced cell cycle reentry (Nourse et al., 1994). Neurons in the adult brain generally cannot leave the G₀ phase or reenter the cell cycle successfully. Consistent with this, in the adult rat brain, post-mitotic neurons are generally negative for p27Kip1. Interestingly, the dense cell population in the (proliferative) SGZ we studied does express p27Kip1. In addition, it is not unlikely that this p27Kip1-positive, presumed progenitor, population also contains other cell types of e.g. glial and endothelial origin (Durand et al., 1998; Durand et al., 1997). However, our present confocal analysis showed that P27Kip1-positive cells and GFAP in the SGZ do not colocalize, although a close association with the processes of GFAP-expressing astrocytes was seen. This is consistent with recent studies.
showing that astrocytes and radial glia are intimately involved in the production of new neurons (Anthony et al., 2004; Gotz et al., 2002) at least during development. The close association of GFAP-positive processes to p27Kip1-positive cells suggests a local control of proliferation though the release of growth factors. Since p27Kip1 expression decreases during G1, and Ki-67 expression rises in the late G1 phase, only a narrow overlap between these markers was expected, which is in agreement with the general absence of colabeling for p27Kip1 and Ki-67 in our study, where only a very few cells appear double positive. Thus the population of p27Kip1-positive cells likely represents a resting population of progenitor cells, in agreement with its proposed role as key regulator in cell division in proliferative cell populations (van Lookeren Campagne and Gill, 1998).

Stress and corticosteroids not only influence cell birth, but also cell death (Almeida et al., 2000; Hassan et al., 1996; Lee et al., 2002; Lucassen et al., 2001). We showed before that the amount of apoptotic cells in the dentate gyrus increased after acute stress, but was remarkably decreased after chronic stress (Heine et al., 2004a). These differences in cell death can now be better appreciated considering the chronic stress-induced G1 arrest. In G1, cells can still decide to continue progressing through the cell cycle, or choose to exit through apoptosis. During the exposure to anti-mitotic signals, the proportion of cells in G1 that have passed the restriction point already, are likely to be much more susceptible to cell death (Lundberg and Weinberg, 1999), which would agree with the increase in number of apoptotic cells in the acutely stressed rats (Heine et al., 2004). On the other hand, in case of a chronic stress-induced G1 arrest, fewer cells will cross this restriction point and consequently, less cycling cells are left to engage in apoptosis. Furthermore, another possibility is that the decreased cell death reflects a decrease in the number of young neurons produced under chronic stress, since a significant proportion of these newborn cells dies (Dayer et al., 2003; Gould et al., 1999).

Furthermore, cell cycle exit and initiation of cell differentiation occurs during the G1 phase as well (Sherr and Roberts, 1999). P27Kip1 appears to play an important role in this decision, as accumulation of p27Kip1 to a sufficient amount, prevents cells from reentering the cell cycle and promotes oligodendrocyte differentiation (Casaccia-Bonnefil et al., 1997; Durand et al., 1997; Tikoo et al., 1998), retinal development (Cunningham et al., 2002) and differentiation of neuroblastoma cells (Borriello et al., 2000; Matsuo et al., 2001; Matsuo and Thiele, 1998; Perez-Juste and Aranda, 1999). Earlier we reported that 3 weeks of chronic stress decreased in particular the proliferation rate of newborn cells, but did not affect the number of cells differentiating into neurons (Heine et al., 2004a). However, increased p27Kip1 levels could have prevented cycling cells from reentering
the cell cycle, and rather induced a higher proportion of the decreased population of proliferating cells to differentiate. Moreover, loss of the G1 checkpoint in p27Kip1-null mice was shown to cause transit-amplifying progenitors (type C cells) in the SVZ to undergo extra rounds of cell division, albeit at the expense of lineage progression (Doetsch et al., 2002). Therefore, the chronic stress-induced increase in p27Kip1 levels may not only cause cells to arrest in the G1, but might also have caused more cycling cells to enter G0 prematurely.

An unresolved issue is how and via what pathway chronic stress increases the number of p27Kip1-expressing cells while decreasing proliferation rate in the SGZ. In addition to increased corticosterone levels, stress causes changes in e.g. synaptic transmission (Alferez et al., 2003; Karst and Joels, 2003), the serotonin response (Fontenot et al., 1995; Graeff et al., 1996; van Riel et al., 2003), NGF (Scaccianoce et al., 2000), BDNF (Smith et al., 1995), VEGF protein expression (Heine et al., unpublished observation), and affects mitogen-activated protein kinase (MAPK) / ERK expression (Meller et al., 2003; Trentani et al., 2002), which all could regulate hippocampal cell proliferation (Brezun and Daszuta, 2000; Gould, 1999; Harada et al., 2004; Jin et al., 2002; Lee et al., 2002; Nacher et al., 2001; Nozaki et al., 2001). Moreover, since the GR and MR are not found on precursor cells, this implies, that GR signaling must have gone via alternative or indirect routes, e.g. through neighboring cells.

In summary, this report shows that the chronic stress-induced decrease in cell proliferation is paralleled by a significant increase in the number of p27Kip1 expressing cells. Although more research is needed to understand the subsequent molecular mechanisms involved, we conclude that chronic stress causes cycling cells in the adult hippocampus to arrest in G1 phase.

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