

Immunohistochemical colocalization of 7B2 and 5HT in the neuroepithelial bodies of the lung of *Rana temporaria*

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Abstract. The neuroendocrine cell population of the lung of *Rana temporaria* has been studied by means of immunocytochemistry. Serotonin (5HT)- and polypeptide 7B2-immunoreactive neuroepithelial bodies have been observed in the epithelial lining of the lung. 5HT- but not 7B2-immunoreactive isolated endocrine cells have also been observed.

Key words: Lung – Neuroepithelial bodies – Immunocytochemistry – 7B2 Protein – Serotonin – *Rana temporaria* (Anura)

Introduction

The discovery of putative endocrine cells in the lungs of vertebrates is credited to Feyrter (1938), who identified “clear cells” dispersed in the epithelia of various organs including the lung. Fröhlich (1949) confirmed these findings and described both single and clustered clear cells spread throughout the epithelium of the airways. The cell clusters have been given a variety of names, suggesting different functional properties or embryological origins. Lauweryns et al. (1972) proposed the term “neuroepithelial bodies” (NEBs) for the clustered cells. Pulmonary “endocrine” cells have been described in Amphibia (Rogers and Haller 1980; Goniakowska-Witalinska 1980b, 1981, 1982; Goniakowska-Witalinska and Cutz 1990; Matsumura 1985) by scanning and transmission electron microscopy. The presence of fluorogenic amines in pulmonary “endocrine” cells of different species of Amphibia has been shown by the formaldehyde-induced fluorescence technique (Rogers and Haller 1978; Wasano and Yamamoto 1978; Goniakowska-Witalinska 1980a). Only two studies of endocrine cells of amphibian lung in which immunohistochemical techniques are used have been reported to date (Cutz et al. 1986; Scheuermann et al. 1989).

7B2 is a protein of 23KDa isolated from human and porcine pituitary gland (Hsi et al. 1982), where it is localized in secretory granules (Marcinkiewicz et al. 1988). Human and porcine cDNA encoding 7B2 has been cloned and sequenced (Martens 1988; Brayton et al. 1988). 7B2 has also been detected in the human gastrointestinal tract (Suzuki et al. 1985, 1988) and pancreas (Suzuki et al. 1987, 1988), and in neural and endocrine tissues of Amphibia (Ayoubi et al. 1990, 1991). The function of 7B2 is not known yet, but it could be involved in cellular secretory processes (Ayoubi et al. 1991).

The aim of the present study was to investigate the occurrence of 7B2 in the endocrine cells and NEBs in the lung of the frog, *Rana temporaria*, and to determine whether it is colocalized with 5HT or not.

Materials and methods

Six adult specimens of frog (*Rana temporaria*) were anesthetized with ether and their lungs were fixed in Bouin's fluid for 24 h. The pieces were dehydrated and embedded in paraffin; then 3- μ m-thick sections were cut. An indirect immunocytochemical method using the avidin-biotin (ABC) technique (Hsu et al. 1981) was applied to paraffin sections. Endogenous peroxidases were blocked by exposure to 0.3% hydrogen peroxide in methanol for 30 min, and non-specific reaction was blocked with normal swine serum (1:20, University of Navarra, Pamplona, Spain, code NP-1) for 30 min at room temperature. Sections were then incubated with the primary antisera directed against either 5HT, (Immunonuclear 43H2TR 1:1000–1:30000) or 7B2, (RPMS, Histochemistry Department ref Nr 2012 1:1000–1:10000). This primary incubation was performed overnight at 4° C in a moist chamber. After thorough rinsing with TRIS-HCl buffer saline (TBS) (0.05 M; pH 7.36; 0.55 M NaCl), secondary antiserum (Biotinylated swine anti-rabbit immunoglobulins, 1:200, Dakopatts, Glostrup, Denmark, code E353) was applied for 30 min at room temperature.

Sections were washed with TBS, and then avidin-biotin peroxidase complex solution (ABCComplex, 1:100, Dakopatts, code K355) was applied for 30 min. Immunoreacted sections were washed in TRIS-HCl buffer (0.05 M; pH 7.36; 0.55 NaCl), and the peroxidase reaction was developed in a solution of 3,3'-diaminobenzidine tetrahydrochloride (0.03% w/v TRIS-HCL, Sigma, St Louis, USA, code D-5637) and H₂O₂ (0.006%). The sections were