Summary. The aim of this work was to analyze the proteins in the cerebrospinal fluid (CSF) of spontaneously hypertensive rats, to study their possible role in the relationship between hydrocephalus, arterial hypertension and alterations in the subcommissural organ. Brains from control Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) sacrificed with chloral hydrate were used. Antiserums against some cerebrospinal fluid protein bands and Reissner’s fiber (RF) were used for immunohistochemical study of the SCO. Ventricular dilation was observed in the lateral and third ventricle of the SHR. Third ventricle ependyma showed immunoreactive material (IRM) for antibody against 141kDa protein band anti-B1 and 117 protein band anti-B2 and the SCO of the SHR showed a decrease of the IRM when compared with WKY rats. An alteration in the expression of anti-RF was found to compare the SCO of the WKY and SHR groups. Our results demonstrate that hydrocephalus and hypertension are interconnected in this kind of rat which produce alterations in SCO secretions and some proteins of the CSF.

Key words: Cerebrospinal fluid, Protein, Hypertensive rats, Subcommissural organ, Ventricular dilatation

Introduction

The subcommissural organ (SCO) is a cerebral structure associated with the circulation and composition of the CSF, which secretes glycoprotein into the CSF. Most of this secretion condenses and forms the RF, and the rest is found in a soluble form in the CSF (Rodriguez et al., 1993; Castañeyra-Perdomo et al., 1998a). The relationship of the SCO and the RF with the circulation and composition of CSF has been reported by several authors such as Sever et al. (1993) who have suggested that the SCO, by means of a specific ion regulation, controls the electrolyte distribution and the pressure mechanisms of the CSF. On the other hand, alterations of the SCO in the hydrocephalus have been reported; Takeuchi et al. (1987) describe agenesis of the SCO and the PC in hydrocephalic mice, Irigoin et al. (1990) and Rodriguez et al. (1992) found alterations in the secretion of the SCO in induced hydrocephalus rats. In addition, Castañeyra-Perdomo et al. (1994) found alterations in the SCO of hydrocephalic human foetuses, where the affectation of SCO was different according to the type of hydrocephalus, and described large cytological and structural alterations of the SCO in the hydrocephalic foetus that were accompanied by other corporal malformations. Recently, Fernández-Llebrez et al. (2004) have reported that the Msx1 gene is necessary for the synthesis of the SCO glycoproteins; this gene has been suggested as being required for keeping the aqueduct open and could explain a type of hydrocephalus.

Cerebrospinal fluid (CSF) is a functional system closely connected with the brain, and the changes in the...
protein composition of CSF could mean an alteration of the brain as an expression of encephalic disorders. Analysis of brain-specific proteins in CSF is complicated by the fact that most CSF proteins are derived from the plasma and have a high degree of individual variability (Davidsson et al., 2002). Therefore, alterations in CSF composition characterize many pathological processes of the central nervous system (Bonadio, 1992). The human CSF (Akert and Steiner, 1970) contains various proteins, two of the main ones are albumin (70%) and gammaglobulin (10 to 15%), and with an overall albumin: globulin ratio of about 5:1. The CSF absolute protein concentration is age-dependent, the CSF mean protein concentrations range from 15-45 mg/dl, but this is similar in different species; the protein concentrations in ferret CSF range from 28.0 to 68.0 mg/dl, with a mean of, 31.4 mg/dl and with the highest level being reported for cat and dog CSF (Bonadio, 1992; Platt et al., 2004). It has been demonstrated that the CSF protein composition is altered in the hydrocephalus (Bonadio, 1992) and that spontaneously hypertensive rats experience a progressive increase in brain ventricle size (Ritter et al., 1988). In the present work we study the interrelationship between hydrocephalus and arterial hypertension to explore their relationship with variations in the subcommissural organ secretion and cerebrospinal fluid proteins.

Material and methods

Ten normotensive male rats (Wistar-Kyoto rats; WKY), and ten spontaneously hypertensive male rats (SHR; Leticia S/A, Barcelona, Spain) were used. The WKY rats were divided into two control groups formed of five animals each: the WKY-26 group which was composed of rats sacrificed 26 weeks after birth and the WKY-38 group which was composed of rats sacrificed 38 weeks after birth. The SHR rats were also divided into two groups: SHR-26 and SHR-38 groups sacrificed at the same age as the controls. The rats were anesthetized with chloral hydrate (200µl/100g of body weight). Animals were kept under 12:12 light-dark conditions and food and water were provided ad libitum. The rats were fixed by vascular perfusion with Bouin’s fluid, dehydrated and embedded in paraffin under standard conditions. Brains were cut into four serial coronal sections; one of the serial coronal sections was stained by the Klüver-Barrera method.

Cerebrospinal fluid (CSF) from the cistern magna of each animal was extracted and processed by electrophoresis according to Laemmli (1970) (sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE, 5%-15% gradient).

Antiserum was raised in mice using the protein band of 141, 117, 48, 43 and 39 kDa, as antigens (method similar to that described by Rodriguez et al., 1993, 2001). Three gel bands of each molecular weight were cut, macerated in PBS and emulsified with complete Freund’s adjuvant and injected subcutaneously into 10 sites on the male mouse back. Twenty days later each mouse received the equivalent of two protein bands emulsified with incomplete Freund’s adjuvant in 4 to 8 subcutaneous injections, and fourteen days later each mouse received the equivalent of two-protein bands in an intraperitoneal injection without adjuvant. Seven days later the mice were killed by intracardial exsanguinations. For laboratory purposes, the antiserum obtained was named anti-B1, anti-B2 and anti-B3 against 141, 117 and 48 kDa proteins respectively. Hardly any or no immunoreactive material with the antibodies against 43 and 39 kDa bands was found.

The sections at the level of the SCO of the WKY-26 and 38, and SHR-26 and 38 rats were incubated simultaneously with anti-B 1:500, anti-B 2 1:300, anti-B 3 1:100 and anti-Reissner’s fiber (AFRU Rodriguez et al., 1984) in 1:2000 dilutions respectively in PBS-Tritton. Incubation was for 24 h at room temperature followed by “DAKO StreptABC complex/HRP Duet, Mouse/Rabbit” procedure. The peroxidase reaction

Fig. 1. Electrophoresis of the CSF. 141: band 1, 117: band 2, 48: band 3, 43: band 4, 39: band 5. WKY: control Wistar-Kyoto rats. SHR-26: spontaneously hypertensive rats of 26 weeks. SHR-38: spontaneously hypertensive rats of 38 weeks.
product was visualized through nickel intensified diaminobenzidine reaction.

The primary antibody was omitted to validate the controlled method specificity.

**Results**

**Electrophoresis**

In the electrophoresis study, we found a total of five protein bands: 141, 117, 48, 43 and 39 kDa (Fig. 1), in the CSF of the SHR rats that were not present in the CSF of WKY rats.

**Ventricular dilatation**

Our results with Klüver-Barrera method showed that the lateral and third ventricles of the SHR-26 and SHR-38 rats were larger in size than those of normal size of the control WKY rats which were of normal size (Fig. 2B,C).

**Immunohistochemistry**

Subcommissural organ

The IRM anti-B1 in the SCO of the WKY rats was

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**Fig. 2.** Panoramic view of transversal section of the rats brain, stained by the Klüver-Barrera method. **A.** WKY at the level of anterior commissure. Bar: 400 µm. **B.** SHR at level of the optic chiasma. Bar: 400 µm. **C.** SHR at level of the posterior commissure. Bar: 600 µm. 3V: III ventricle; AC: anterior commissure; F: fornix; LV: lateral ventricle; OP: optic chiasma; PC: posterior commissure.
Fig. 3. Photographs of the SCO rostral part of the WKY and SHR rats immunostained with anti-B1. Coronal view of the SCO and ependyma of the third ventricle, (A, WKY and D, SHR). Bar: 100 µm. Lateral parts of the SCO (B, WKY and E, SHR). Bar: 50 µm. Ependymal and hypendymal layers of the SCO (C, WKY and F, SHR). Bar: 10 µm. 3V: III ventricle; Ep: ependyma cells; Hy: hypendymal cells; PC: posterior commissure; SCO: subcommissural organ; Small arrowheads: apical part of the ependymal cells; Large arrowheads: subapical part of the ependymal cells.
located mainly in the apical, subapical and intermediate parts of the ependymal cells and also in the perinuclear part of some hypendymal cells (Fig. 3A-C). In the SCO of the SHR rats, the IRM was present in the apical and intermediate parts of several ependymal cells (Fig. 3D-F). In general the reaction was qualitatively weaker in the SCO of the SHR than the WKY rats (Fig. 3C, F).

The anti-B2 immunoreactive material was found in the ependymal layer of the SCO of WKY and SHR. But anti-B2 expression qualitatively increased in the SCO of the SHR rats (Fig. 4A-D). The SCO of WKY rats showed IRM anti-B2 in several cells of the hypendymal layer (Fig. 4C, D).

No IRM was found in the SCO with antibody against the band 3.

Material immunoreactive anti-B1, anti-B2 and anti-B3 was also found in the ependyma of the third ventricle and no significant variations were found when comparing the WKY and SHR groups, an increase of IRM for anti-B2 was only found in the ependyma of the third ventricle of the SHR (Fig. 4C, D).

In WKY rats, the AFRU immunoreactive material was found in all the SCO located in the different parts of the ependymal, hypendymal cells and even in its peripheral prolongation. The IRM mainly decreased in the apical, subapical and perinuclear parts of the ependymal cells and in the hypendymal layer in the hypertensive rats.

**Discussion**

Ritter et al. (1988) found that brain ventricle size was normal in animals with experimental hypertension, however a progressive increase of ventricular size from

![Fig. 4](image.png)
4 to 56 weeks of age was found in the spontaneously hypertensive rats (Ritter and Dinh, 1986). In agreement with a previous study (Castañeyra-Perdomo et al., 1998b), we did not find qualitative ventricular dilation in SHR rats of 15 weeks of age but, we did find ventricular dilation, which can be qualitatively observed after 26 weeks of postnatal age as it is clearly present in our 38 week old SHR rats.

The structural alterations of the subcommissural organ have been described in the hydrocephalus foetus (Castañeyra-Perdomo et al., 1994), rats (Irigoin et al., 1990) and mice (Takeuchi et al., 1987; Pérez-Figares et al., 1998; Fernández-Llebrez et al., 2004; Ramos et al., 2004). In the present work, we have found brain ventricular dilation and CSF protein bands in SHR rats which were not present in WKY rats, moreover the SCO and ventricular ependymal cells were positive for antibody against CSF protein band 1 (141 kDa) and band 2 (117 kDa) but no immunostaining was found in SCO anti-B3 (48 kDa). Besides, proteins of 150 and 45 kDa have been found in the CSF of the human hydrocephalus of two month old neo-natals, and the human SCO was also immunostained with antibodies against those proteins (Rodríguez et al., 1993, 2001). The antiserum against the 150 kDa band immunostained similar structures in rats (Rodriguez et al., 1993).

In concordance with a previous work (Castañeyra-Perdomo et al., 1998b) we observed a decrease in AFRU immunoreactive material in the SCO of the hypertensive rats and also that the secretory material stored in SCO is partially depleted in rats with induced hydrocephalus (Irigoin et al., 1990). These hypertensive animals showed an increase in ventricular size; therefore the variations of the IRM for the glycoproteins of the RF and proteins bands could be the cause and/or consequence of the hypertension and hydrocephalus.

The variations in the secretor activity in the SCO in SHR rats and the structural alterations of the SCO in hydrocephalus, described above, support the possibility that the SCO, the hydrocephalus and the variations in the proteins of the CSF could be connected with physiopathological aspects of this type of hypertension.

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**Fig. 5.** Photographs of the SCO subcommissural part of the WKY and SHR rats immunostained with anti-RF (AFRU). Coronal view of the SCO and ependyma of the third ventricle. (A, WKY and C, SHR). Bar: 100 µm. Medial parts of the SCO (B, WKY and D, SHR). Bar: 50 µm. Ependymal and hypendymal layers of the SCO. 3V: III ventricle; Ep: ependyma cells; Hy: hypendymal cells; PC: posterior comissure.
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