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REGULAR ARTICLE

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Quantification of the secretory glycoproteins of the subcommissural organ by a sensitive sandwich ELISA with a polyclonal antibody and a set of monoclonal antibodies against the bovine Reissner's fiber

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Abstract The subcommissural organ (SCO) is an ependymal brain gland that releases glycoproteins into the ventricular cerebrospinal fluid where they condense to form the Reissner's fiber (RF). We have developed a highly sensitive and specific two-antibody sandwich enzyme-linked immunosorbent assay (ELISA) for the quantification of the bovine SCO secretory material. The assay was based on the use of the IgG fraction of a polyclonal antiserum against the bovine RF as capture antibody and a pool of three peroxidase-labeled monoclonal antibodies that recognize non-overlapping epitopes of the RF glycoproteins as detection antibody. The detection limit was 1 ng/ml and the working range extended from 1 to 4000 ng/ml. The calibration curve, generated with RF glycoproteins, showed two linear segments: one of low sensitivity, ranging from 1 to 125 ng/ml, and the other of high sensitivity between 125 and 4000 ng/ml. This assay was highly reproducible (mean intra- and interassay coefficient of variation 2.2% and 5.3%, respectively) and its detectability and sensitivity were higher than those of ELISAs using exclusively either polyclonal or monoclonal antibodies against RF glycoproteins. The assay succeeded in detecting and measuring secretory material in crude extracts of bovine SCO, culture medium supernatant of SCO explants and incubation medium of bovine RF; however, soluble secretory material was not detected in bovine cerebrospinal fluid.

Key words Subcommissural organ · Reissner's fiber · ELISA · Glycoproteins · Cerebrospinal fluid · Monoclonal antibodies · Bovine

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Introduction

The subcommissural organ (SCO) is an ependymal brain gland located in the roof of the third cerebral ventricle of vertebrates. It secretes *N*-linked sialylated glycoproteins into the ventricular CSF that primarily aggregate to form the so-called Reissner's fiber (RF), which runs along the cerebral ventricles and the central canal of the spinal cord up to its caudal end (Oksche et al. 1993; Rodríguez et al. 1992). Thus, RF is regarded as virtually pure mature secretory material of the SCO. On the other hand, there is some evidence indicating that a part of the SCO secretory material remains soluble in the ventricular CSF (Rodríguez et al. 1993).

Crude extracts of bovine SCO analyzed by Western blotting with an antiserum against the bovine RF (generically named AFRU) (Rodríguez et al. 1984) showed three secretory bands of apparent molecular weight 540, 450 and 320 kDa. On the other hand, AFRU in blots of bovine RF extracts revealed eight electrophoretic bands with molecular weight ranging between 450 and 57 kDa (Nualart et al. 1991; Pérez et al. 1996). Thus, both the intracellular secretion of the SCO and the RF are composed of a mixture of several molecular forms.

The function of the SCO as well as the biological activity of its secretion are not yet completely understood. In this respect, it has been recently reported that soluble material from bovine RF may modulate neuronal aggregation and promote cell survival in cortical and spinal cultured cells (Monnerie et al. 1995, 1996, 1997). The availability of a reliable method for quantifying the SCO secretion would enable some of the important challenges to be faced concerning the biology of the SCO, such as the purification of its secretory proteins and the analysis of the factors regulating the secretory activity of this gland.

Early attempts to standardize a method to quantify the SCO secretions were based on the use of AFRU. Thus, by using a competitive enzyme-linked immunosorbent assay (ELISA) with AFRU as the only antibody, Lehmann et al. (1993) were able to detect secretory material released by

SCO explants into the culture medium. A sandwich ELISA using two polyclonal antibodies (raised in rabbit and rat) against the bovine RF was used for the detection of soluble secretory material released by the rabbit SCO into the CSF (Rodríguez et al. 1993). To quantify antigens, the most suitable method is a two-antibody sandwich assay; however, ideally, either monoclonal antibodies (MAbs) that bind to independent epitopes on the antigen or affinity-purified polyclonal antibodies must be used (Harlow and Lane 1988).

We have recently raised a set of MAbs against the bovine RF glycoproteins. Three of these MAbs (named 2A5, 3E6 and 3B1) were characterized by immunocytochemistry, Western blotting and different variants of ELISA, showing a high specificity and affinity for the secretory glycoproteins of the bovine RF and SCO (Pérez et al. 1995, 1996). In the present work, we have used these MAbs in combination with AFRU to develop a sandwich ELISA for the quantification of the bovine SCO secretion. This assay has been validated by its use in the analysis of various biological samples previously reported as containing SCO-derived soluble secretory material.

Materials and methods

The variant of sandwich ELISA assayed, named AFRU/MAbs ELISA, was based on the use of a rabbit polyclonal antibody against bovine RF glycoproteins (AFRU) as the first antibody (capture antibody) and a pool of three MAbs as the second antibody (detection antibody).

Standard RF solution

Bovine RF glycoproteins were used as the antigen for preparing the standard curves. For this, RF was collected by perfusing bovine spinal cord according to the procedure previously described by Rodríguez et al. (1984) (Fig. 1a). The extraction medium was 50 mM ammonium bicarbonate, pH 8, containing 1 mM ethylenediaminetetraacetate (EDTA), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 μM pepstatin and 1 μM leupeptin as protease inhibitors. The RF collected from a total of 10 m spinal cord was transferred to 1 ml extraction medium, sonicated for 15 s and cooled down in ice. This sonication-cooling cycle was repeated several times up to complete dissolution of the RF. Extracts were aliquoted and frozen at –20°C until used.

Determination of protein concentration in the RF extract used for the preparation of the calibration curves was performed by the bicinchoninic acid method (BCA, Pierce, Rockford, USA) (Smith et al. 1995) using bovine serum albumin (BSA; Sigma, St. Louis, USA) as the standard. Serial dilutions of RF solution from 10000 to 0.1 ng/ml were used for standard curves.

The protein concentration of the extract used in the present investigation was equivalent to 17.5 μg protein/m RF (Table 1), this yield being quite constant between the different extracts analyzed. However, when the same extract of RF was analyzed by the method of Bradford (1976) the yield estimated was 10 μg protein/m RF.

Test samples

Samples from four different sources were analyzed for the presence of SCO/RF-secretory material.

1. Crude extracts of bovine SCO. The SCOs used in the present work were kindly provided by Dr. E.M. Rodríguez (Valdivia, Chile). Blocks of tissue including the SCO and the posterior commissure were dissected out at the slaughterhouse approximately 20 min after death and immersed in acetone until they were brought to the laboratory where the tissue pieces were lyophilized. Desiccated material was sent to our laboratory, where the SCO extracts were prepared.

The extraction medium was ammonium bicarbonate (see above). Seven extracts, each one containing material from 200 SCOs, were prepared from a pool of 1400 SCOs. The SCOs were homogenized in 50 ml extraction medium with the use of a homogenizer at 8000 rpm. The extract was subjected to 6 cycles of sonication of 15 s each. Then, it was centrifuged at 4000×*g* for 15 min. The supernatant was centrifuged again at 50000×*g* for 90 min. The latter supernatant was regarded as the crude extract of bovine SCO. All procedures were performed at 4°C. The protein concentration of the extract was determined by the method of Bradford and BCA. Aliquots were stored at –20°C. In addition, an extract of bovine nervous tissue containing non-secretory ependyma from the lateral ventricle plus corpus callosum was prepared in the same way to be used as a negative control for the ELISA.

2. Culture medium supernatant of bovine SCO explants. Explants from adult bovine SCO were prepared according to the procedure described by Cifuentes et al. (1995) with minor modifications.

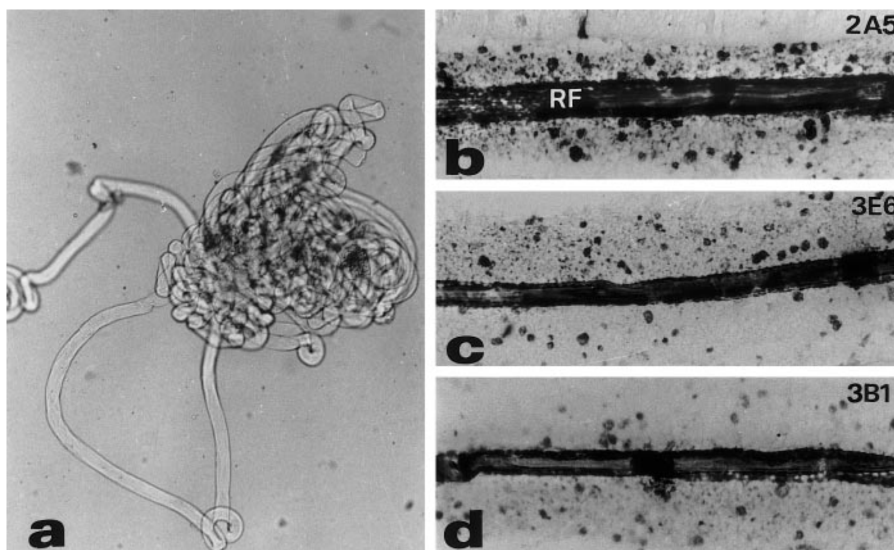

Fig. 1 **a** Phase-contrast micrograph of fresh bovine Reissner's fiber as obtained after perfusing the central canal of the spinal cord. ×14. **b–d** Sagittal sections through the central canal of bovine spinal cord immunostained with MAbs 2A5 (**b**), 3E6 (**c**), and 3B1 (**d**). Note the strong labeling of the Reissner's fiber (*RF*). ×87.5

Table 1 Protein concentration in various preparations of bovine RF with different methods of determination (*BCA* bicinchoninic acid method)

RF preparation	Protein concentration (μg/m RF)[a]	Method	Reference
Urea extract (FRU)[b]	11	Bradford	Rodríguez et al. 1984
Aqueous extract	3.5	Bradford	Rodríguez et al. 1984
Ammoniun bicarbonate extract[b]	10	Bradford	Present report
	17.5	BCA	Present report
Medium conditioned by RF[c]	33–41	Fluoraldehyde	Monnerie et al. 1996
	1.4–37.5	AFRU/MAb ELISA[d]	Present report

[a] For comparative purposes, values of protein concentration from the cited sources were converted into micrograms protein rendered per meter RF
[b] Extracts in which RF was completely dissolved
[c] Prepared by incubating portions of RF in PBS for 5 days at 37°C
[d] Two-antibody sandwich assay used in the present work. The protein concentration of the RF solution used for the calibration curve was determined by BCA

Ten explants per SCO were obtained. Explants were kept in RMPI medium containing 10% fetal calf serum for 2 weeks with a change of the medium every other day. After this time, groups of 10 explants were transferred into individual wells of a 24-well culture plate containing 1 ml medium. A total of ten wells were prepared, each one containing the secretory tissue derived from one bovine SCO. Culture medium supernatants conditioned for 3 days were analyzed for the presence of SCO-secreted material. The negative control was culture medium conditioned by explants of bovine non-secretory ependyma from the lateral ventricle.

3. Soluble secretory material spontaneously released from bovine RF was obtained following the method of Monnerie et al. (1996). The RF collected from 12-cm-long fragments of the bovine spinal cord was incubated in 1 ml phosphate-buffered saline (PBS) for 5 days at 37°C without agitation. The incubation medium was centrifuged (10 000×*g*, 5 min) and the supernatant saved, aliquoted and frozen at –20°C until analyzed by ELISA. Four samples prepared from RF of randomly chosen fragments of spinal cord were analyzed.

4. Bovine CSF was obtained from the lateral ventricle of approximately 120-day-old fetuses (n=2) and from the cisterna magna of adults (n=20). In both cases, freshly sacrificed animals (a few minutes postmortem) were used. Samples from each individual specimen (both fetuses and adults) were saved and frozen for analysis. Principles of animal care and specific national laws were followed.

Furthermore, in an attempt to concentrate the putative SCO secretory material, the bulk of cisternal CSF was pooled and submitted to immunoaffinity chromatography in a MAb-Sepharose column before being analyzed by ELISA. Two columns were prepared by coupling 5 mg of the MAbs 2A5 or 3E6 to 1 ml 4B-Sepharose. About 250 ml CSF, after being centrifuged at 1500×*g* for 10 min and dialysed against PBS, were applied to the column at a flow rate of 20 ml/h. Subsequently, the column was washed with 10 bed-volumes of PBS and then the bound material was eluted with 0.1 M triethanolamine buffer, pH 11.5, containing 0.15 M NaCl. Fractions of 0.5 ml were collected in 1 M TRIS buffer, pH 6.8, dialyzed against PBS and stored at –20°C until assayed by ELISA for the presence of SCO secretory material. All these procedures were carried out at 4°C. The chromatography schedule was repeated 4 times with each of the columns used, so that a total of about 2 l adult bovine CSF was processed.

ELISA procedure

Microtiter plates (Costar, Cambridge, USA) were coated with 20 μg/ml of the IgG fraction of AFRU (raised in our laboratory according to Rodríguez et al. 1984) in 0.1 M sodium bicarbonate buffer, pH 9.5, by incubating overnight at 4°C. As a negative control of the capture antibody, unspecific rabbit IgG at the same concentration was used. After removing the coating buffer, the wells were washed and the unspecific binding sites blocked by incubation with 200 μl/well PBS containing 0.25% BSA and 0.05% Tween 20. The wells were subsequently washed, and then standard and test samples diluted in blocking buffer were added and incubated for 3 h. The blank calibrator was blocking buffer. After washing, the wells were incubated with a pool of three peroxidase-conjugated MAbs (2A5, 3E6, 3B1; at 1 μg/ml each) in blocking buffer for 1 h. The peroxidase reaction was developed with a solution containing 0.01% tetramethyl benzidine (Sigma) and 0.0015% perhydrol (Merck) in acetate buffer, pH 6.0. After 10 min of development, the chromogen reaction was stopped by the addition of 1 M sulfuric acid. Optical density was determined at 450 nm using a computerized ELISA reader (Microplate Reader 2001, Biowhittaker, Walkersville, USA). Incubations were performed at 25°C, and 50 μl of each solution per well was used unless otherwise stated. In all assays, each of the antigen concentrations as well as the test samples were tested in triplicate.

Serial dilutions of the test samples showed a linearity similar to that of the standard curve. To estimate the concentration of secretory material in the test samples, the lower dilution with a value of absorbance falling in the linear region of the curve was routinely used. On the other hand, when the amount of secretion detected in the sample was high, the high-sensitivity segment of the standard curve was used. For culture medium of SCO explants, the low-sensitivity region of the standard curve had to be used.

Immunocytochemistry

Pieces of bovine spinal cord containing the central canal were fixed by immersion in Bouin's fluid and embedded in paraffin. Sagittal sections, 8 μm thick, were stained according to the unlabeled enzyme method of Sternberger et al. (1970). Sections were incubated at room temperature for 18 h in undiluted culture medium from each of the hybridoma cell lines producing MAbs 2A5, 3E6 and 3B1. They were subsequently incubated with anti-mouse IgG (raised in our laboratory; dilution 1:50) for 30 min and a monoclonal mouse PAP (Sigma; dilution 1:200) for 45 min. This was followed by the diaminobenzidine reaction. The supernatant medium of myeloma cell culture was used as control.

Results

All three MAbs used strongly stained the bovine RF (Fig. 1b–d) (cf. Pérez et al. 1995) and recognized native solubilized bovine RF (Pérez et al. 1996). The AFRU/MAbs ELISA (Fig. 2) showed a detection limit (three

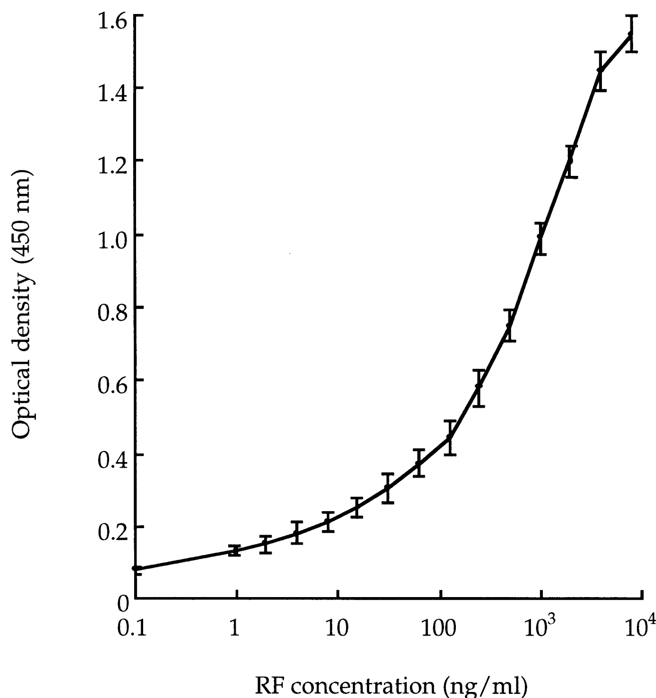

Fig. 2 Typical calibration curve for two-antibody sandwich ELISA AFRU/MAbs for RF glycoproteins (one in three serial dilutions from 4000 to 1 ng/ml). Standard deviations shown as *bars*

standard deviations above the mean for the blank calibrator) of 1 ng/ml (0.05 ng/well).

Sensitivity of the assays, regarded as the ability to discriminate significantly between small differences of antigen concentrations (Bergmeyer et al. 1983), was defined as the slope of the calibration curve within the linear range. The effective working range of AFRU/MAbs ELISA was from 1 to 4000 ng/ml. The standard curve (Fig. 2) may be divided into two parts according to the slope exhibited, namely, a low-sensitivity segment corresponding to a range of antigen concentrations of 1–125 ng/ml (r=0.984) and a high-sensitivity one between 125 and 4000 ng/ml (r=0.995).

This assay is highly specific for the SCO/RF secretion since it did not show any cross-reactivity with compounds present in samples other than SCO and RF preparations, such as crude extracts of bovine cerebral tissue (non-secretory ependyma plus corpus callosum), bovine serum or bovine CSF. In order to check the suitability of this ELISA for detecting and measuring intracellular and extracellular SCO secretory material, test samples from different sources were analyzed.

Soluble extracts of bovine SCO

The amount of secretion detected in the crude extracts of bovine SCO showed a great variation between the different extracts assayed, with values ranging between 0.38 and 0.88 µg secretory proteins per SCO (Table 2). When the same sample of SCO extract was analyzed several times in one assay and in consecutive assays, the average intra-assay and interassay coefficients of variation were only 2.2% and 5.3%, respectively.

Table 2 Concentration of soluble secretory material detected by sandwich ELISA AFRU/MAbs in different ammonium bicarbonate extracts of bovine SCO

Extract number	Secretory proteins (µg/SCO)
1	0.46
2	0.88
3	0.71
4	0.86
5	0.47
6	0.38
7	0.47
Mean	0.60±0.2

Culture medium conditioned by SCO explant

The amount of soluble secretory proteins accumulated in the culture medium after an incubation period of 3 days ranged between 107 and 188 ng/well containing 1 ml medium, the mean being 137±29 ng/ml. Since ten explants obtained from one SCO were cultured per well, this value corresponds to the secretory material released by one bovine SCO into the culture medium.

Incubation medium of RF

In the present investigation, samples of incubation medium of RF, obtained by the procedure described by Monnerie et al. (1996), were analyzed by AFRU/MAbs ELISA for the presence of material spontaneously released from the RF. The concentration of secretory proteins measured in the different samples showed a noticeable variation, with values ranging between 0.17 and 4.58 µg/ml. The mean was 1.77±2.00 µg/ml, equivalent to 14.75 ±16.66 µg/m RF.

Bovine CSF

Samples of ventricular CSF from bovine fetuses and cisternal CSF of adult bovines when analyzed directly by AFRU/MAbs ELISA gave absorbance values undistinguishable from the background. Considering the possibility that the amount of soluble SCO secretion in the bovine CSF was under the detection limit of the assay (1 ng/ml), large volumes of CSF were subjected to affinity chromatography in order to concentrate the putative secretory material. When the same procedure was applied to other preparations containing soluble SCO secretion, such as bovine SCO extracts and culture medium supernatant of bovine SCO explants, the elution fractions contained a concentration of secretion that represented an enrichment higher than 500-fold in relation to the original sample

(unpublished results). However, the elution fractions of CSF did not show any detectable specific signal when analyzed by AFRU/MAbs ELISA.

Discussion

Assay features

In the present study we describe a sensitive and specific ELISA reliable for detection and quantitation of soluble secretion derived from the bovine SCO/RF system. It is a sandwich assay based on a high-avidity polyclonal antibody and three monoclonal antibodies against the bovine RF glycoproteins.

In other ELISA formats we have used only one of the MAbs as the detection antibody (unpublished results). We also developed assays in which one of the MAbs was used as the capture antibody and a different MAb as the detection antibody (Pérez et al. 1996). The detectability of both variants of ELISA was much lower than that of the AFRU/MAbs ELISA described here. Likewise, the detection limit reported by Lehmann et al. (1993) for their competitive assay (4.2 ng/ml) exceeded by about four times that of our sandwich assay (1 ng/ml).

The sensitivity of the AFRU/MAbs assay, especially at the high concentration range, was only slightly higher than that shown by the sandwich ELISA based on the MAbs 2A5 and 3E6 (Pérez et al. 1996) but markedly higher than that of the competitive ELISA described by Lehmann et al. (1993) as demonstrated when comparing the slopes of the respective calibration curves.

The high detectability and sensitivity of the AFRU/MAbs assay may be accounted for by the following binding features of the antibodies used: (a) both AFRUs and the three MAbs employed display a high affinity for the SCO secretory material (Rodríguez et al 1984, Pérez et al 1996); (b) the epitopes of the three MAbs used as the detection antibody are present on every molecular constituent of the RF (Pérez et al. 1996) as well as on the three secretory forms of the bovine SCO (540, 450 and 320 kDa); and (c) the three MAbs used are directed against non-overlapping epitopes on the RF glycoproteins (Pérez et al. 1996), whereby the signal produced by the pool is additive in relation to that generated by each one of the MAbs separately.

Determination of protein in bovine RF extracts

Rodríguez et al. (1984) established that the amount of protein rendered by bovine RF extracts depends on the extraction medium used for its dissolution. Thus, whereas extracts of RF in a medium containing urea rendered about 11 μg protein/m, aqueous extracts rendered only 3.5 μg/m. Obviously, this variation reflects that RF dissolves to a different extent according to the medium used. We have found that ammonium bicarbonate extracts in which RF is completely dissolved by sonication rendered an amount of protein very similar to the urea extracts. These yield data were obtained by using the method of Bradford for the quantitation of protein in the extracts.

On the other hand, we have proved that the protein concentration measured in a given extract of RF depends greatly on the quantification method used (Table 1). Actually, we have found the Bradford assay to underestimate the RF-protein concentration as compared to the results obtained by other methods of quantification, viz. BCA assay (present report), absorbance at 280 nm and absorbance at 205 nm (unpublished results). This is probably due to a low affinity of Coomassie blue for the RF glycoproteins as it has been shown to occur when this dye is used for staining RF or SCO proteins on acrylamide gels. Therefore, the type of assay employed appears to be of paramount importance when determining the concentration of proteins in RF extracts.

Quantification of SCO/RF secretory glycoproteins from different sources

Soluble extracts of bovine SCO

The marked variation observed between different soluble extracts of bovine SCO for secretion content when analyzed by AFRU/MAbs ELISA is a striking finding. Such differences cannot be attributed to impreciseness of the method since the interassay coefficient of variation for the same extract was negligible.

There is evidence indicating that a portion of the intracellular secretory material of bovine SCO is strongly associated with membranes (Cifuentes et al. 1995). This membrane-bound secretion can be extracted by the use of detergents and then demonstrated by immunoblotting with AFRU. Unfortunately, this solubilization treatment is not compatible with the reactivity of the MAbs in ELISA (unpublished results). With the method of extraction used in the present work, it is probable that only a portion of the total secretory material present in the bovine SCO is being recovered and, in consequence, detected. Furthermore, we have proved that secretory material in crude extracts of SCO shows a strong tendency to aggregate (probably with other proteins present in the extract) and to precipitate; this results in a decrease of the soluble detectable material. All these factors could account for the differences found in the amount of secretory material detected between different SCO extracts.

Culture medium conditioned by bovine SCO explants

The culture conditions used in the present investigation have proved to support the viability of the SCO explants for a period of several months. The concentration of secretion detected in the culture medium is much lower than that reported by Lehmann et al. (1993), who, working with bovine explants cultured in a serum-free medium and using a competitive ELISA, measured an amount of

secretion equivalent to aproximately 500 ng/SCO and ml culture medium conditioned for 3 days. The differences in the amount of secretion in the culture medium might either be the result of a different rate of release or be related to the method of quantification used. In the first case, we may assume that the fetal bovine serum used in our experiments might decrease the secretory activity of the cultured SCO. If, on the contrary, the discrepancy were related to the type of ELISA used, it would make clear the importance of assay format selection in quantifying SCO secretory material.

Incubation medium of bovine RF

Monnerie et al. (1995, 1996, 1997) have performed pioneer work in which effects of RF glycoproteins on cultured neurons were shown. In these studies, two different sources of RF glycoproteins were used for the treatment of the cultures: (a) a portion of RF added directly to the culture wells and (b) soluble RF proteins obtained by incubation of segments of RF in culture medium ("medium conditioned by RF"). These authors reported that 12 cm RF when incubated in 1 ml PBS for 5 days at 37°C rendered 4–5 µg protein/ml (33–41 µg/m RF) as determined by the Pierce fluoraldehyde assay. Assuming that RF is pure secretory product of the SCO, the concentration of RF proteins determined by a general method of protein quantification can be directly compared to the values obtained by a specific method such as ELISA. The amount of assayable secretory proteins detected by ELISA in the present study represents a mean yield much lower than that reported by Monnerie et al. (1996). This significant discrepancy can be attributed to differences in the analytical methods used or in the way in which the samples have been prepared and stored. In fact, we have recently noticed that treatments such as freezing highly modify the amount of assayable secretory material in the sample (unpublished observation).

On the other hand, our present results show an extremely high intersample variation (0.17–4.58 µg/ml). The wide variability observed in the amount of protein spontaneously released from different segments of RF as measured by ELISA is in sharp contrast to the constancy in protein yield found among extracts with completely dissolved RF (Table 1). Therefore, under the conditions of incubation used, it would appear that whereas some fragments of RF dissolve almost completely, others contribute only a negligible amount of soluble material to the medium. An alternative explanation is that the perfusion of a 12-cm segment of central canal does not warrant the collection of 12 cm of RF; this is due to the fact that RF undergoes fragmentation during the perfusion procedure and some of these fragments may be retained within the central canal (E.M. Rodríguez, personal communication). So, there may be considerable variations in the amount of RF actually collected from individual fragments of spinal cord. Irrespective of the reasons for this variability, such a circumstance must be taken into consideration when using RF proper or a medium conditioned by RF as a source of soluble RF glycoproteins for experimental purposes.

Bovine CSF

We found no significant signal in any of the samples of bovine CSF analyzed. These negative results clearly contrast with those reported by Rodríguez et al. (1993) in the rabbit. These authors, by using a sandwich assay with two polyclonal antibodies against the bovine RF, were able to detect in cisternal CSF of adult rabbits concentrations of soluble SCO secretory material ranging between 9 and 23 ng/ml.

The absence of a specific signal using AFRU/MAbs ELISA on bovine CSF may have two reasons: (a) the assay used is unable to detect the SCO-derived soluble material present in the CSF and (b) there is no SCO-soluble secretory material in the bovine CSF. With regard to the first possibility, the putative soluble material in the CSF might consist either of secretory molecules released by the SCO remaining soluble instead of condensation into a RF, or molecules released from the RF. The absence in the bovine CSF of a soluble SCO secretion may represent an important difference from the situation described by Rodríguez et al. (1993) for the rabbit. In this case we should have to assume that the occurrence of soluble SCO secretory material in the CSF is a species-dependent feature.

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