Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1763 (2006) 1184–1191
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbamcr

Review

Calcium transients and calcium signalling during early neurogenesis in the amphibian embryo *Xenopus laevis*

Catherine Leclerc [a,*], Isabelle Néant [a], Sarah E. Webb [b], Andrew L. Miller [b], Marc Moreau [a]

[a] *Centre de Biologie du Développement, UMR 5547 et GDR 2688, Université Paul Sabatier, 118 route de Narbonne 31062 Toulouse, France*
[b] *Calcium Aequorin Imaging Laboratory, Department of Biology, HKUST, Clear Water Bay, Kowloon, Hong Kong, PR China*

Received 6 July 2006; accepted 8 August 2006
Available online 10 August 2006

Abstract

Development of the vertebrate embryonic nervous system is characterized by a cascade of signalling events. In *Xenopus*, the initial step in this cascade results from signals emanating from the dorsal mesoderm that divert the fate of the ectoderm from an epidermal to a neural lineage. These signals include extracellular antagonists of the bone morphogenetic protein (BMP). Experiments performed with isolated ectoderm suggest that epidermis is induced by BMP, whereas neural fates arise by default following BMP inhibition; however, we show that this mechanism is not sufficient for neural determination. Ca^{2+} imaging of intact *Xenopus* embryos reveals patterns of Ca^{2+} transients in the dorsal ectoderm but not in the ventral ectoderm. These increases in intracellular calcium concentration ($[Ca^{2+}]_i$), which occur via the activation of dihydropyridine (DHP)-sensitive Ca^{2+} channels, are necessary and sufficient to orientate the ectodermal cells toward a neural fate. On the one hand, the treatments that antagonize the increase in $[Ca^{2+}]_i$, inhibit neuralization, while on the other hand, an artificial increase in $[Ca^{2+}]_i$, whatever its origin, neuralizes the ectoderm. Using these properties, we have constructed a subtractive cDNA library between untreated ectoderm and caffeine-treated ectoderm. The caffeine stimulates an increase in $[Ca^{2+}]_i$ and thus orientates the cells towards the neural pathway. We have identified early Ca^{2+} target genes expressed in neural territories. One of these genes, an arginine methyl transferase, controls the expression of the early proneural gene, Zic3. Here, we discuss an alternative model where Ca^{2+} plays a central regulatory role in early neurogenesis. This model integrates the activation of a Ca^{2+}-dependent signalling pathway due to an influx of Ca^{2+} through DHP-Ca^{2+} channels. While Ca^{2+} is required for neural determination, epidermal determination occurs when Ca^{2+}-dependent signalling pathways are inactive.

Keywords: Calcium; DHP-channel; Neural determination; *Xenopus laevis*; Gene expression

1. Introduction

In the amphibian, the formation of the nervous system is initiated very early in development (9 h post fertilization), during gastrulation, with a process called neural induction. This is the first phase in neurogenesis. Neural induction in vertebrate species may exhibit a degree of morphological and signalling similarities, however, only a few species have been studied in great detail. Most current information regarding Ca^{2+} signalling during neural induction comes from amphibian development, and this shall be used as a model to discuss the phenomenon. The classical experiments of Spemann and Mangold in 1924 [1], using the urodele amphibian model system, show that neural induction involves an interaction between the dorsal mesoderm and the ectoderm, which leads to a diversion of the epidermal lineage towards the neural fate. During this process, the ectoderm of the embryo becomes regionalized to form the highly specialized and interconnected regions found later in the adult nervous system [2]. The cells develop in defined temporal and spatial patterns as a result of several classes of signalling molecules and a precise local control of gene expression. Thus, immature ectoderm cells are faced with a series of binary choices, the first of which is to become an epidermal or a neural cell.

In the last 10 years it has been suggested that neurogenesis results from the opposing action of ventralizing signals (e.g. Bone Morphogenetic Proteins, BMP) coming from the ectoderm, and dorsalizing signals from the dorsal mesoderm (e.g. noggin, chordin, follistatin, XnR3 and Cerberus) (reviewed by [3]). However, there is increasing evidence to suggest that

* Corresponding author.
E-mail address: leclerc@cict.fr (C. Leclerc).

0167-4889/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2006.08.005

antagonizing BMP signalling is not sufficient to explain neural induction and that FGF signalling is also required [4,5].

In this review, we will outline our hypothesis that another signalling pathway, involving transient rises in intracellular calcium concentration ($[Ca^{2+}]_i$), controls the binary choice (i.e., epidermis or neural tissue) of determination. We will describe (i) the Ca^{2+} signals that occur during neural induction and their essential characteristics; (ii) a possible downstream effector that fits into the signalling cascades involved; and (iii) a new model to explain the role of Ca^{2+} in neural induction and thus re-evaluate the concept of "by default" neural induction.

1.1. Ca^{2+} is involved in the choice between neural and epidermal fate

The several layers of ectoderm cells above the blastocoel in late blastula- or early gastrula-stage embryos are called the animal cap. Animal caps can be dissected and kept for several days in physiological medium. These cells are multipotent and exhibit plasticity where, without the addition of inducing factors, they give rise to epidermal cells (i.e., atypical epidermis). When appropriate neural inducers such as noggin are added to the culture medium, animal cap cells express a variety of neural markers. This assay is therefore a good model to estimate the neural inducing activity of activators or inhibitors.

As early as 1964, Barth and Barth [6] suggested that Ca^{2+} is important to trigger neuralization in *Rana pipiens* ectoderm. More recently, the dissociation of animal caps in Ca^{2+} and Mg^{2+}-free medium has been shown to orientate the cells toward a neural fate [7–10]. Frequently, the explanation given for this neuralization by dissociation phenomenon is that the epidermal inductors (BMPs) are being diluted from their receptors. This theory was taken as fact for many years because it was reported that when BMP4 is added at a high concentration during dissociation, the expression of neural markers is totally abolished [11]. Recently, it has been shown that cell dissociation induced a sustained activation of the Ras/MAPK pathway, which causes the phosphorylation of Smad1 at sites that inhibit the activity of this transcription factor. This demonstrates that BMP ligands continue to signal in dissociated cells [12]. In addition, we have shown that the dissociation of animal caps in Ca^{2+}-free medium triggers an increase in $[Ca^{2+}]_i$ (Fig. 1A). This increase is due to an efflux of Ca^{2+} from internal stores resulting from the inversion of the gradient of concentration in Ca^{2+} between intra- and extracellular compartments [13]. To discriminate whether during cell dissociation, the increase in $[Ca^{2+}]_i$ is a cause or a consequence of neural induction, we have loaded animal cap cells with the Ca^{2+} chelator BAPTA. Under these conditions, the neuralization by dissociation is blocked (i.e., the neural marker NCAM is not expressed, Fig. 1B and Ref. [13]). This demonstrates that, at least in animal caps, a Ca^{2+}-dependent signal is necessary to trigger neuralization of the ectoderm and to inhibit epidermal determination.

1.2. DHP-sensitive Ca^{2+} channels and neural induction

The neuralizing protein noggin has been shown to be a binding partner of BMPs and an antagonist of BMP signalling

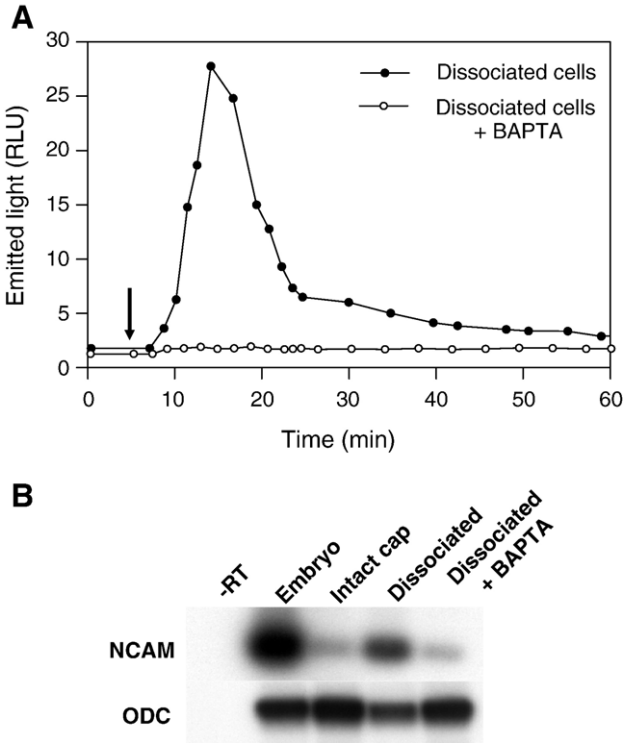

Fig. 1. Neuralization by dissociation of ectodermal cells is associated with a Ca^{2+} signal. (A) Recording of internal Ca^{2+} concentration during dissociation of *Xenopus* ectodermal cells in medium free of divalent ions (filled circles) and after preincubation of the ectodermal cells with the membrane permeant form of BAPTA (open circles, BAPTA-AM, 0.4 mM in the external medium). To measure Ca^{2+}, the cells were previously loaded with aequorin as previously described [13]. Arrow indicates substitution of normal saline medium by Ca^{2+}—Mg^{2+}-free medium. (B) Expression of the pan-neural marker NCAM in animal caps was measured by RT-PCR. Dissociated caps differentiated into neural cells expressing NCAM. BAPTA-AM loaded caps before dissociation (dissociated+BAPTA) show a dramatic reduction in NCAM expression. Intact cap: animal caps not dissociated; sibling control embryo (stage 20; 21 h. post-fertilization) served as positive control (embryo) and PCR on the same RNA without reverse transcription was done to check the absence of genomic DNA (−RT). Ornithine decarboxylase (ODC) gene is used as control.

[14]. Addition of the neural inducer noggin to animal caps from amphibians (the urodele, *Pleurodeles* or the anuran, *Xenopus*) triggers an increase in $[Ca^{2+}]_i$. This increase has a duration of 10–20 min and represents a rise to about 15% above that of the resting level of $[Ca^{2+}]_i$ [15,16]. It is completely inhibited by antagonists of dihydropyridine (DHP)-sensitive Ca^{2+} channels, such as nifedipine or nimodipine, thus indicating an influx of Ca^{2+} from an external source.

The animal caps directly stimulated by specific agonists of DHP-sensitive Ca^{2+} channels, such as S(−)Bay K 8644, present a transient increase in $[Ca^{2+}]_i$ of 20 min duration. This increase is sufficient, even in an active BMP context, to trigger not only the expression of neural markers but also the formation of neurons and glial cells [16]. Conversely, the blockade of DHP-sensitive Ca^{2+} channels inhibits neural induction [17]. In addition, methyl-xanthines such as caffeine or theophyline, which are known to release Ca^{2+} from internal stores, are potent neural inducers [16,18]. These experiments suggest the crucial role played by Ca^{2+} since, whatever its provenance, it triggers neuralization of the ectoderm.