Int. J. Dev. Biol. 45 (S1): S83-S84 (2001)

Short Report

Temperature affects adult neurogenesis in the lizard brain

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ABSTRACT Postnatal and adult neurogenesis have been reported to occur in the brain of lizards. The aim of this study was to know if adult neurogenesis is a temperature-dependent process. We have shown, by using 5'-bromodeoxiuridine immunocytochemistry, that at low temperatures the number of newly generated neurones was significantly decreased (70%-90%) in several telencephalic areas of the lizard *Psammodromus algirus*. On the other hand, the migration process was also affected by low temperatures. This study provides evidence for a potential temperature-modulation of adult neurogenesis in lizards as it may occur during seasonal changes.

Introduction

Adult neuronal generation has been demonstrated in the brain of some species of lizards (López-García *et al.,* 1988, Peñafiel *et al.,* 1996; Pérez Cañellas and García-Verdugo, 1996). The new neurones are generated in the ependymal layer of the lateral ventricles and migrate to their destination mainly along the radial glial processes or following other tangential pathways (Peñafiel *et al.,* 1996). An interesting question is to know which factors are involved in the control of adult neurogenesis in vertebrates. For example, in the adult avian brain some studies have focused on the effect of the gonadal hormone levels in neuronal formation (Brown *et al.,* 1993). In lizards, the capacity of regeneration of the previously lesioned medial cortex is affected by temperature and photoperiod (Font *et al.,* 1991; Ramírez *et al.,* 1997). However, there are no studies on the effect of temperature during normal (i.e. not after lesion) neurogenetic activity through all telencephalic areas. In the present study we have analyzed if low temperature affects the rate of proliferation of stem cells in the ventricular zone and also the migration process of newly generated neurones.

Material and Methods

Adult lizards *Psammodromus algirus* were used in this study. Three groups of animals were exposed to different temperature conditions: lizards from group A (control group, n=8) were injected with 5'-bromodeoxiuridine (BrdU) (see below), maintained at room temperature (20 °C) and sacrificed after short (2 days, n=4), intermediate (18 days, n=2) or long (33 days, n=2) survival times. Lizards from group B (n=8) were first acclimated to 10 °C for 5 days, then injected with BrdU and maintained at this low temperature for 2 days (n=4), 18 days (n=2) or 33 days (n=2). Lizards from group C (n=4) were injected with BrdU, left 4 days at room temperature, then exposed to 10 °C and sacrificed after 2 days (n=2) and 33 days (n=2). During these experiments all animals had free access to water and food and also were deprived of light.

Each lizard received two doses (at 24 hours interval) of BrdU (SIGMA, 100 μg/g, i.p.) diluted in 0.1M phosphate buffered saline pH 7.3 (PBS). After the respective postinjection survival time, animals were deeply anaesthetized with urethane (1 μg/g, i.p.) and transcardially perfused with PBS followed by Carnoy fixative. The brains were then removed from the skull and immersed in the same fixative overnight at room temperature, paraffin embedded and transverse sections cut at 10 μm.

Before immunocytochemistry, deparaffined and hydrated sections were treated with 2N HCl in PBS containing 0.3% Triton X-100 during 20 minutes for DNA denaturation, rinsed in 0.1M borate buffer pH 8.5, and washed in PBS. Then, the sections were incubated with: 1) the monoclonal antibody anti-BrdU (Boehringer Manheim) diluted 3 μg/ml in PBS containing 5% normal serum bovine and 0.3% Triton X-100 for 18 hours; 2) the secondary antibody, rabbit anti-mouse IgG (SIGMA) diluted 1:35 for 1 hour; and 3) the mouse monoclonal peroxidase-antiperoxidase complex (PAP; SIGMA) diluted 1:100 for 1 hour. After each incubation, the sections were rinsed three times with PBS for 10 minutes each. The immnunoreaction product was visualized with 0.05% 3-3'-diaminobenzidine tetrahydrochloride and 0.03% H_2O_2. Finally, the sections were counterstained with hematoxylin, dehydrated and coversliped.

Quantitative study

Only the lizards sacrificed after 2 days postinjection, from both groups A and B, were used for the quantitative analysis. BrdU labeled nuclei were counted in evenly spaced hemisections in those areas that exhibited adult neurogenesis: olfactory bulbs (OB), rostral telencephalon (Rt), medial (MC), dorsal (DC) and lateral (LC) cortices, dorsal ventricular ridge (DVR), septum (SEP), dorsal striatum (St) and nucleus sphericus (NS). In all cases labeled nuclei were located on the ependyma of each brain area. The mean number of BrdU-labeled nuclei for each area was the average of four animals used in each group. On the other hand, the percentage of decreasing of cell proliferation in group B as compared to control group A was also calculated. X^2 analysis was made to test if there were significant differences ($p<0.05$) for each group.

Results and Discussion

BrdU immunocytochemistry revealed labeled nuclei in all processed animals, but significant differences were found in number and location of these labeled cells depending on the different conditions. Group A showed a elevated number of labeled nuclei (Fig. 1A). In those animals sacrificed after 2 days of survival postinjection, all labeled cells were found in the ventricular zone of the studied brain areas. After 18 days, many labeled nuclei were found migrating between the ventricular zone and their final destination. After 33 days most of labeled cells already reached their destination, mainly in the principal cell layer of the corresponding

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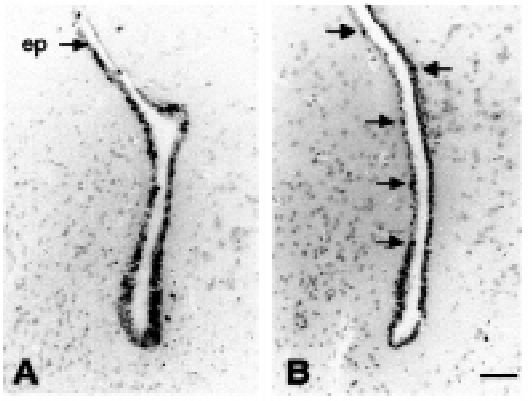

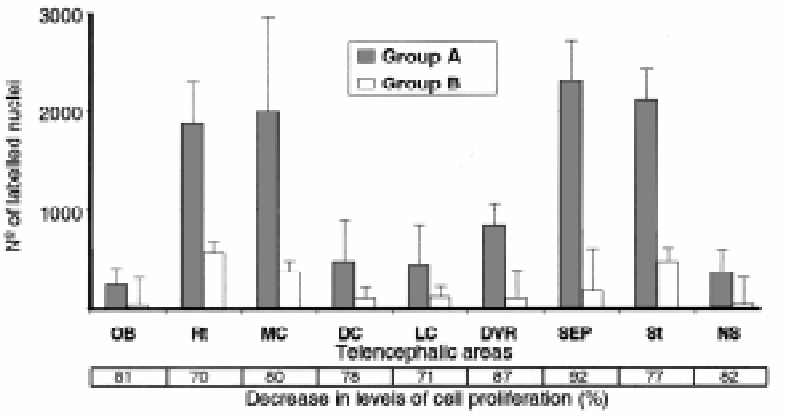

Fig. 1. (Left). BrdU-immunostained sections at level of the sulcus ventralis/terminalis corresponding respectively to lizards of group A (maintained at 20°C after BrdU injection) and B (acclimated to 10°C before BrdU injection). *In A, many labeled nuclei were found in ventricular zone as compared with B. Scale bar, 66 μm. ep, ependyma.*

Fig. 2. (Right). Quantitative analysis of BrdU-labeled nuclei in the ventricular zone of lizards maintained at 20°C (group A) and at 10°C (group B) and sacrificed after 2 days postinjection. *Data represent the mean of the total number of labeled nuclei in the different telencephalic areas. Numbers below the graph indicate the percentage of decrease from group B with respect to group A.*

area, and some labeled nuclei could be seen still on the ependymal layer or migrating.

However, the number of labeled cells in the brains of lizards from group B, exposed to low temperature before BrdU injection, was very low (Fig. 1B). The labeled cells were located in the ventricular zone, even in lizards sacrificed after 18 or 33 days of survival. These findings demonstrate that the rate of cell proliferation was decreased at low temperatures. On the other hand, the no existence of labeled cells outside ventricular zone at intermediate and long survival times, indicates that the migration process of the newly generated cells was also affected by the low temperature. The results obtained from group C confirmed these findings. The number of labeled nuclei in ventricular zone after 2 days of survival and before being exposed to low temperature, was very similar to the obtained in group A. However, no labeled cells were found in their final destination after 33 days of survival. Occasionally some few migrating cells could be seen at short distance from ventricular zone, probably those that started their migration process before acclimation to low temperature. These results indicate an inhibition of the migration process.

The quantitative analysis revealed a remarkable and significant decrease (70% - 90%) of labeled nuclei at low temperature.

These findings indicate that postnatal neurogenesis in lizards seems to be affected by variations of temperature, and also suggest the existence of temperature-dependent mechanisms involved in the neuronal migration processes. This is in agreement with previous studies on regeneration of the specifically lesioned medial cortex of the lizard *Podarcis hispanica* (Ramírez *et al.,* 1997). After lesion with 3-acetylpiridine of the medial cortex granule neurones, the ependyma is activated and a reactive neurogenesis takes place that leads to the regeneration of this cellular layer. Under low temperature conditions the lizards cannot repair its lesioned medial cortex, and summer-like conditions (high temperature and long photoperiod) permited reactive neurogenesis and neuronal migration processes. A similar case is the high vocal center of avian that shows the largest peak of neurogenesis in late summer, when male songbirds learn new songs (Nottebohm *et al.,* 1994).

In the present study, we have analyzed the effect of temperature in the adult neurogenesis process but not the photoperiod factor. Certainly, both cell proliferation and migration processes might be also affected by photoperiod as shown in the regeneration of lesioned medial cortex of adult lizard. It is noteworthy that number of labeled cells in group A was in general lower than the obtained in other lizards not deprived of light (data not shown).

We conclude here that low temperatures decrease the rate of cell proliferation in ventricular zone of adults lizards and also prevent the migration process of the newly generated neurones. Considering seasonal changes, the neuronal formation might be restricted to the summer period, when lizards have their major activity (matching, breeding).

Acknowledgemets

This research was supported by DGES (PM98/0223) and by Junta de Andalucia (CTS-0161).

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