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REVIEW ARTICLE

HYPEROSMOLALITY ALTERS MACROPHAGE SYNTHESIS AND REGULATION; ROLE OF PHOSPHOLIPASE C ENZYMES

Manoj G Tyagi

Department of Pharmacology, Christian Medical College, Vellore 632002, Tamilnadu

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ABSTRACT

High NaCl stimulates activity of the osmoprotective transcription factor TonEBP/OREBP by enhancing its phosphorylation, transactivating ability, and localization to the nucleus. PLC γ plays an important role in these mechanisms. On the other hand, macrophages play important roles in both innate and adaptive immune responses. One major function of macrophages is to monitor their environment for invading pathogens like bacteria or viruses and consequent elimination by phagocytosis. In addition, phagocytosing macrophages are essential for the clearance of apoptotic cells and cell debris. Foreign antigens are identified and internalized by pattern-recognition receptors on the cell surface, including scavenger receptors and Toll-like receptors. This review examines the influence of NaCl induced hyperosmolality and the role of Phospholipase C (PLC) in macrophage synthesis and regulation.

Key words: PLC, macrophages, Hyperosmolality, TonEBP, NaCl, p53

INTRODUCTION:

The Interstitial fluid in the renal medulla has high NaCl, which energizes the urinary concentration. TonEBP/OREBP protects renal medullary cells from this high NaCl and also contributes to urinary concentration by transactivating genes that code for urea transporters and aquaporin 2 water channel. It transduces the extracellular NaCl gradient signal into the cell to start indirect events, which eventually affect late chemotactic responses. One possible candidate of this late mechanism in salt-dependent chemotaxis might be the NaCl-induced expression of chemokines [1], [2], which are capable of enhancing cell motility in an autocrine/paracrine way. A co-relation with the IP3 system has been suggested with the involvement of PLC γ. Recent studies support the hypothesis that macrophages are not only essential for efficient immune responses, but are also regulators of an extrarenal salt balance system, which controls blood pressure [3]. Macrophages function may open new avenues to treat anomalies like the ischemia, hypertension and metabolic disorders [4].

The phospholipase C enzyme subtype PLC γ is an important enzyme for cancer biology. Two mammalian subtypes of PLC-γ isozymes have been identified. PLC-γ1 mRNA is widely detected in various tissues. It is abundantly expressed in embryonal cortical structures, neurons, oligodendrocytes and astrocytes [5]. Unlike PLC-γ1, PLC-γ2 mRNA is expressed in the limited areas of anterior pituitary and cerebellar Purkinje and granule cells [6]. PLC hydrolyzes phosphatidylinositol 4,5–bisphosphate to generate 2 second messengers, inositol 1,3,4–triphosphate and diacylglycerol. While inositol 1,3,4-triphosphate regulates Ca^{2+} efflux, it and diacylglycerol activates the enzyme PKC. On the other hand, the β family of PLC, which consists of 4 isoforms, PLC β1–β4, is regulated by heterotrimeric G proteins [7]. PLC β2 is primarily expressed in hematopoietic cells, whereas PLC β3 and PLC β1 are expressed in a variety of cells and tissues. PLC β4 is predominantly expressed in neuronal cells [8]. While all of the PLC β isoforms can be activated by the members of the Gq family of α subunits, PLC β2 and β3 can also be potently activated by Gβγ .The expression of PLC-γ2 is primarily limited to cells of haematopoietic lineage [9]. In this review article the possible roles of the phospholipase C enzymes are discussed in hyperosmolality induced alteration of macrophage synthesis and survival.

Hyperosmolality and macrophage survival:

It has been shown by previous studies that the 600 mOsm culture extended the macrophage half-life from 44, which is consistent with the duration of macrophage primary culture [10], to approximately 102 days. At the molecular

*Corresponding author: Dr. Manoj G Tyagi | E-mail: nirajkhatri22@yahoo.com

level, it maintained the low levels of expression of p53 and Bax whereas it increased the level of Bcl-2. p53 is a transcription factor situated at the cross-roads of several signaling pathways that are essential for cell-growth regulation and apoptosis [11]. In normal conditions, the level of p53 in the cells is usually limited by a constant degradation of the protein by the ubiquitin/proteasome pathway and play an important role in several cell types [12]. When normal mouse macrophages were put in culture in isosmotic conditions, it was found that the p53 level is slightly increased until cell death. Consistently, in isosmotic cultures, Bcl-2, a pro-survival protein is down-regulated while Bax, a pro-apoptotic gene transactivated by p53, is overexpressed [13]. However, hyperosmolarity prevents p53 and Bax regulation as well as Bcl-2 inhibition. This suggests that hyperosmolarity prevents apoptosis and allows macrophage half-life to be extended. These might be the initial events in a cell transformation process. During inflammation, there is normally an influx of macrophages and a high rate of epithelial cell death can occur. Recent data strongly suggests that hyperosmolarity appears to play a key role both in the recruitment and the survival of macrophages.

The Salt-dependent chemotaxis is not restricted to RAW264.7 macrophage-like cells, since both murine bone marrow derived macrophages as well as peritoneal macrophages migrate towards excess NaCl. However, it is important to note here that not all motile cells of the myeloid lineage recognize a high NaCl concentration as a chemo-attractant, since it has been demonstrated that LPS-stimulated bone marrow derived dendritic cells do not migrate towards 40mM excess NaCl [14]. Although it was not excluded that other motile cells show a chemotactic response toward a hypertonic NaCl stimulus, the results are consistent with the notion that salt-dependent chemotaxis is a macrophage-specific function. In the central nervous system, the mechanisms for hyperosmolality-induced increases in SNA from the PVN are Ang II/AT1R dependent,and the negative regulatory action of full form macrophage inhibitory factor (MIF) quenches Ang II/AT1R-stimulated increases in neuronal discharge and elevations in arterial pressure. Findings from the latter study were consistent with the earlier *in vitro* observation that the Ang II-increased firing response of PVN neurons is blunted by macrophage inhibitory factor (MIF), which is likely to be activated by continual AT1R stimulation [15].Thus, an Ang II activity-dependent feedback mechanism may exist to afford protection against overstimulation by Ang II, which is presumed to operate *in vivo*. However, crucial further experiments are required that should include determining whether HS-induced induction of endogenous MIF in the PVN can be prevented by AT1R blockade and provide tempering over HS-induced sympatho-excitation. Also, it now becomes imperative to determine the role that MIF plays in the PVN for chronic elevations of sympathetic nerve activity and arterial pressure observed during dehydration [16]. The demonstration that HS conditions also produce an increase in MIF mRNA expression in the PVN opens up the possibility that MIF is a major central nervous system regulator of sympathetic outflow in conditions of high salt levels.

TonEBP and NaCl induced macrophage migration:

To assess whether TonEBP is involved in salt-dependent chemotaxis was investigation of migration behavior in RAW264.7 cells with a stable TonEBP overexpression. TonEBP protein expression was strongly increased following a high NaCl concentration. TonEBP overexpressing RAW264.7 cells displayed a high amount of TonEBP protein without an additional salt stimulus. However, upon stimulation with excess NaCl protein expression did not increase much further in these cells. Concerning migration behavior, if TonEBP played a positive role in salt-dependent chemotaxis, the migration response was expected to be much higher and earlier in TonEBP overexpressing than in wildtype RAW264.7 cells.Nevertheless, when comparing migration capacity of RAW264.7 wildtype cells to TonEBP overexpressing cells over 20 hours, no significant difference in migration kinetics was observed. These results indicate that TonEBP is not responsible for salt-dependent chemotaxis. In macrophages, TonEBP additionally binds to two sites of the Vegfc promoter and thus regulates vascular endothelial growth-factor C (VEGF-C) expression [3]. VEGF-C release by macrophages resulted in a local lymphangiogenesis of preexisting lymph capillaries by binding VEGFR3 receptors. In addition, VEGF-C increased interstitial endothelial nitric oxide synthase (eNOS) expression via activation of VEGFR2 receptors. The subsequent release of nitric oxide is thought to compensate blood pressure following a dietary high salt loading [17,18]. The newly discovered TonEBP/VEGF-C mediated regulation mechanism was designated extrarenal, since it does not directly involve blood pressure or volume regulation by the kidney. Instead, macrophages were recognized to control local tissue environment and blood pressure in the skin interstitium by driving lymphatic vessel hyperplasia and increasing interstitital eNOS expression. Failures in this regulatory axis are associated with the development of salt-sensitive hypertension in both rodents [3] and humans, as elevated VEGF-C serum levels in hypertensive patients or following a high-salt diet [19] have been reported. These results emphasize clinical importance of a homeostatic

macrophage function, which might be beneficial for prospective therapy of hypertension.

Phospholipase C γ and macrophage function:

It has been shown that PLC-γ1 contributes to high NaCl–induced increase of the transcriptional activity of TonEBP/OREBP by increasing its transactivating activity and by increasing its nuclear localization. PLC-γ1 plays a central role in signal transduction by cleaving phosphatidyl inositol into diacylglycerol and the second messenger inositol triphosphate. PLC-γ1 lipase activity contributes to high NaCl–induced activation of TonEBP/ OREBP by increasing its nuclear localization but not its transactivating activity. It can also signal independently of its lipase activity. Macrophages infiltrate to the sites of Na^+ and Cl^- overload in the skin which display a hypertonic microenvironment, indicating that the salt-gradient may be the driving force of macrophage cell attraction [20,21].These recruited macrophages sense the interstitial electrolyte composition and subsequently upregulate the transcription factor, tonicity enhancer binding protein, which is an essential transcription factor required for the expression of osmoprotective genes in response to hypertonicity-induced osmotic stress [22, 23]. A probable mechanism is that PLC-γ1 can act as a guanine nucleotide exchange factor (GEF), independent of its lipase activity. For example, it acts through its SH3 domain as a GEF for the GTPase dynamin-1 in mediation of clathrin-dependent endocytosis of the EGF receptor [24]. Additionally, its GEF activity can activate phosphatidylinositol 3-kinase (PI3K-1A). For example, NGF triggers localization of PLC-γ1 to the nucleus, where it acts as a GEF for the GTPase, phosphatidylinositol-3-kinase enhancer (PIKE), and PIKE, in turn, activates nuclear PI3K [25,26]. This signaling cascade may contribute to high NaCl–induced activation of TonEBP/OREBP because deletion of the PLC-γ1 SH3 domain and inhibition of PI3K-1A both reduce that activation. They speculated that a urea-activatable and tyrosine-phosphorylated upstream receptor or nonreceptor tyrosine kinase recruited and activated PLC-γ, and additionally may activate another tyrosine kinase effector, PI3-K. PI3-K phosphorylates membrane phosphoinositides at the D-3 position. These phospholipids act as second messengers that mediate the activation of Akt. Phosphoinositide-dependent kinase I (PDKI) and PDKII phosphorylate Akt at residues Thr308 and Ser473 and activate Akt. Activated Akt inhibits activation of capsase 9- and cytochrome c-induced apoptosis [27,28] and nicely depicted in Fig.1. Thus, the most favored hypothesis is that the alteration of ionic and solute concentrations in the cell activates PLC-γ and PI3-K, and then the activated PI3-K phosphorylates Akt and inhibits apoptosis in MDCK cells [29].

Macrophages as lipid modulators:

The scavenger cells i.e the macrophages infiltrate and reside in nearly every tissue, including adipose. Along with the observation that macrophages accumulate within adipose tissue with obesity [30], there has been a great interest on the effect of lipids on macrophage function and activation. Macrophages take up lipids via scavenger receptors, such as the CD36 and the scavenger receptor A (SR-A). This process is not subject to a negative feedback mechanism and therefore where there is excess lipid present macrophages can become loaded with lipid and form pro-atherogenic foam cells [31]. In conditions of over nutrition, where the adipose tissue is overwhelmed with nutrients resulting in different amounts of cellular stress [32], macrophages accumulate within adipose tissue and subsequently switch from an alternative activated (M2) phenotype to a classically activated (M1), suggesting that excess fat can enhance the activation of inflammatory signaling pathways [33]. This has also been demonstrated by several *in vitro* experiments, where incubation of macrophage with free fatty acids led to the activation of Toll-like receptor 4 signaling, NF-kB activation and subsequently fatty acid-induced insulin resistance [34]. The JNK signaling pathway has additionally been shown to be involved in the activation of inflammatory M1 macrophages and the development of obesity and insulin-resistance [32]. This was also shown by the deletion of JNK1 in hematopoietic-derived cells, which subsequently resulted in protection against diet-induced inflammation and insulin resistance without affecting obesity [35].

CONCLUSION : There appears to be a co-relation between NaCl induced hyperosmolality and macrophage synthesis and survival. These may have critical implications for cardiovascular disorders like the hypertension and cardiac failure. Do hypervolemic hypertensive episodes have major influence on the macrophage function and the role of phospholipase C mediated mechanism in these mechanisms needs to be fully explored. Some studies suggest the involvement of the phospholipase C mediated signal transduction in hyperosmolar mediated macrophage function modulation.

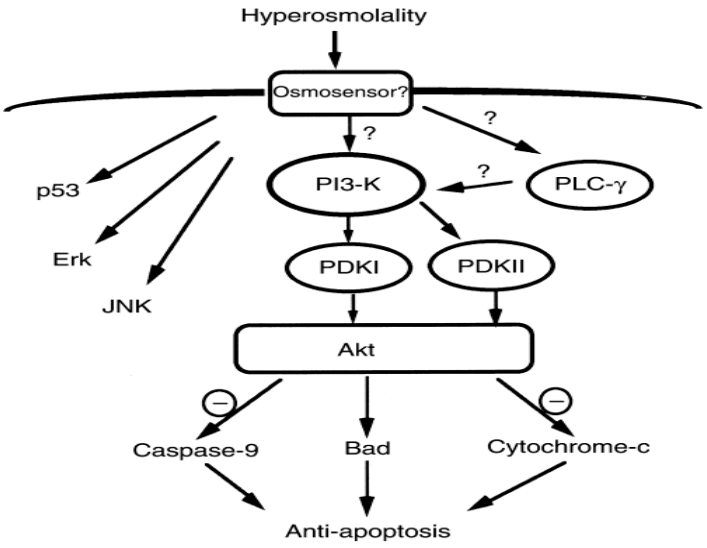

Figure 1: Courtesy Terada Y *et al* Kidney International, (2001)

REFERENCES:

1. Kojima R, Taniguchi H, Tsuzuki A, Nakamura K, Sakakura Y, et al. (2010) Hypertonicity-induced expression of monocyte chemoattractant protein-1 through a novel cis-acting element and MAPK signaling pathways. J Immunol 184: 5253–62
2. Kostyk AG, Dahl KM, Wynes MW, Whittaker LA, Weiss DJ, et al. (2006) Regulation of chemokine expression by NaCl occurs independently of cystic fibrosis transmembrane conductance regulator in macrophages. Am J Pathol 169: 12–20
3. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, et al. (2009) Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nat Med 15: 545–52
4. Jonathan Jantsch, Katrina J. Binger, Dominik N. Müller and Jens Titze.Macrophages in homeostatic immune function. Front. Physiol., 05, 146, 2014
5. Rhee, S.G., Bae, Y.S. 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. J. Biol. Chem. 272:15045-15048.
6. Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem 70:281–312.
7. Kano, M., Hashimoto K, Watanabe M, Kurihara H, Offermanns S, Jiang H, Wu Y, Jun K, Shin HS, Inoue Y, Simon MI, Wu D. Phospholipase C beta4 is specifically involved in climbing fiber synapse elimination in the developing cerebellum. Proc. Natl. Acad. Sci. U. S. A. 1998, 95:15724-15729.
8. Jiang, H.P., Lyubarsky A, Dodd R, Vardi N, Pugh E, Baylor D, Simon MI, Wu D. 1996, Phospholipase C b4 is involved in modulating the visual response in mice. Proc. Natl. Acad. Sci. U. S. A. 93:14598-14601.
9. Wu, D., Huang, C., Jiang, H. 2000. Roles of phospholipid signaling in chemoattractant-induced responses. J. Cell Sci. 113:2935-2940.
10. Müller S, Quast T, Schröder A, Hucke S, Klotz L, et al. (2013) Salt-Dependent Chemotaxis of Macrophages. PLoS ONE 8(9): e73439.
11. Labi V, Erlacher M, Kiessling S, Villunger A: BH3-only proteins in cell death initiation, malignant disease and anticancer therapy. Cell Death Differ. 2006, 13:1325-1338
12. N Sawhney, MK Patel, M Schachter and AD Hughes. Inhibition of proliferation by heparin and expression of p53 in cultured human vascular smooth muscle cells. Journal of Human Hypertension (1997) 11, 611–614
13. Hussain SP, Harris CC: p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. J Nippon Med Sch 2006, 73:54-64.
14. Muller S. Salt dependent chemotaxis of macrophages. PhD dissertation. University of Bonn, 2014.
15. Li H, Gao Y, Freire CD, Raizada MK, Toney GM, Sumners C. Macrophage migration inhibitory factor in the PVN attenuates the central pressor and dipsogenic actions of angiotensin II. FASEB J. 2006; 20:1748–1750.
16. E Colombari, D S.A. Colombari, Hongwei Li, P Shi, Y Dong, N Jiang, M K. Raizada, C Sumners, D Murphy, and J F.R. Paton. Macrophage Migration Inhibitory Factor in the Paraventricular Nucleus Plays a Major Role in the Sympathoexcitatory Response to Salt. Hypertension. Nov 2010; 56(5): 956–963.
17. Leonard AM, Chafe LL, Montani JP, Van Vliet BN.Increased salt-sensitivity in endothelial nitric oxide synthase-knockout mice. Am J Hypertens. 2006 Dec;19(12):1264-9.
18. Kang Y, Dempo Y, Ohashi A, Saito M, Toyoda H, Sato H, Koshino H, Maeda Y, Hirai T.Nitric oxide activates leak K+ currents in the presumed cholinergic neuron of basal forebrain.J Neurophysiol. 2007 Dec;98 (6):3397-410.
19. Cohen, DM, Gullans, SR, Chin, WW: Urea signaling in cultured murine inner medullary collecting duct (mIMCD3) cells involves protein kinase C, inositol 1,4,5-trisphosphate (IP_3), and a putative receptor tyrosine kinase. J Clin Invest 1996 97:1884–1889

20. Zhang, Z, Yang, XY, Soltoff, SP, Cohen, DM: PI3-K signaling in the murine kidney inner medullary cell response to urea. Am J Physiol. 2000 278:F155–F164

21. Halterman, J. A., Kwon, H. M., and Wamhoff, B. R. (2012). Tonicity-independent regulation of the osmosensitive transcription factor TonEBP (NFAT5). Am. J. Physiol. Cell Physiol. 302, C1–C8

22. Tyagi M G. NFAT-5 / TonEBP and its Role in Physiological Regulation and Aging Process. 2014 RJPBCS 5(4), 450-456

23. Choi JH, Park JB, Bae SS, Yun S, Kim HS, Hong WP, Kim IS, Kim JH, Han MY, Ryu SH, Patterson RL, Snyder SH, Suh PG. (2004) Phospholipase C-gamma1 is a guanine nucleotide exchange factor for dynamin-1 and enhances dynamin-1-dependent epidermal growth factor receptor endocytosis. J Cell Sci 117:3785–3795.

24. Ye K (2005) PIKE/nuclear PI3-kinase signaling in preventing programmed cell death. J Cell Biochem 96:463–472.

25. Ye K, Aghdasi B, Luo HR, Moriarity JL, Wu FY, Hong JJ et al. (2002) Phospholipase C gamma 1 is a physiological guanine nucleotide exchange factor for the nuclear GTPase PIKE. Nature 415:541–544.

26. Fruman, DA, Meyers, RE, Cantley, LC. Phosphoinositide kinases. Annu Rev Biochem 1998 67:481–507

27. Kennedy, SG, Kandel, ES, Cross, TK, Hay, N: Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. Mol Cell Biol 1999 19:5800–5810

28. Terada Y, Inoshita S, Hanada S, Shimamura H, Kuwahara M, Ogawa W, Kasuga M, Sasaki S, Marumo F.Hyperosmolality activates Akt and regulates apoptosis in renal tubular cells. Kidney Int. 2001; 60(2):553-67.

29. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112, 1796–808.

30. Nagy L., Szanto A., Szatmari I., Szeles L. (2012). Nuclear hormone receptors enable macrophages and dendritic cells to sense their lipid environment and shape their immune response. Physiol. Rev. 92, 739–789

31. Odegaard, JI., and Chawla, A. (2013). The immune system as a sensor of the metabolic state. Immunity.38, 644–654.

32. Lumeng C. N., Bodzin J. L., Saltiel A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J. Clin. Invest. 117, 175–184

33. Pal, D., Dasgupta, S., Kundu, R., Maitra, S., Das, G., Mukhopadhyay,S., Ray S, Majumdar SS, Bhattacharya S (2012). Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat. Med. 18, 1279–1285

34. Han, M. S., Jung, D. Y., Morel, C., Lakhani, S. A., Kim, J. K., Flavell, R. A., et al. (2013). JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science 339, 218–222.

35. Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM, Karin M. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. Cell Metab. 2007 Nov;6(5):386-97.

