INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-3, July-Sept-2012 Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

*Received: 05th July-2012* *Revised: 19th July-2012* *Accepted: 21st July-2012*

*Research article*

# APPLICATION OF WESTERN BLOTTING TECHNIQUE FOR EVALUATING THE EXPRESSION OF VASOPRESSIN RECEPTORS IN THE HEART CELLS; IMPORTANCE IN THE CARDIOVASCULAR SYSTEM

Manoj G Tyagi

Department of Pharmacology, Christian Medical College, Vellore 632002, TN, India

*Author for Correspondence: E-mail: tyagi239@yahoo.co.in*

**ABSTRACT:** Vasopressin, a posterior pituitary hormone is responsible for water reabsorption by the kidneys and maintenance of cardio-vascular homeostasis. Vasopressin receptors are characterized as $VR_1$ (V1a), $VR_2$ (V2), and $VR_3$ (V1b). VR1, which is abundant in vascular smooth muscles, causes vasoconstriction by increasing intracellular calcium via the phosphatidylinositol bisphosphonate pathway and a positive inotropic effect in cardiac muscle. $VR_2$ has also been shown to be expressed in the heart. There is emerging role for vasopressin receptors in health and disease. This study describes the application of Western blotting to elucidate the importance of vasopressin receptors in the heart cells.

**Key words:** Vasopressin, receptor, Western blotting, myocytes, heart

## INTRODUCTION

The association of cardiac dysfunction with elevated plasma AVP levels has important prognostic implications (Holmes *et al* 2003). Indeed, experimental left ventricular hypertrophy in rats as a result of aortic stenosis increases AVP in magnocellular nuclei of the hypothalamus and heightens plasma AVP levels (Antunes-Rodrigues *et al* 2004). Interestingly, AVP is secreted from isolated, pressure-overloaded buffer-perfused rat hearts; it has been localized in endothelial and vascular smooth muscle cells of arterioles and perivascular tissue (Xu and Gopalakrishnan, 1991). Therefore, AVP activity in the myocardium and coronary vasculature may be influenced by an intrinsic cardiac AVP system.

Among neurohormonal targets for therapy in CHF, arginine vasopressin (AVP) has attracted much recent interest. Indeed, it is increased AVP secretion in heart failure, and its potential to promote hyponatremia and other effects that can lead to CHF progression, that makes CHF a topic of interest for this article. There is greater emphasis on the role of AVP as it relates to CHF and the potential benefits of AVP antagonism as a new therapeutic option for patients with CHF are being investigated (Parmley, 1995).AVP belongs to the family of vasoactive and mitogenic peptides involved in physiological and pathological cell growth and differentiation. Its physiological activities are mediated through three receptor subtypes coupled to different G proteins: VR 1(V1a), VR2 (V2), and VR3 (V1b) (Gutkowska *et al* 2007, Gassanov *et al* 2007). $VR_1$, which is abundant in vascular smooth muscles, causes vasoconstriction by increasing intracellular calcium via the phosphatidylinositol bisphosphonate pathway and a positive inotropic effect in cardiac muscle. Prolonged VR1 stimulation leads to the synthesis of proteins involved in cellular hypertrophy in vascular and myocardial tissues (Nakamura *et al* 2000, Hiroyama *et al* 2007). $VR_2$, which is considered a renal receptor located on renal tubular epithelial cells, mediates water retention, as well as cAMP formation, and alters aquaporin (AQP) expression. $VR_3$, which is localized in pituitary, pancreatic beta cells, and the adrenal medulla, induces the release of hormones. The expression of AVP in the heart has been described by recent studies. This study describes the Western blotting technique for evaluating the expression of vasopressin receptors in the ventricular myocytes of the Wistar albino rat.

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

## MATERIALS AND METHODS

**Preparation of ventricular myocytes:** The ventricular myocytes can be isolated and cultured by a modified technique described earlier (Engelmann *et al* 1990). Wistar albino rats weighing between 200-220g were used for the study. The rats were anesthetized with sodium pentobarbital 40 mg/kg i.p injection. The ventricles were removed and rinsed, minced on ice, and digested with 125 U/mL type II collagenase (Calbiochem, USA) in Joklik's modified Eagle's medium (GIBCO) supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 250 ng/mL amphotericin, and 50 µmol/L $CaCl_2$ at 37°C. Cells were harvested after digestion and resuspended in ice-cold collagenase-free digestion medium containing 0.5% fetal bovine serum. Additional 5 ml of KHB was added at least three times at 12 minutes intervals with out removal of supernatants. In between the addition of Kreb's buffer the myocytes were disaggrated using a wide mouthed pipette. Disaggregated tissue was filtered through 230µm nylon mesh. The cell suspensions were then centrifuged at 40g for 2 minutes. The supernatant fraction was discarded and the cell pellets were resuspended gently in Kreb's buffer and again the cell suspension centrifuged at 50g for 2 minutes. The centrifugation was repeated until the preparation was composed of 90 % viable ventricular myocytes. The freshly prepared myocytes were used for the experiments.

### Western Blotting Technique

For Western blot analysis, ventricular myocytes are suspended in Tris HCl buffer containing a cocktail of protease inhibitors and insoluble materials removed by centrifugation at 4°C. Protein content was determined in homogenized samples using a detergent-compatible protein assay (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (20µg) from heart cells was separated by 10 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a Tris-Glycine-SDS buffer system (Bio-Rad, USA), and the separated proteins were transferred to nitrocellulose membranes (Hybond-C, Amersham-Pharmacia, USA). Molecular size calibration was achieved with Broad Standard Solution (Bio-Rad Laboratories, USA). The nitrocellulose blots were blocked overnight with 5% nonfat milk in Tris-buffered saline (20 mM Tris·HCl, pH 8.0, 140 mM NaCl, 1.1 % BSA, and 0.1% Tween 20) and then probed with Rat anti-$VR_1$, anti-$VR_2$, or anti-$VR_3$ antibody (Alpha diagnostics International, USA, 1:800 dilutions) for 2 h at room temperature. Antibody incubations and washes were performed in Tris-buffered saline. Detection is realized by enhanced chemiluminescence with an ECL kit (Amersham-Pharmacia Biotech, United Kingdom) and an appropriate peroxidase-conjugated secondary antibody according to the protocol provided by the manufacturer. Densitometry of the bands was quantitated by Image J software. Differences in protein loading were corrected by densitometric quantification. Figure 1 describes the effect of vasopressin analogues on $V_2$ receptor protein expression in ventricular myocytes.

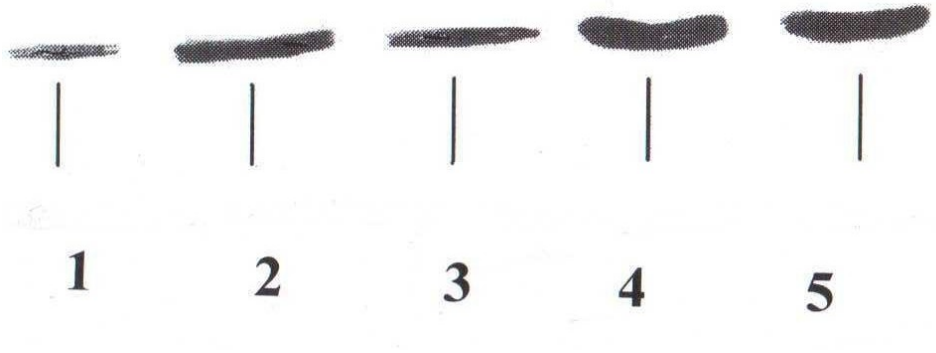


**Figure 1 : Effect of vasopressin analogues, lysine vasopressin (LVP) and arginine vasopressin (AVP) on $V_2$ receptor protein expression in cardiac myocytes. Lane 1 (Control), Lane 2 (LVP, 200nM), Lane 3 (LVP, 400nM), Lane 4 (AVP, 200nM), Lane 5 (AVP, 400nM).**

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

## DISCUSSION

In this study a novel culture technique for ventricular myocytes and the modified Western blotting technique has been described for the expression of vasopressin receptors. Vasopressin has been show to modulate the actions of the heart and may cause bradycardia after intravenous injection and affect physiological actions like the renal absorption of water and anti-nociception (Sulthana *et al* 2011, Tyagi, 2011, Tyagi *et al* 1992). Vasopressin has also been shown to be involved in cardioprotective actions after ischemia reperfusion injuries (Nazari *et al* 2011). There is also expression of vasopressin receptors in the heart. Thus, long-lasting $VR_2$ activity in the presence of agonist has been described in rat cardiomyocytes subjected to adenoviral transfer of the $VR_2$ gene (Laugwitz *et al* 1999). It has been reported, in addition, that $VR_2$ overexpression in the rat myocardium increases cardiac contractility *in vivo* (Hupf et al 1999), consistent with the notion that cAMP is the most important modulator of cardiac contractility . Therefore, we can hypothesize that cardiac-specific enhancement of $VR_2$ signaling may play a role during significant changes in the contractility and histological structure of the ventricular myocardium during the early neonatal period. Indeed, in cardiomyocytes and many other cell types, cAMP mediates the action of a number of different receptors and modulates many cellular functions as diverse as movement, growth, metabolism, and synaptic plasticity (Hunter, 2000). Stimulation of the V1A receptors in vascular smooth muscle could therefore increase systemic vascular resistance, increasing impedance to ventricular emptying (i.e, afterload) and thereby adversely affect ventricular function in heart failure (Pruszczynski *et al* 1984). Sustained increases in afterload also contribute to myocardial remodeling and progressive failure. Direct stimulation of the myocyte over time may have the same effect. The AVP targets in newborn rats were $VR_1$ and $VR_2$ expressed in cardiomyocytes. Another novel finding in the several studies is the identification and full characterization of $VR_2$ in newborn hearts, confirmed by RT-PCR at the transcript level, with specific $VR_2$ protein shown by immunoblotting and localized by immunofluorescence in newborn cardiomyocytes, as well as by cAMP production in these cells in response to DDAVP treatment. Thus there is greater implication of vasopressin's role in the heart. There is general agreement with those of Kaufmann *et al* (Kaufmann *et al* 2003), who noted $VR_2$ expression in the human heart. In addition, these authors demonstrated activation of nitric oxide (NO) production in human endothelial cells by DDAVP, suggesting vasodilatation through $VR_2$.

Western blotting techniques can be used to elucidate the expression of vasopressin receptors in specific regions of the heart. There is a likelihood of vasopressin *per se* and its receptors contributing to the pathophysiology of cardiovascular diseases, apart from their roles in maturation and normal physiological functioning. Thus, in conclusion this study describes a novel western blotting technique to elucidate vasopressin receptors in cardiac myocytes.

## REFERENCES

Antunes-Rodrigues J**,** de Castro M, Elias LL, Valenca MM, McCann SM.(2004) Neuroendocrine control of body fluid metabolism. Physiol Rev 84: 169–208

Engelmann GL, McTiernan C, Gerrity RG, Samarel AM (1990). Serum-free primary cultures of neonatal rat cardiomyocytes: cellular and molecular applications.Technique ;2:279-291

Gassanov N, Jankowski M, Danalache B, Wang D, Grygorczyk R, Hoppe UC, Gutkowska J (2007). Arginine vasopressin-mediated cardiac differentiation: insights into the role of its receptors and nitric oxide signaling. J Biol Chem.282: 11255–11265

Gutkowska J, M Miszkurka, B Danalache, N Gassanov, D Wang and M Jankowski (2007).Functional arginine vasopressin system in early heart maturation**.** AJP – Heart. October**,** 4, 293, H2262-H2270

Hupf H**,** Grimm D, Riegger GA, Schunkert H (1999). Evidence for a vasopressin system in the rat heart. Circ Res 84: 365–370.

Hunter T (2000). Signaling—2000, and beyond. Cell 100: 113–127

Holmes CL**,** Landry DW, Granton JT (2003)**.** Vasopressin and the cardiovascular system. Receptor physiology. Crit Care 7: 427–434.

Hiroyama M, Wang S, Aoyagi T, Oikawa R, Sanbe A, Takeo S, Tanoue A (2007).Vasopressin promotes cardiomyocyte hypertrophy via the vasopressin V1A receptor in neonatal mice. Eur J Pharmacol. 559:89 –97

Kaufmann JE**,** Iezzi M, Vischer UM (2003)**.** Desmopressin (DDAVP) induces NO production in human endothelial cells via $V_2$ receptor- and cAMP-mediated signaling. J Thromb Haemost.1: 821–828

Laugwitz KL, Ungerer M, Schoneberg T, Weig HJ, Kronsbein K, Moretti A, Hoffmann K, Seyfarth M, Schultz G, Schomig A (1999). Adenoviral gene transfer of the human $V_2$ vasopressin receptor improves contractile force of rat cardiomyocytes. Circulation 99: 925–933

Tyagi MG (2011). Vasopressin hormone receptor density and gene expression in lithium induced polyuria rats. Ind.J.Appl.Biol.Pharm.Tech. Vol-2, Issue-4, 81 to 86

Tyagi MG, S. Ramaswamy, DG. Shewade, and KD. Tripathi (1992) Vasopressin acts independently of calcium to induce anti-nociception in mice. Medical Sci. Res. 20 (4), 131-132

Nakamura Y, Haneda T, Osaki J, Miyata S, Kikuchi K (2000). Hypertrophic growth of cultured neonatal rat heart cells mediated by vasopressin V1A receptor. Eur J Pharmacol 391: 39–48

Nazari A, Sadr SS, Faghihi M, Imani A, Moghimian M (2011). The cardioprotective effect of different doses of vasopressin (AVP) against ischemia-reperfusion injuries in the anesthetized rat heart. Peptides, 32(12):2459-66

Parmley WW (1995)**.** Neuroendocrine changes in heart failure and their clinical relevance. Clin Cardiol 18: 440–445

Pruszczynski W, Vahanian A, Ardaillou R, Acar J (1984). Role of antidiuretic hormone in impaired water excretion of patients with congestive heart failure. J Clin Endocrinol Metab. 58: 599–605

Sulthana A, Nidhi Tyagi and MG Tyagi (2011). Tramadol Potentiates Vasopressin Induced Analgesia in Albino Mice. Recent Research in Science and Technology, 3(6): 64-65

Xu YJ**,** Gopalakrishnan V (1991). Vasopressin increases cytosolic free [$Ca^{2+}$] in the neonatal rat cardiomyocyte. Evidence for $V_1$ subtype receptors. Circ Res. 69: 239–245.