

Title for CLAS Undergraduate Research Symposium

Student 1, Faculty 1
Department of Research



Introduction

Vasopressin hormone assists with the regulation of homeostasis of water and solutes in the blood by reabsorbing molecules in kidney tubules (3). In addition, more recent research indicates vasopressin may play a role in various social behaviors across species (2). Vasopressin responses are mediated through arginine vasopressin receptors (AVPR's). Three known subtypes of AVPR's have been described with each subtype exhibiting a unique tissue distribution. AVPR V1A receptors are expressed in smooth muscle cells, hepatocytes, and platelets (1). AVPR V1B receptors are expressed in cells of the anterior pituitary (4). AVPR V2 receptors are expressed in the kidney tubules and the lungs, and all 3 subtypes are expressed in various regions of the brain (1).

Materials and Methods

Frozen brain tissue from adult male Wistar rats was sectioned at 20 μm on a cryostat and mounted on Gold plus slides (Fisherbrand®). Sections were covered with hydrogen peroxide solution to block endogenous peroxidase and incubated with protein block to prevent nonspecific binding. Following an overnight incubation at 4°C in a 1:200 dilution of AVPR V2 primary antibody (ABCAM®), sections were incubated with biotinylated goat polyclonal secondary antibody to rabbit IgG (ABCAM®). Sections were then covered with streptavidin peroxidase followed by visualization with DAB substrate (brown stain). The sections were then counterstained with hematoxylin (purple stain).

Abstract

Vasopressin hormone, found in most mammals, plays a key role in the homeostasis of water and solute concentration in the blood. There are three known subtypes of vasopressin receptors, each with unique tissue distributions. Arginine vasopressin receptor 2 (AVPR V2) has been identified in mammals in the kidney, lungs, and various regions of the brain. The purpose of this study was to determine whether the AVPR V2 receptor was present in rat brain, and if present, to identify the specific brain region and cell type expressing the receptor. Frozen, preserved rat brain was sectioned on a cryostat and processed with rabbit polyclonal AVPR V2 and biotinylated goat polyclonal secondary antibody to rabbit IgG. Staining of antibody treated sections revealed AVPR V2 receptors were present on Purkinje cell axons and cell bodies in the cerebellum. These data suggest that the cerebellum of rats may be an important regulatory site of vasopressin functions.



Figure 1:
Cerebellum of male rat brain treated with primary and secondary antibodies. [400x]

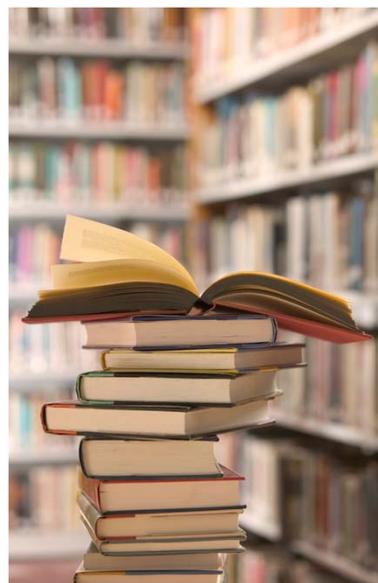


Figure 1:
Cerebellum of male rat brain treated with primary and secondary antibodies. [400x]

Results

Sections treated with AVPR V2 receptor primary antibody and biotinylated goat polyclonal secondary antibody to rabbit IgG resulted in staining of cells in the cerebellar region of the male rat brain. In particular, Purkinje cell bodies and axons were noticeably stained with DAB substrate (**Figures 1 and 2**). Sections incubated without primary antibody (**Figure 3**) and without secondary antibody (**Figure 4**) did not stain with DAB substrate.

Discussion

Previous studies have found AVPR V1A and V1B receptors in cerebellar tissue (2, 4); however only one study to date has found AVPR V2 in the cerebellum (5). Results of our immunochemical analysis of the adult male rat brain also show that AVPR V2 receptors are present in the cerebellum. In addition, our study indicates that AVPR V2 receptors are located primarily on the large Purkinje cell bodies and axons. These data suggest that the cerebellum of rats may be an important regulatory site of vasopressin functions and that the Purkinje cells play an important part in vasopressin regulation.

Literature Cited

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