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Short Report

Bovine floor plate cells express the sco-spondin gene

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ABSTRACT A good body of evidence points to a chemical relationship between compounds secreted by both, the floor plate (FP) and the subcommissural organ (SCO; a ventricular brain gland whose function has not been fully established). Since SCO-spondin is the major SCO secretory glycoprotein, we investigated whether this protein is also expressed in the FP. For this purpose, RNA from bovine FP was analyzed using PCR primers which revealed the expected SCO-spondin-product. The sequence identity of this PCR-fragment to the corresponding SCO-spondin region was established by southern blot and sequencing. FP-translation of SCO-spondin was demonstrated by western blot analysis and immunocytochemistry. These results support previous evidence ascribing CNS developmental properties to SCO-spondin.

Introduction

The FP, located in the midventral line of the neural tube (Bronner-Fraser, 1994), participates in neuronal differentiation and patterning of the CNS (Placzek *et al.,* 1990). Its rostralmost region displays distinct secretory features resembling the SCO, a highly specialized brain gland located in the roof of the third ventricle. The SCO secretes high molecular weight glycoproteins (Nualart *et al.,* 1991) into the cerebrospinal fluid (CSF), where they aggregate into a threadlike structure known as Reissner's fiber (RF). Antisera against RF-glycoproteins (AFRU, Rodríguez *et al.,* 1984) immunostain FP cells and also react with proteins in FP extracts. Thus, it has been proposed a chemical relationship between the secretory compounds synthesized by both the SCO and the FP (Yulis and Muñoz, 2001). SCO-spondin, the major protein component of RF has been recently fully characterized at the molecular level (Meiniel, 2001). On the basis of the molecular features and some *in vitro* effects (Monnerie *et al.,* 1998), it has been postulated that SCO-spondin may participate in CNS development. In the present report we demonstrate that the SCO-related material detected in the FP corresponds to an expression product of the SCO-spondin gene.

Material and Methods

Adult SCO and embryonic FP (2.5 month-old) from bovine were used in the present study. Depending on the experiment, tissue blocks containing either structure were processed for immunocytochemistry, western blotting and PCR. *Immunocytochemistry*: A standard PAP method was applied to FP *in situ* and *in vitro* sections, using an antibody raised against a 15 mer synthetic peptide deduced from the 3'-end of SCO-spondin (anti-P15; Nualart *et al.,* 1998). *Western blot*: FP and SCO protein extracts were analyzed using AFRU as first antibody (enhanced chemiluminescent system, Pierce). *FP organ culture:* FP explants were obtained and cultured during 2 and 20 d. The incubation medium, modified after Placzek *et al.* (1990), was changed every 3 d. *RT-PCR, southern blot and sequencing*: SCO and FP DNaseI-digested total RNA was subjected to RT-PCR under standard conditions using a primer pair derived from the 3'-end of SCO-spondin. The PCR-products amplified from both tissues were further analyzed by southern blotting and sequencing.

Results

The anti-P15 serum and the PCR primers used in the present study may be considered specific for SCO-spondin gene products, because they derive from a 3'-end region of SCO-spondin, which is unique along the SCO-spondin sequence and it lacks homology with other proteins. The immunocytochemical staining of fresh and cultured bovine FP using anti-P15 revealed specific reaction in the FP cells (Fig. 1 A,B). The antibody AFRU was used to analyze blots of the SCO and FP. In the SCO three immunoreactive bands were detected: 540, 450 and 320 kDa (Fig. 2). In the fresh FP three polypeptides were immunostained: 540 kDa, 200 and 112 kDa (Fig. 2). To demonstrate that the SCO-spondin mRNA is transcribed in the FP, we analyzed RNA samples from fresh and cultured bovine FP, and the expected 233 bp PCR-fragment was efficiently amplified (Figs. 3, 4A). This transcriptional activity was maintained by the FP *in vitro* even after 20 d of culture (Fig. 3). The identity of the PCR-fragment amplified from FP with the corresponding SCO-spondin region was confirmed by southern blot analyses (Fig. 4B) and sequencing.

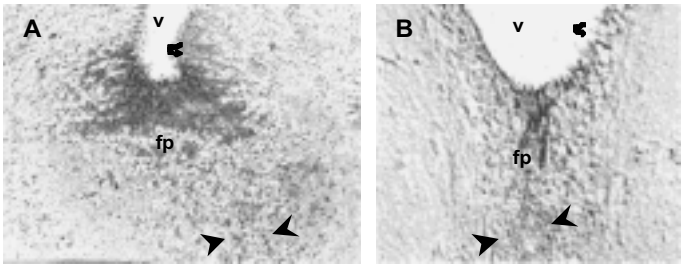

Fig. 1. Immunocytochemistry of bovine FP *in situ* (A) and *in vitro* (B) using anti-P15 serum. *The FP cells show an even immunostaining throughout the cell body. v, ventricle; fp, floor plate cells; arrowheads, basal processes; arrows, ependymal cells. 2.5-month-old embryos. PAP method.*

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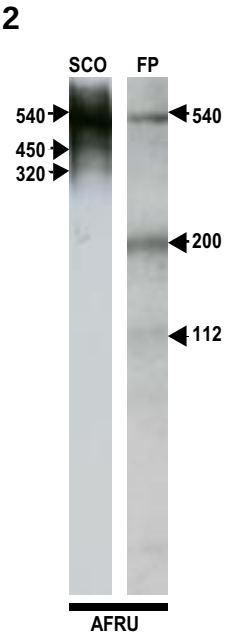

Fig. 2. Western blot analysis of adult SCO and embryonic FP using AFRU as primary antibody. *In both, SCO and FP samples, a 540 kDa compound was detected. The SCO sample shows also components of 450 and 320 kDa, which were not detected in the FP. In FP extracts, two additional immunoreactive compounds of 200 and 112 kDa were detected. SCO, subcommissural organ; FP, floor plate. Molecular weights are indicated.*

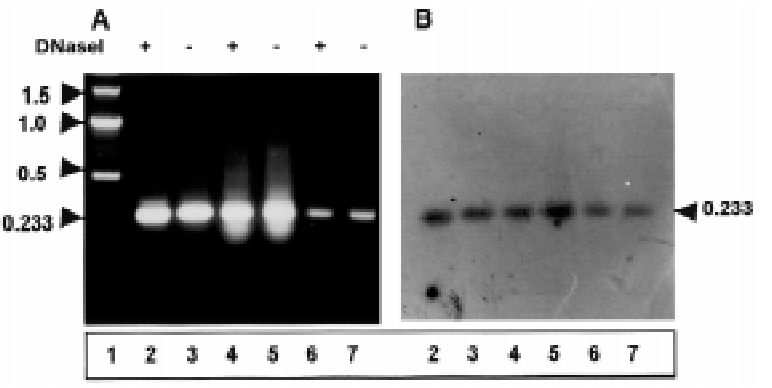

Fig. 4. RT-PCR southern blot analysis of RNA from bovine FP and SCO. *The sequence identity of the 233 bp PCR products to that of SCO-spondin, was examined by means of southern blot analysis. Lanes: 1, DNA size markers (1kb; Sigma); 2 and 3, adult SCO (1 µg); 4 and 5, fresh FP (5 µg); 6 and 7, FP explants cultured for 20 d (2.5 µg). DNaseI, DNase I.* **(A)** *Ethidium bromide-stained agarose gel.* **(B)** *Southern blot of the gel shown in (A) using a ^{32}P-labeled SCO-spondin cDNA clone (2.5 kb) as probe. The blot was exposed to X-ray film overnight, at -70 ºC.*

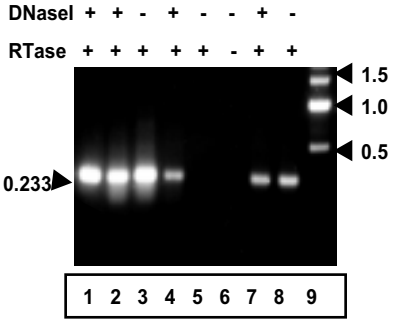

Fig. 3. PCR analysis of reverse-transcribed RNA from bovine FP and SCO. *The expected 233 bp PCR product was amplified using a SCO-spondin derived amplimer pair with total RNA templates. Adult SCO (1 µg, control +, lane 1), fresh FP (5 µg, lanes 2 and 3), FP explants cultured for 2 d (2.5 µg, lane 4), no RNA (control -, lane 5), FP explants cultured for 20 d (2.5 µg, control -, lane 6), and FP explants cultured for 20 d (2.5 µg, lanes 7 and 8),. Lane 9, DNA size markers (1kb; Sigma). After 20 d of culture, FP cells continue to express the SCO-spondin mRNA. RTase, SuperScript II RNaseH- reverse transcriptase; DNaseI, DNase I.*

Conclusions

The SCO-spondin mRNA is expressed by FP cells, where it seems to be fully transcribed and translated, as suggested by the presence in FP blots of a 540 kDa AFRU-immunopositive compound. This large protein is a highly glycosylated precursor form of the 450 kDa SCO-spondin secreted by the SCO. The presence of the 540 kDa compound in both, SCO and FP, but the absence of the expected 450 kDa SCO-spondin in the FP suggests that both tissues process the 540 kDa precursor in a different way. This is further supported by the presence in FP blots of 200 and 112 kDa AFRU-immunoreactive compounds. The presence in the conditioned medium of FP explants of a 540 kDa AFRU-immunoreactive compound suggests the possibility that the FP cells might have the property to release to the fetal CSF the 540 kDa precursor proper (data not shown). Considering that SCO-spondin (I) contains functional domains known to be involved in CNS development; (ii) it exhibits *in vitro* effects on primary neuronal cultures; (iii) it is expressed in FP cells, it may be concluded that this compound is involved in CNS development.

Acknowledgments

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