DEVELOPMENTAL EXPRESSION OF FK506 BINDING PROTEINS AND CALCINEURIN IN RAT BRAIN.

ABIGAIL R. CHARTERS*, MASAKAZU KOBAYASHI# and STEVE P. BUTCHER*.

*Fujisawa Institute of Neuroscience, Department of Pharmacology, 1 George Square, Edinburgh, EH8 9JZ, U.K. #Fujisawa Pharmaceutical Co. Ltd., 2-1-6 Kashima, Yodogawa-Ku, Osaka 532, Japan.

The immunosuppressant FK506 exerts its pharmacological effects through the high affinity interaction with a subclass of immunophilins, the FK506 binding proteins (FKBPs) [1]. The high levels of these binding proteins and their mRNAs in rat brain suggests a physiological role for FKBPs in the mammalian central nervous system. The major intracellular target for FK506 is a 12 kDa protein (FKBP-12). Although this protein possesses peptidyl-prolyl cis-trans isomerase activity, the clinical effects of FK506 are mediated through protein phosphatase 2B (calcineurin) [2]. The FK506-FKBP-12 complex inhibits the activity of calcineurin, and calcineurin and FKBP-12 are co-localised in adult rat brain [3]. We have previously reported the regional and subcellular distribution of FKBPs in rat brain [4,5]. In this study, we have investigated both the expression and ontogeny of FKBPs and calcineurin in rat brain.

Monoclonal antibodies against recombinant human FKBP-12 were raised by the Biotechnology Group of Fujisawa Pharmaceutical Company Ltd., Japan [6]. These were used in Western Blotting experiments to study the presence of FKBP-12 in whole brain extracts from rat pups at various stages of development. Brains were dissected, the tissue homogenised and the protein was subjected to SDS-PAGE, transferred onto PVDF membrane prior to blotting with 3F4-70 monoclonal antibody (Fujisawa Pharmaceutical Co., Japan). The bands were detected by the Amersham ECL detection method. The specificity of the monoclonal antibody was determined as described previously [4].

It has been shown that FKBP-12 is present in substantial quantities in rat brain before birth. It remains present throughout all ages tested in this study (P0 to adult) and the amount present appears to remain stable. In addition to FKBP-12, two other putative FK506 binding proteins have been found in brain. These proteins have molecular weights of 35 and 44 kDa, and are recognised by the monoclonal antibody employed in the present study. In the developing rat brain, the relative amount of 35 kDa protein appears to decrease with age. The 44 kDa protein is present in only very small quantities (Fig. 1). It has also been found that the β-subunit of calcineurin is present from birth in a commercially available monoclonal antibody (Upstate Biotechnology Inc.).

The amounts of α-subunit of calcineurin (measured using a commercially available polyclonal antibody purchased from Upstate Biotechnology Inc.) are very low in the period before and immediately following birth, but start to increase after postnatal day 7.

The results presented in this study demonstrate that the expression of FKBPs and calcineurin follows a developmental pattern. This may be important when considering the mechanisms by which FK506 may exert its clinical effects and may prove to be a useful tool when attempting to elucidate the interactions of calcineurin with the immunophilins.


Abbreviations used: FKB, FK506 binding protein; PVDF, polyvinylidene difluoride.

Figure 1.
Developmental expression of FKBPs in rat brain. Homogenates of rat brain were subjected to SDS-PAGE on 12.5% homogeneous gels and transferred onto PVDF membranes for Western blotting. Incubation with primary antibody was performed for 1 h at 25 °C. Detection was by Amersham ECL method. Lanes are labelled as number of postnatal days.