Arch. Biol. Sci., Belgrade, 64 (2), 427-433, 2012 DOI:10.2298/ABS1202427P

DIFFERENT MODULATORY EFFECTS OF AMMONIUM IONS ON ANGIOTENSIN VASCULAR ACTIONS IN ISOLATED RAT AORTIC AND RENAL ARTERIES

I. RADUCU POPESCU[1], W. BILD[1,2], A. CIOBICA[2,3] and VERONICA BILD[1,2]

[1] *Gr. T. Popa University of Medicine and Pharmacy,* Iaşi 700115, Romania
[2] *Center of Biomedical Research of the Romanian Academy, Iasi Branch,* Iasi 700115, Romania
[3] *Alexandru Ioan Cuza University,* Iaşi, 700506, Romania

Abstract - In the present study, we were interested in the vascular effects of angiotensin II on perfused rings of the rat thoracic aorta and renal artery. Our results demonstrated different modulator alterations of these preparations induced by ammonium ions. Unlike the aortic rings, which exhibited only a reduction of angiotensin-induced contractility by NH_4Cl, the renal artery preparations showed both activation of vasoconstriction and inhibition of vasorelaxation in the ring pre-contracted with phenylephrine or noradrenalin. These results are interpreted as a modulation by the ammonium ions of vascular reactions induced by the stimulation of the vasoconstrictor AT_1 receptor on the one side and AT_2 vasodilator receptors on the other. The potentiation of renal vasoconstriction accompanied by the reduction of angiotensin vasodilation by NH_4Cl suggests the possibility of involvement from the blood flow and renal vascular tonus disturbances induced by ammonium ions during hyperammonemia of various causes.

Keywords: Ammonia, angiotensins, vascular smooth muscle

INTRODUCTION

Ammonia (NH_3) is the simplest nonionic molecule of any nitrogenous compound that has an essential role in many biological processes. Being a weak base, NH_3 is formed by the natural deamination of endogenous molecules and occurs in micro-molecular concentrations in human blood as result of its diffusion across the cell membranes. As part of the nitrogen cycle, NH_3 fulfils the role of a component for amino acids, nucleotide and protein synthesis. Having many endogenous and exogenous origins, most of the NH_3 is detoxified in mammals, mainly by its conversion to urea in the liver with participation of the five urea cycle enzymes (Visek, 1984).

In the renal tubular cells, NH_3 is protonated and converted to ionic form (NH_4^+) by reaction with secreted H^+ ions. The resulting ammonium ion rises within cells by trapping H^+ because of the more acid intracellular environment and highly permeable membranes. Under such circumstances, NH_3 equilibrates between the extra- and intracellular spaces, inducing a large variety of biochemical and neuropathological alterations.

In the biological fluids, NH_3 exists as such or predominantly as ammonium ions (NH_4^+), depending on the pH of solutions. The effects of NH_3/NH_4^+ in biological systems are as irritants, and they are responsible for some of the toxic actions that interface with energy metabolism, particularly in the brain. Elevated blood NH_4^+/NH_3 has been linked to hepatic coma, decreased neural excitability, epileptic seizures, convulsions induced by high-pressure oxygen breathing and fatigue states (Mutch and Banister, 1983).

Ammonium ions inhibit synaptic transmission by postsynaptic action, producing an inhibitory effect on glutamate-induced firing (Fan et al., 1990). Chronic hyperammonemia intensifies neuronal apoptosis and increases the serotonin turnover inducing the alterations of sleep patterns seen in hepatic encephalopathy (Szerb and Butterworth, 1992).

Several electrophysiological and biochemical mechanisms have been proposed to explain the deleterious effects of NH_4^+/NH_3 on central nervous system (CNS) functions, beginning with normal processes of uptake, storage or release of neurotransmitters, and finishing with the disruption of neuron-astrocyte trafficking of amino acids or neuroamines in the brain (Butterworth, 1998).

Acute ammonia toxicity is mediated through an increased accumulation of nitric oxide which combines with free radicals to form a highly toxic compound, peroxynitrite (Konopacka, 2006). A hydrogen bond formed between the superoxide anion and ammonium ion accelerates electron transfer from the radical anion to oxygen. The production of reactive nitrogen intermediates protein tyrosine nitration alters astrocyte function and contributes to ammonia neurotoxicity (Schliess et al., 2002).

Hyperammonemia exerts also a calcium channel blocking action that enhances the effects of central nervous system depression and of certain opioid analgesics (Kuta et al., 1984).

A number of investigators have reported the influences of hyperammonemia on hormones of metabolisms. A decreased glucose tolerance and an inhibitory action on the pancreatic beta cells were found (Barej and Harmeyer, 1979). Experiments on dairy cattle provided evidence that the increased production of ammonia reduced fertility and reproduction by lowering the plasma LH, progesterone and steroid hormone (Jordan and Swanson, 1979).

The relationship between hyperammonemia and the hormonal renin-angiotensin-system has been predominantly studied at renal level. NH_4Cl administration is associated with metabolic acidosis and with the increased plasma renin-angiotensin and aldosterone secretion (Schambelan et al., 1987, Györke et al., 1991). Infusion of NH_4Cl for 60 min suppressed plasma renin activity (Kisch et al., 1976).

While angiotensin II (Ang II) has potent effects on renal ammonia production and secretion rates by proximal tubule segments (Chobanian and Julin, 1991), potentiated by NH_4Cl acidosis (Nagami et al., 2004), the effect of ammonium ions on the vascular smooth muscle in general and the renal artery in particular, are still under investigations. Using helical strips of isolated rabbit aortas, Furtado et al. (1987) have signaled that a brief exposure to NH_4Cl did not affect the resting aortic tension but modified the responsiveness of the precontracted strips induced by norepinephrine. Ammonium ions cause relaxation of isolated large canine arteries, independent of the presence of the endothelium (Feletou et al., 1989), and amplify the K^+-induced contraction of the rat aorta by facilitating transmembrane Ca^{++} influx (Tanaka et al., 1996). On the other hand, the vascular tone and spontaneous twitch contractions of rat portal isolated vein were augmented by ammonium chloride and abolished in calcium-free solution or in the presence of 1μM nifedipine (Wakabayashi et al., 1992).

The influence of ammonium ions on the renal vascular actions of angiotensin peptides have been studied less. The aim of the present work was to determine their comparative actions on the contractility of rat aorta and renal arteries induced by angiotensin II.

MATERIALS AND METHODS

The subjects (n=24) were experimentally naive, male Wistar rats, weighing approximately 200-250 g at the beginning of the experiment. The animals were housed in a temperature- and light-controlled room (at 22°C and a 12-h cycle starting at 08:00 h), and were fed and allowed to drink water *ad libitum*. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimen-

tation and Animal Health and Welfare Act of Romania, and all procedures complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC) (Hogas et al., 2011, Hritcu et al., 2011). This study was approved by the local Ethics Committee and efforts were made to minimize animal suffering and to reduce the number of animals used.

The thoracic aorta was dissected and cut into 3-4 mm wide rings. The aortic rings, either intact or with the endothelium removed by gentle rubbing of the vascular lumen with a rough steel wire, were mounted in an organ bath with 4 ml Krebs-Henseleet saline containing mM: NaCl – 118; KCl – 4.7; $CaCl_2$ – 2.52; $MgSO_4$ – 1.64; $NaHCO_3$ – 24.88; KH_2PO_4 – 1.18; glucose – 5.55 at 37°C and bubbled with 95% O_2 and 5% CO_2.

The renal arteries were collected from extrarenal and intrarenal territories using the same technical procedure. The rings were stretched with 2 g of preload and left to balance for 60 min before the beginning of the experiment. The presence of the functional endothelium was checked pharmacologically with carbachol (10^{-6}M) administration, which releases the relaxant endothelial NO on the precontracted rings with phenylephrine (10^{-6}M). The isometric tension of the vascular smooth muscle was continuously recorded with a force transducer (Experimetria, Budapest, Hungary) connected to a computer data acquisition system. The reactivity of the preparations was tested by electric and pharmacological stimulation using angiotensin II (10^{-7} - 10^{-5} M), phenylephrine (10^{-7} - 10^{-6} M) and noradrenaline (10^{-7} M). In order to avoid tachyphylaxis, the administration of Ang II was repeatedly washed-out and readministered only after more than 60 min. The vascular reactivity to Ang II was examined on resting rings and rings that were precontracted with phenylephrine or noradrenaline.

Electrical field stimulation (EFS) was produced using an Exp-MCU stimulator (Experimetria, Budapest, Hungary) with a wave pulse of 10 volts and duration of 0.5 msec at frequency ranging for 4-20 Hz in increments of 4 Hz. After equilibration, a frequency response curve was performed as control for each administration. The vascular effects of the ammonium ions on the electrical and pharmacological stimulation were recorded before and after short exposure (10 min) of the preparations to NH_4Cl in various doses (5-50μM).

Data acquisition was made using an analog-digital convertor (National Instruments NiDaq 12DD713, USA).

The experiments were performed in series, and the results are presented as representative individual traces, tables and percentile graphs.

Data analysis

The changes in contractility on the vascular smooth muscle were statistically analyzed using one-way analysis of variance (one-way ANOVA). The results were expressed as the mean ± SEM. *Post hoc* analyses were performed using Tukey's honestly significant difference test in order to compare the different doses of NH_4Cl + Ang II used. F values for which $P<0.05$ were regarded as statistically significant (Padurariu et al., 2011).

RESULTS

In the first series of experiments, the reactivity of rings from the thoracic aorta and renal artery, both normal and without endothelium, was tested. The stimulation was performed with either EFS or pharmacologically, using phenylephrine, noradrenaline and angiotensin II. The vasoconstrictor reactions were more intense for the renal arteries, accompanied by slow spontaneous variations of the basal vascular tonus. The enhanced responses were observed both for electrical and pharmacological stimulations.

Unlike the vascular reactions of the thoracic aorta, which were exclusively vasoconstrictor indifferent to the nature of the stimulus, the renal artery demonstrated biphasic responses for angiotensin II. Besides its well-known vasoconstrictive action angiotensin II induced a relaxation in the rings of renal

artery which were precontracted with phenylephrine or noradrenalin.

The reactivity of renal and aortic rings was studied comparatively under the influence of short time exposure to ammonium ions. The preparations, incubated for 10 min with NH_4Cl in variable amounts (0.5 – 50 μM), demonstrated different reactions in the renal arteries for angiotensin II. While the vasoconstriction of the aortic rings induced by angiotensin II was strongly inhibited by NH_4Cl, the reactivity of the renal artery preparations increased significantly in the presence of ammonium ions (Fig. 1).

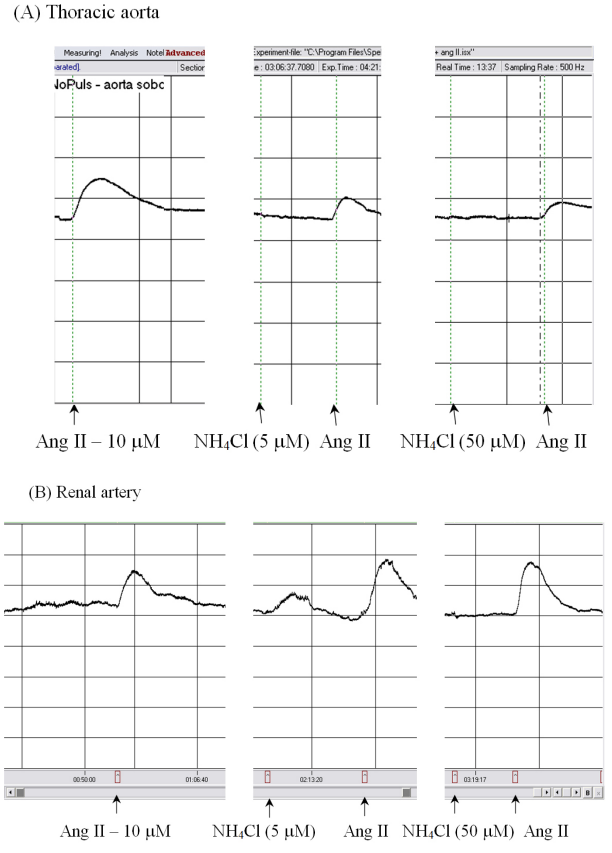

Fig. 1. Influence of ammonium ions on vasoconstrictor effects of angiotensin II on rat thoracic aorta (A) and renal artery (B).

Averages and variations of the results are presented in Fig. 2. As described in the case of rat thoracic aorta, we noticed a significant decrease of the contraction process in the NH_4Cl 5 μm + Ang II group

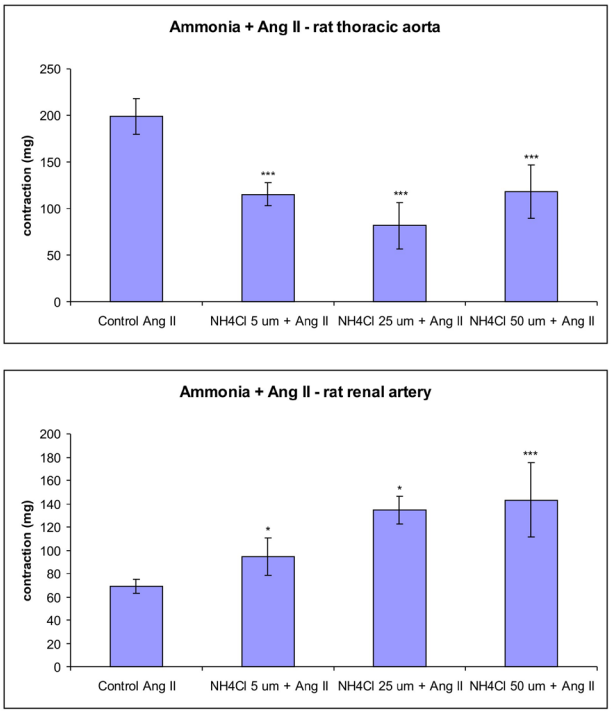

Fig. 2. Averages of inhibitory modulators effects of NH_4Cl on angiotensin II vasoconstriction at the level of rat aortic rings (A) - *** p<0.0001 vs. Ang II control group, and renal artery (B) - *p= 0,002 vs. Ang II control group, *** p<0.0001 vs. Ang II control group. The values are mean ± S.E.M. (n=6 animals per group).

(F(1.10)=53, p<0.0001), as well as the NH_4Cl 25 μm + Ang II group (F(1.10)=51, p<0.0001) and NH_4Cl 50 μm + Ang II group (F(1.10)=51, p<0.0001), in comparison with the control Ang II group (Fig. 2A). However, *post-hoc* analysis did not reveal any significant differences between the NH_4Cl 5 μm + Ang II vs. NH_4Cl 25 μm + Ang II groups (p= 0.068), or between the NH_4Cl 5 μm + Ang II vs. NH_4Cl 50 μm + Ang II groups (p = 0.87), and Ang II vs. NH_4Cl 25 μm vs. NH_4Cl 50 μm + Ang II groups (p = 0.083).

Additionally, regarding the renal artery, we demonstrated a significant increase of the contraction process in the NH_4Cl 5 μm + Ang II group (F(1.10)=7, p<0.02), as well as the NH_4Cl 25 μm + Ang II group (F(1.10)=7, p<0.02) and NH_4Cl 50 μm + Ang II group (F(1.10)=117, p<0.0001), in comparison with the control Ang II group (Fig. 2B). However, in this case the *post-hoc* analysis showed significant differ-

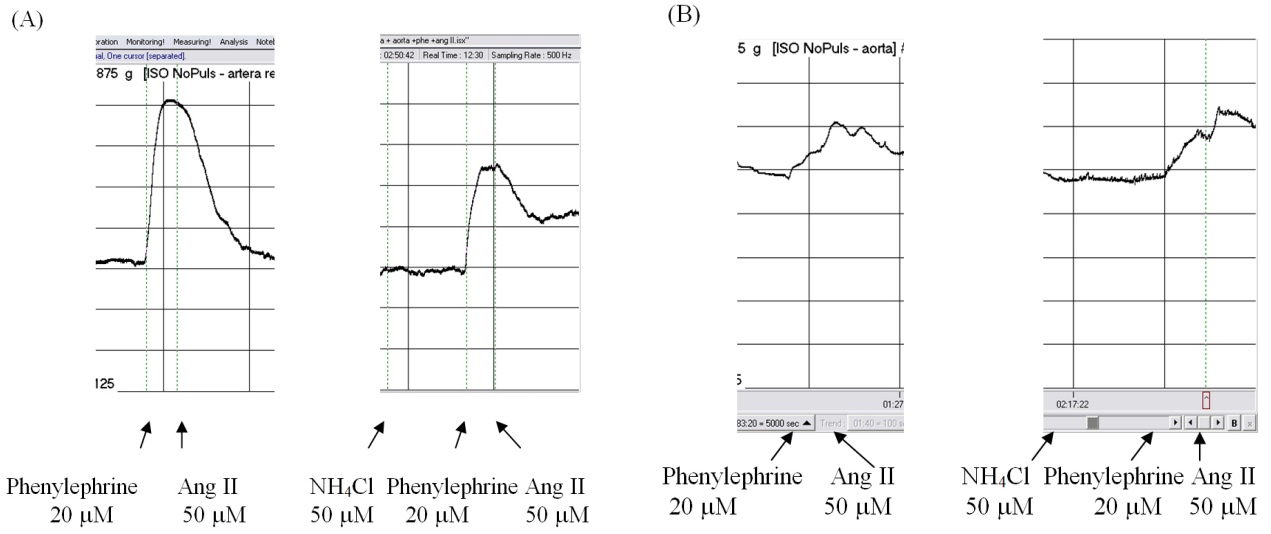

Fig. 3. Different modulatory properties of NH_4Cl upon the vascular effects of angiotensin II in the rat isolated renal artery (A) and thoracic aorta (B).

ences between the NH_4Cl 5 μm + Ang II vs. NH_4Cl 25 μm + Ang II groups (p= 0.006), as well as between the NH_4Cl 5 μm + Ang II vs. NH_4Cl 50 μm + Ang II groups (p = 0,002), but not for the Ang II vs. NH_4Cl 25 μm vs. NH_4Cl 50 μm + Ang II groups (p = 0.34).

The vasodilating effects produced by angiotensin II on rings that were precontracted with phenylephrine or noradrenalin were significantly inhibited by NH_4Cl and intensified on the precontracted ring (Fig. 3). The pH of the solution was kept within a variation range of 7.38 – 7.42.

DISCUSSION

Our results are in agreement with previous literature data (Furtado et al., 1987, Feletou et al., 1989, Tanaka et al., 1996). They offer an important contribution to the study of the different modulated properties of ammonium ions within the thoracic aorta and renal artery of the rat. The mechanisms through which these effects might occur seems to be very complex, beginning with the reactivity of the specific receptors and the accompanying electro-chemical membrane reactions, and ending with cellular metabolic reactions.

Even if both vascular structures are conductance vessels, with different densities and sensitivities of receptor formations, the alpha-adrenergic receptors tested with phenylephrine or noradrenalin demonstrated no significant changes of reactivity in the presence of ammonium ions, while angiotensin receptors were largely influenced in their presence, mainly in what concerns the renal artery rings.

The comparative study of the effects of NH_4Cl on the contractility of aortic or renal artery rings in the presence of angiotensin II demonstrated the inhibition of its vasoconstrictive action in the thoracic aorta and a significant potentiation of the contractility of the renal arteries. Most of the previous studies have demonstrated that the vascular effects of angiotensin II are mainly constrictive due to the stimulation of AT_1 receptors and protein kinase C, the activation of IP_3 and cytosolic calcium ion release, accompanied by detectable vasodilating properties (Timmermans et al., 1993). The vasodilating actions of angiotensin II rely on the stimulation of the AT_2 receptors (Toda and Miyazaki, 1981) that activate the synthesis and release of NO-cGMP, prostaglandins and plasma kinins (Israel et al., 2000, Katada and Majima, 2002). While AT_1 receptors interact with G_5 proteins induc-

ing phosphorylation of tyrosine kinase and activation of nitrogen-activated protein kinase (Touyz and Berry, 2002), AT_2 receptors are involved through G_i proteins in the activation of dephosphorylating phosphatases (Nouet and Nahmias, 2000). As the vasorelaxation induced by angiotensin II was obvious in our experiments only in the renal vessels precontracted with phenylephrine or noradrenalin, this has also allowed the demonstration of the modulator properties of NH_4Cl on the AT_2 receptors in the renal artery. Unlike the vasoconstriction that was potentiated by NH_4Cl, its vasodilating effects, previously indicated by Tirapelli et al. (2006) in the isolated rat carotid and Grbovic et al. (2008) in the isolated renal artery, were strongly inhibited in the presence of the ammonium ions.

The functional significance of these changes of renal vascular reactivity by the excess ammonium ions is insufficiently established. The potentiation of the constriction of the renal artery produced by an increased reactivity of the AT_1 receptors, accompanied by the inhibition of the relaxation induced by the AT_2 receptors, might contribute to the faulty renal blood flow in hyperammonemia of various causes.

The above findings provide further evidence for different modulated properties of ammonium ions in the thoracic aorta and renal artery in the rat. Although promising, these studies require further replication and new investigations using more advanced techniques and methods.

REFERENCES

Barej, W. and *J. Harmeyer* (1979). Effect of hyperammonaemia on blood glucose and plasma insulin levels in sheep. *Q J Exp Physiol Cogn Med Sci.* **64**, 31-37.

Butterworth, R.F. (1998). Effects of hyperammonaemia on brain function. *J Inherit Metab Dis.* **21**, 6-20.

Chobanian, M.C. and *C.M. Julin* (1991). Angiotensin II stimulates ammoniagenesis in canine renal proximal tubule segments. *Am J Physiol.* **260**, 19-26.

Fan, P., Lavoie, J., Lé, N.L., Szerb, J.C. and *R.F. Butterworth* (1990). Neurochemical and electrophysiological studies on the inhibitory effect of ammonium ions on synaptic transmission in slices of rat hippocampus: evidence for a postsynaptic action. *Neuroscience* **37,** 327-334.

Feletou, M., Harker, C.T., Komori, K., Shepherd, J.T. and *P.M. Vanhoutte* (1989). Ammonium ions cause relaxation of isolated canine arteries. *J Pharmacol Exp Ther.* **251,** 82-89.

Furtado, M.R. (1987). Effect of NH_4Cl on the contractility of isolated vascular smooth muscle. *Life Sci.* **41**, 95-102.

Grbovic, L., Djokic, J., Radenkovic, M. and *S. Pesic* (2008). Analysis of the vasorelaxant action of angiotensin II in the isolated rat renal artery. *J Pharmacol Sci.* **106**, 376-384.

Györke, Z.S., Sulyok, E. and *J.P. Guignard* (1991). Ammonium chloride metabolic acidosis and the activity of renin-angiotensin-aldosterone system in children. *Eur J Pediatr.* **150**, 547-549.

Hogas, M., Ciobica, A., Hogas, S., Bild, V. and *L. Hritcu* (2011), The effects of two doses manganese administration on short-term spatial memory and anxiety-like behavior in rats. *Arch. Biol. Sci., Belgrade* **63**, November 2011.

Hritcu, L., Stefan, M., Costica Misaila, Ciobica, A. and *G. Dumitru* (2011). Effects of bacterial lipopolysaccharide exposure on immune responsiveness in a rodent model of Parkinson's disease. *Arch. Biol. Sci., Belgrade* **63**, 99-105.

Israel, A., Cierco, M. and *B. Sosa* (2000). Angiotensin AT(2) receptors mediate vasodepressor response to footshock in rats. Role of kinins, nitric oxide and prostaglandins. *Eur J Pharmacol.* **394**, 103-8.

Jordan, E.R. and *L.V. Swanson* (1979). Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *J Anim Sci.* **48,** 1154-1158.

Katada, J. and *M. Majima* (2002). AT(2) receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. *Br J Pharmacol.* **136,** 484-491.

Kisch, E.S., Dluhy, R.G. and *G.H. Williams* (1976). Regulation of renin release by calcium and ammonium ions in normal man. *J Clin Endocrinol Metab.* **43,** 1343-1350.

Konopacka, A., Freśko, I., Piaskowski, S., Albrecht, J. and *M. Zielińska* (2006). Ammonia affects the activity and expression of soluble and particulate GC in cultured rat astrocytes. *Neurochem Int.* **48,** 553-558.

Kuta, C.C., Maickel, R.P. and *J.L. Borowitz* (1984). Modification of drug action by hyperammonemia. *J Pharmacol Exp Ther.* **229**, 85-90.

Mutch, B.J. and *E.W. Banister* (1983). Ammonia metabolism in exercise and fatigue: a review. *Med Sci Sports Exerc*. **15**, 41-50.

Nagami, G.T. (2004). Ammonia production and secretion by S3 proximal tubule segments from acidotic mice: role of ANG II. *Am J Physiol Renal Physiol.* **287**, 707-712.

Nouet, S. and *C. Nahmias* (2000). Signal transduction from the angiotensin II AT_2 receptor. *Trends Endocrinol Metab.* **11,** 1-6.

Padurariu, M., Ciobica, A., Mavroudis, I., Fotiou, D. and *S. Baloyannis* (2012), Hippocampal neuronal loss in the CA 1 and CA3 areas of Alzheimer's disease patients. *Arch. Biol. Sci., Belgrade.* in press.

Schambelan, M., Sebastian, A., Katuna, B.A. and *E. Arteaga* (1987). Adrenocortical hormone secretory response to chronic NH4Cl-induced metabolic acidosis. *Am J Physiol.* **252,** 454-460.

Schliess, F., Görg, B., Fischer, R., Desjardins, P., Bidmon, H.J., Herrmann, A., Butterworth, R.F., Zilles, K., and *D. Häussinger* (2002). Ammonia induces MK-801-sensitive nitration and phosphorylation of protein tyrosine residues in rat astrocytes. *FASEB J.* **16**, 739-741.

Szerb, J.C. and *R.F. Butterworth* (1992). Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. *Prog Neurobiol.* **39**, 135-153.

Tanaka, H., Wakabayashi, I., Sakamoto, K. and *E. Kakishita* (1996). Mechanism of the potentiating effect of NH4Cl on vasoconstriction in rat aorta. *Gen Pharmacol.* **27,** 535-538.

Timmermans, P.B., Wong, P.C., Chiu, A.T., Herblin, W.F., Benfield, P., Carini, D.J., Lee, R.J., Wexler, R.R., Saye, J.A. and *R.D. Smith* (1993). Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev.* **45,** 205-51.

Tirapelli, C.R., Fukada, S.Y., de Godoy, M.A. and *A.M. de Oliveira* (2006). Analysis of the mechanisms underlying the vasorelaxant action of angiotensin II in the isolated rat carotid. *Life Sci.* **78,** 2676-2682.

Toda, N. and *M. Miyazaki* (1981). Angiotensin-induced relaxation in isolated dog renal and cerebral arteries. *Am J Physiol.* **240,** H247-H254.

Touyz, R.M. and *C. Berry* (2002). Recent advances in angiotensin II signaling. *Braz J Med Biol Res.* **35,** 1001-1015.

Visek, W.J. (1984). Ammonia: its effects on biological systems, metabolic hormones, and reproduction. *J Dairy Sci.* **67**, 481-498.

Wakabayashi, I., Hatake, K. and *K. Sakamoto* (1992). Ammonium ion increases the tone of rat portal vein. *Gen Pharmacol.* **23**, 1189-1192.