Original Research
ISSN (Print): 2455-1244
ISSN (Online): 0971-913X
Journal of Endocrinology and Reproduction, *Vol 24(1), 43-52, June 2020, DOI: 10.18311/jer/2020/27223*

Nitric Oxide Modifies Hepatic and Cardiac Proton Gradient during Immersion-Stress in the Air-Breathing Fish (*Anabas testudineus* Bloch): Role of H^+-ATPase and H^+/K^+-ATPase

R. Gayathry[1], Valsa S. Peter[2] and M. C. Subhash Peter[1,2] *

[1]Department of Zoology, Inter-University Centre for Evolutionary and Integrative Biology, School of Life Science, University of Kerala, Kariavattom, Thiruvananthapuram - 695581, Kerala, India; subashpeter@yahoo.com
[2]Inter-University Centre for Evolutionary and Integrative Biology, School of Life Science, University of Kerala, Kariavattom, Thiruvananthapuram - 695581, Kerala, India

Abstract

Fishes have evolved complex and multi-step physiological mechanisms to drive ion homeostasis in challenging environments. Induction of stress that disturbs ion homeostasis in fishes evokes recovery response for their survival. Nitric Oxide (NO) as gasotransmitter modulates many physiological mechanisms including ion transport in osmoregulatory epithelia in teleosts. However, little is known about the role of NO in the transport of H^+ ions that creates proton gradient with the help of H^+-dependent ATPases like H^+-ATPase and H^+/K^+-ATPase, particularly in hepatic and cardiac tissues of bony fish. We, thus, quantified H^+-ATPase and H^+/K^+-ATPase in these tissues after *in vivo* treatments of NO donor, Sodium Nitro-Prusside (SNP) or NOS inhibitor, L-NAME, in both non-stressed and immersion-stressed air-breathing fish, *Anabas testudineus* Bloch. We found that elevated NO availability by SNP treatment lowered H^+-ATPase-driven H^+ transport in both hepatic and cardiac tissues of immersion-stressed fish. In contrast, NO depletion by L-NAME treatment elevated H^+-ATPase activity in these tissues of stressed fish, pointing to a direct role of H^+-ATPase in NO-mediated proton gradient regulation during stress condition. H^+/K^+-ATPase that drives H^+ transport against K^+ reduced its activity in cardiac tissue by SNP and L-NAME treatments. But L-NAME treatment in stressed fish imposed a higher H^+ transport in cardiac tissue of these fish. Overall, the data indicate that NO has a vital role in the regulation of H^+-ATPase-driven proton gradient in both cardiac and hepatic tissues of immersion-stressed fish.

Keywords: Air-Breathing Fish, Immersion-Stress, Nitric Oxide, Sodium Nitroprusside, H^+-ATPase, H^+/K^+-ATPase, L-NAME

1. Introduction

Teleost fishes have evolved complex and multi-step physiological mechanisms to drive ion homeostasis in challenging environments. Fishes are excellent models to understand the mechanisms related to stress as they are exposed to constant stress conditions in their natural habitats. Fishes respond to stressful conditions by evoking an array of stress-sensitive pathways, which include the release of stress hormones like catecholamines and corticosteroids[1]. This is followed by the metabolic changes and osmoregulatory disturbances in order to maintain homeostatic state[1-3]. Maintenance of ionic homeostasis is a complex process during stress, which mainly relies on active and passive transporters[4]. In teleosts, the heart and liver appear as the main targets of stress due to their involvement in maintaining whole body energetics. Heart is considered as one of the major targets of stressors in teleosts. It has been reported that post-stress cortisol production correlates with the myocardial remodeling at

*Author for correspondence

least in salmanoid fishes[18]. In addition, heart is a major producer of Nitric Oxide (NO) and also a major target of its actions.

The gasotransmitter, NO, has regulatory role in physiological processes in teleosts including ion transport, osmoregulation and metabolic regulation[19,20]. The cardiac physiologyof NO has been widely assessed in teleosts and data concomitant to the NO system with cardiac system have been accumulating[21-23]. Studies have suggested that presence of two NOS isoforms (nNOS and iNOS) within the myocardium of fish and the constitutively expressed nNOS are present in myocytes, endothelial cells, and neurons in the myocardium[24]. Evidence is also available for the expression of iNOS in myocytes, small vessel endothelium, vascular smooth muscle cells and immune cells that influence the heart. A canonical eNOS seems to be absent in teleosts[25], nevertheless, various studies reported the eNOS-like immunoreactivity in the heart of several teleost species[23,26-29]. Still, the occurrence of the NO endothelial system in fish has been questioned by many authors. It has also been suggested that NO does not have a main role in the vasodilatory control of arteries in fish[30]. Somehow, NO generated from nitric oxide synthase isoforms bound to cellular membranes may regulate the function of key membrane-associated enzymes. NO has been shown to affect ionic transport across various transmembrane transporters in different tissues of teleosts[31-33]. However, the role of NO in ion transporter function in fish cardiac tissue is poorly studied.

Vacuolar H^+-ATPases form an important class of ATP-driven proton pumps that are present in the intracellular and plasma membranes of eukaryotic cells and play diverse roles in both normal and abnormal cellular processes[5]. The electrochemical H^+ gradient created by H^+-ATPases is mainly involved in acid-base transport, and contribute to the overall body homeostasis[6]. The regulatory role of these transporters on acid-base balance is well explained in osmoregulatory tissues of teleosts[7-9]. H^+/K^+ATPase is a P-type transporter which is responsible for ATP-dependent exchange of H^+ for K^+ across plasma membranes[10]. This transporter is expressed at highly in mammalian gastric parietal cells, where it functions primarily as a proton pump transporting H^+ in exchange for K^+ into the secretory canaliculi[11,12]. Several studies have pointed to a role for the H^+/K^+ATPase in a variety of other cells including colonic enterocytes and renal tubular cells[13]. The presence of non-gastric HKA has been reported in different tissues of teleosts including air-breathing fish[14-17].

Liver plays an important role in stress acclimation and recently it has been reported that major stress hormone cortisol can mediate rapid and non-genomic action in hepatocytes of teleosts[34]. NO is also an important mediator of liver physiology and has differential effects on the liver depending on its source[35]. In liver biology, eNOS and iNOS are major role players, whereas the role of nNOS is little known. Endothelial NOS is mainly expressed in Liver Sinusoidal Endothelial Cells (LSECs) and endothelial cells of the hepatic artery, portal vein, central vein, and lymphatic vessels[36]. In contrast, iNOS is induced in various liver cells, including LSECs, hepatocytes, Kupffer cells (liver resident macrophages), Hepatic Stellate Cells (HSCs), smooth muscle cells, cholangiocyte and other immune cells[36-38]. Constitutively generated NO maintains the hepatic microcirculation and endothelial integrity, while inducible NO synthase–governed NO production can be either beneficial or detrimental. Given the importance of NO as a signaling molecule for ion transport, examination of the role of NO in the liver would be interesting. In the present study, we examined the modulatory role of NO in proton transporter function during immersion-induced stress in the climbing perch, *Anabas testudineus* Bloch. The purpose of this study was to investigate how donor of NO, Sodium Nitro-Prusside (SNP) and NOS antagonist, L-NAME, modulate H^+-dependent ATPases such as H^+-ATPase and H^+/K^+ATPase in hepatic and cardiac tissues of the air-breathing fish kept at non-stressed or immersion-stressed condition.

2. Materials and Methods

2.1 Collection of Model Fish and Acclimation

Adult healthy climbing perch of both sexes (*Anabas testudineus* Bloch), that belongs to order Perciformes and family Anabantidae, weighing about 35-45 g were, collected from near water bodies and reared in large cement tanks. They were acclimated to laboratory conditions for two months in well water kept at 28±1^{0}C under natural photoperiod (12L/12D). Fish were fed with commercial fish feed daily (1% body weight) and were in the pre-spawning period (March-April). Prior

to experiment, fish were kept in 60 L glass aquaria for three weeks, and food was withdrawn for 24 hr prior to sampling to ensure optimum experimental conditions. The requirements of Institutional Bioethics committee were fulfilled while rearing and experiment the fish and no mortality was recorded during the experiment.

2.2 Experimental Design

Four sets of *in vivo* experiments were conducted. Two experiments tested the dose-responsive *in vivo* effects of NO donor, SNP and NOS inhibitor, L-NAME in non-stressed fish. The other two sets of experiments were conducted to quantify the action of selected dose of SNP and L-NAME in immersion-stressed fish groups. Intraperitonial injections were given to control and treated fish groups and all the fish groups were handled the same manner. Immersion was selected as the mode of induction of stress. Strict care was taken to minimize stress during injection and handling. All fish were sampled concurrently after 30 min injection.

2.2.1 Dose-Dependent in Vivo Effects of SNP and L-NAME

In each experiment, twenty-four laboratory acclimated freshwater climbing perch were assigned as four groups of six each and kept in separate glass tanks (60×30×30cm). The first group, which served as the control, was administered intraperitoneal (*ip*) injections of 0.65% saline. The second, third and fourth groups were injected with varied doses of SNP (2.5, 5 and 10 µg g^{-1}) (Sigma Aldrich) or L-NAME (25, 50 and 100 ng g^{-1}) (Sigma Aldrich) intraperitoneally and kept for 30 min. The different doses of SNP/ L-NAME that were administered were dissolved in 100µL per fish in 0.65% saline and all the treatments were injected between 9.00 and 11.00 a.m.

2.2.2 Effects of in Vivo SNP in Non-Stressed and Stressed Fish

Test groups of non-stressed (N=6) and stressed (N=6) climbing perch were injected intraperitonially with the selected dose of SNP (5µg g^{-1})/ L-NAME (100ng g^{-1}) which was dissolved in 0.65% saline. Control groups of non-stressed (N=6) and stressed (N=6) fish were injected with 0.65% saline only. The control and test groups of stressed fish were subjected to immersion stress after injection, which was accomplished by keeping iron nets just below the surface water for 30 min.

2.3 Sampling and evaluation

Thirty minutes after the injection, fish in each group were netted together and anesthetized in a 0.1% 2-phenoxyethanol solution (SRL, Mumbai) and blood was drawn from the caudal vessels in all experiments. The fish in each group were then sacrificed by spinal transection and liver and heart tissues were excised, kept in ice cold SEI (Sucrose-EDTA-Imidazole) buffer (0.05M; pH 7.1) and stored at -80^{0}C until analysis.

2.3.1 Preparation of Membrane Fraction

Membrane fraction (H_0 fraction) was isolated from heart and liver tissues following the method of Peter *et al.*[39]. Briefly, 10% tissue homogenates were prepared in 0.25M SEI buffer and the homogenate was subjected to differential centrifugation at 700×g for 10 min(4^{0}C) to separate the cell debris and nuclei and the supernatant was collected as homogenization-zero fraction (H_0 fraction).

2.3.2 Determination of Vacuolar H^+-ATPase-Specific Activity

The baffilomycin-sensitive V-H^+ATPase activity was measured as described earlier[39]. The samples in duplicate containing (1.0 µg protein) were added to a 96-well microplate containing 100 mM NaCl, 30 mM imidazole (pH 7.4), 0.1 mM EDTA and 5 mM $MgCl_2$. Baffilomycin A (32 nM) was used as an inhibitor and the reaction was initiated by the addition of 0.3mM ATP and incubated at 37^{0}C for 15 min. The reaction was terminated by adding 8.6% TCA and the liberated inorganic phosphate was measured against phosphate standard at 700 nm in microplate Reader (Synergy HT Biotek). The activity is expressed in µMoles Pi liberated per hr for mg protein.

2.3.3 Determination of SCH28080-Sensitive H^+/K^+-ATPase Activity

SCH28080-sensitive H^+/K^+-ATPase activity were measured as described earlier[16]. 0.1 mM SCH28080, (2-methyl-8-(phenylmethoxy) imidazo [1,2-a] pyridine-3-acetonitrile) was used as inhibitor whereas 0.13 mM KCl was used as promoter to measure H^+/K^+-ATPase activity. Saponin-treated samples in duplicates (1.0 mg protein) were added to a 96-well microplate containing the above reaction mixture and the reaction was initiated by the addition of 0.3 mM ATP and incubated at 37^{0}C for15 min. The reaction was terminated by adding 8.6%

TCA and the inorganic phosphate content was measured in Synergy HT Biotek Microplate Reader. The change in absorbance at 700 nm between promoter and inhibitor assays was calculated using regression analysis and the rate of transport activity of H^+/K^+-ATPase is expressed in µMoles Pi liberated per hr for mg protein.

2.4 Statistical Analysis

Before statistical analysis, data were checked for normal distribution and variance homogeneity. The values were represented as mean ± SEM and the statistical difference among groups was tested by means of one-way analysis of variance (ANOVA) followed by SNK comparison test. Significance between the groups was analyzed with the help of GraphPad software (Instat-3, San Diego) and the level of significance was accepted if $p< 0.05$.

3. Result

3.1 Effect of SNP and L-NAME on Bafilomycin-Sensitive Vacuolar H^+-ATPase Activity in Liver and Heart of Non-Stressed and Immersion-Stressed Fish

Our data suggested that SNP at the two lower concentrations (2.5 and 5 µg g^{-1}) decreased the vacuolar H^+-ATPase activity in the heart (Figure 1A), while its activity increased after treatment with 10 µg $g^{-1}$of SNP (Figure 1A). However, in liver the activity showed a significant increase after the different concentrations of SNP (2.5, 5 and 10 µg g^{-1}) (Figure 1A). In stressed fish, selected concentration of SNP (5µg g^{-1}) decreased the H^+-ATPase-specific activity in both liver and heart of climbing perch (Figure 1B&C).

NOS inhibitor, L-NAME, did not alter the V-H^+ATPase activity in heart and liver of climbing perch at the given concentrations (25, 50 and 100 ngg^{-1}) (Figure 1A). Interestingly, the selected concentration of L-NAME (100ngg^{-1}) caused increase of its activity in both liver and heart of *Anabas* during immersion-stress (Figure 1B&C).

3.2 Effect of SNP and L-NAME on SCH28080-Sensitive H^+/K^+-ATPase Activity in Liver and Heart of Non-Stressed and Immersion-Stressed Fish

In heart, the H^+/K^+-ATPase activity decreased after treatment with different concentrations of SNP (2.5, 5 and 10 µg g^{-1}) (Figure 2A). Similar pattern of response was observed in liver of climbing perch after treatment with the lower concentration of SNP (2.5 and 5 µg g^{-1}), while the higher concentration (10 µg g^{-1}) increased its activity (Figure 2A). On the contrary, the H^+/K^+-ATPase activity remained unaltered after treatment with the selected dose of SNP (5 µg g^{-1}) in heart of stressed fish (Figure 2C), but a significant rise in its activity occurred in liver of immersion-stressed fish at this dose (Figure 2B).

A significant increase in the H^+/K^+-ATPase -specific activity was observed after treatment with the higher dose of L-NAME (100 ng g^{-1}) in heart, whereas it showed a reverse pattern in heart at lower dose (25 ng g^{-1}) (Figure 2A). L-NAME did not alter the H^+/K^+-ATPase activity in liver of climbing perch at given concentrations (25, 50 and 100 ng g^{-1}) (Figure 2A). In the stressed fish, the selected dose of L-NAME (100 ng g^{-1}) further increased the H^+/K^+-ATPase-specific activity in heart (Figure 2C).

Table 1. Table showing the activity pattern of V-H^+ATPase and H^+/K^+-ATPase in hepatic (L) and cardiac (C) tissues of SNP-treated and L-NAME treated *Anabas testudineus* in non-stressed (NS fish) and immersion-stressed (IMR fish) state.

Transporter Activity	Tissue	NS fish		IMR fish	
		SNP	L-NAME	SNP	L-NAME
V-H^+ATPase	H	↑	–	↓	↑
	C	–	–	↓	↑
H^+/K^+-ATPase	H	↓	–	–	–
	C	↓	↓	–	↑

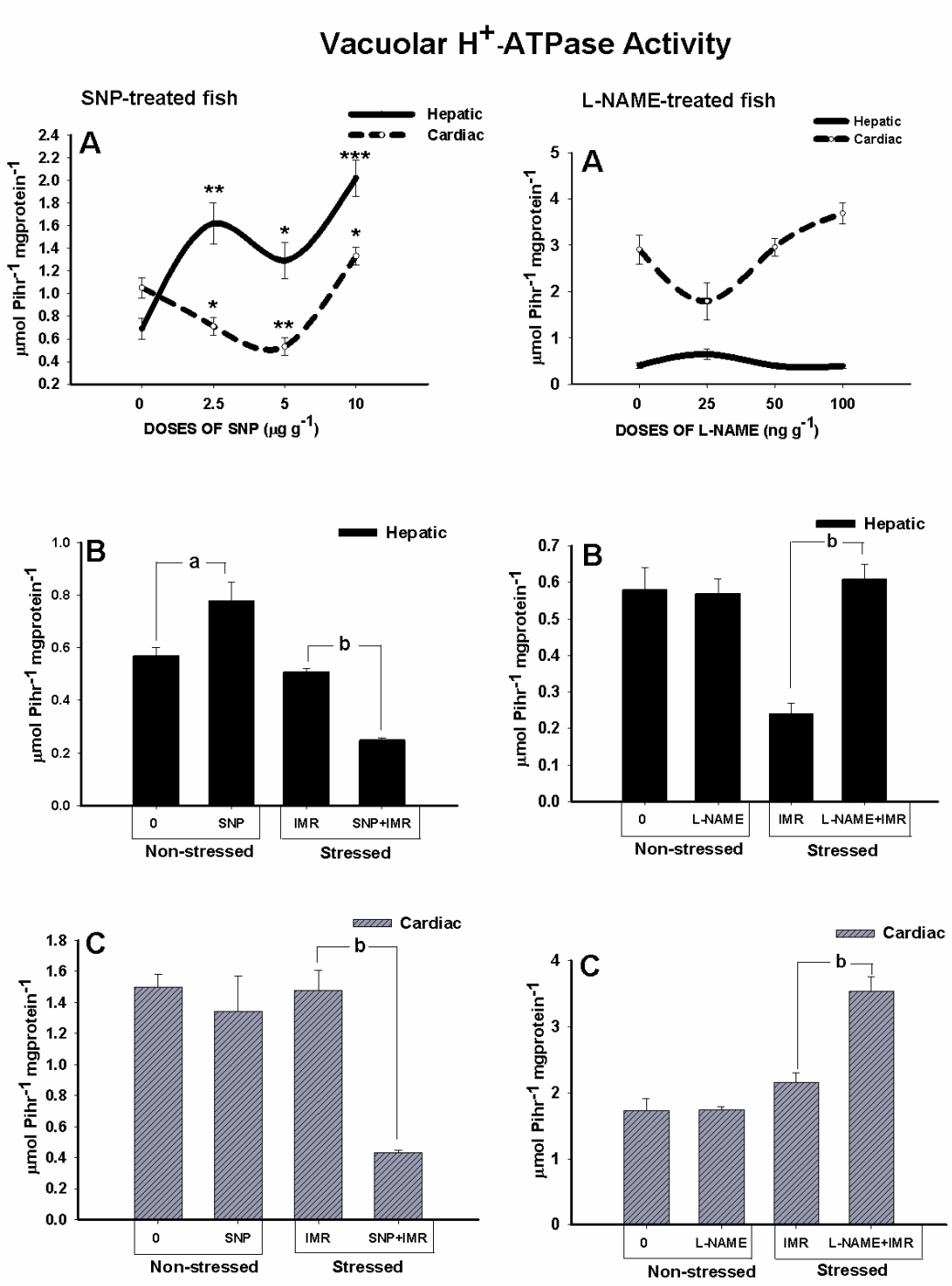

Figure 1. Dose-responsive action of sodium nitroprusside (SNP; 2.5, 5 and 10 µg g^{-1}) and N^{ω}–Nitro- L- Arginine methyl ester(L-NAME; 25, 50 and 100 ng g^{-1}) treatment for 30 min on bafilomycin-sensitive Vacuolar H^+-ATPase activity in the hepatic and cardiac tissues of *Anabas testudineus* **(A)**. The activity pattern of V-H^+-ATPase in the hepatic tissue is presented in "**(B)**" and in cardiac tissue is presented in "**(C)**". These activities were obtained from non-stressed and immersion-stressed fish after SNP treatment (5 µg g^{-1}) and L-NAME treatment (100 ng g^{-1}). Each bar is mean ± SE for 6 fish. In figure 1A, significant levels are represented as"*" (p<0.05), "**" (p<0.01) and "***" (p<0.001) when compared with control fish (0 µg g^{-1}). The significance levels of Figure 1B and C are represented as "**a**" when compared between control and SNP-treated (SNP)/ L-NAME-treated (L-NAME) fish and "**b**" represents significance between immersed control fish (IMR) and SNP-treated stressed (SNP^+IMR)/ L-NAME-treated stressed ($L\text{-}NAME^+IMR$) fish.

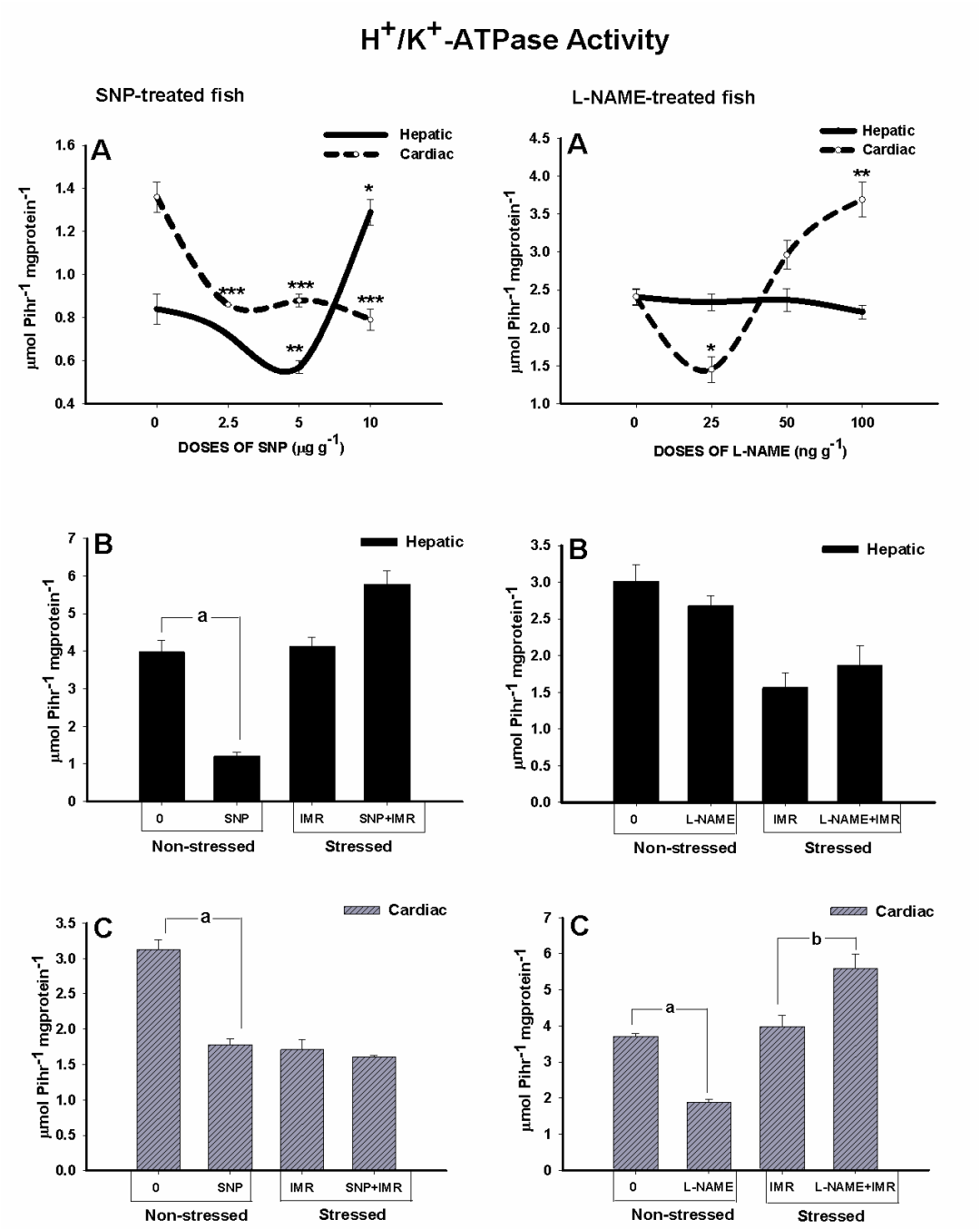

Figure 2. Dose-responsive action of sodium nitroprusside (SNP; 2.5, 5 and 10 µg g^{-1}) and N$^{\omega}$–Nitro- L- Arginine methyl ester(L-NAME; 25, 50 and 100 ng g^{-1}) treatment for 30 min on SCH28080-sensitive H^+/K^+-ATPase activity in the hepatic and cardiac tissues of *Anabas testudineus.* **(A)**. The activity pattern of H^+/K^+-ATPase in the hepatic tissue is presented in "**(B)"** and in cardiac tissue is presented in "**(C)"**. These activities were obtained from non-stressed and immersion-stressed fish after SNP treatment (5 µg g^{-1}) and L-NAME treatment (100 ng g^{-1}). Each bar is mean ± SE for 6 fish. In figure 2A, the significant levels are represented as "*" (p<0.05), "**" (p<0.01) and "***" (p<0.001) when compared with control fish (0 µg g^{-1}). The significance levels of figure 2B and C are represented as "**a**" when compared between control and SNP-treated (SNP)/L-NAME-treated (L-NAME) fish and "**b**" represents significance between immersed control fish (IMR) and SNP-treated stressed (SNP^+IMR)/ L-NAME-treated stressed ($L\text{-}NAME^+IMR$) fish.

However, the activity in liver was not responding to L-NAME treatment after immersion-stress (Figure 2B).

4. Discussion

Homeostatic regulation of ionic gradients across plasma membranes and the membranes of intracellular organelles is critical for most functions in cells[40]. Intrinsic membrane-active transporters like ATPases are of special interest in maintaining ionic homeostasis. Vacuolar H^+-ATPases localized in the plasma membrane mediate proton extrusion from the cell and has a critical role in the membrane transport system of protons in regulation of pH of intracellular and extracellular fluid, and thus play an important role in maintaining physiological homeostasis[9,41-43]. Evidences suggest that intracellular pH could affect the contractile function of the heart, metabolic reactions, ion exchange and calcium homeostasis in higher vertebrates[44]. In cardiac and hepatic tissues, the maintenance of proton gradient are critical and the active proton transporters such as H^+-ATPase and H^+/K^+-ATPase drive the proton homeostasis in these tissues.

The role of NO in blood vessel dilation and various cardiac functions has been well defined. But its integrated role in maintaining proton gradient in this tissue is ambiguous. The data of this study demonstrate that exogenous donor of NO, SNP,can inhibit the bafilomycin-sensitive H^+-ATPase activity in cardiac tissue of fish at their non-stressed state. NO has been reported to mediate stimulation of the activities of active transporters like Na^+K^+-ATPase in the cardiovascular regions of higher vertebrates[45-48]. NO derivative, peroxynitrite has been shown to inhibit this transporter activity in various tissues after treatment with NO donors[49]. Previously it has been reported that SNP treatment decreased the activity of H^+-ATPase in the gills, kidney and intestine of non-stressed *Anabas*[50]. In addition, it has been reported that elevated levels of endogenous NO can inhibit the H^+-ATPase activity in kidney of Wistar rats[51]. In contrast, the SNP treatment in immersion-stressed fish increased the H^+-ATPase activity in cardiac tissue. The total nitrite/nitrate level in heart tissue during immersion stress has been shown lowered after SNP treatment in *Anabas*[52]. This clearly shows a regulatory role of NO in H^+-ATPase-driven proton gradient regulation in cardiac tissue of *Anabas* at basal condition.

In contrast, in metabolic tissue like hepatic tissue, SNP treatment increased the bafilomycin-sensitive H^+-ATPase activity in non-stressed fish. Nitric oxide and its derivatives are known to change the membrane fluidity in the liver tissue[49] and the alteration in membrane fluidity can activate the membrane H^+-ATPase[53]. Treatment of SNP in immersion-stressed fish decreases its activity in hepatic tissue which clearly indicates a regulatory role for NO in H^+-ATPase modulation in this tissue in stressed condition as well. Interestingly, treatment with NOS-inhibitor, L-NAME, did not modulate the activity of H^+-ATPase in cardiac and hepatic tissues of non-stressed fish, where its activity showed reversal in immersion-stressed fish, pointing to a recovery role of NO in proton homeostasis in these fish.

The SCH28080-sensitive K^+-dependent H^+-extrusion ATPases respond to SNP in both hepatic and cardiac tissues which points to the existence of the functional H^+/K^+-ATPase in these cells. The decreased activity of HKA after SNP treatment in hepatic and cardiac tissues of non-stressed fish clearly implies a regulatory role of NO in these tissues. In excitable tissue like cardiac tissue the HKA activity showed a significant decline after L-NAME treatment in non-stressed condition. Similar observation has been found in rat colon after L-NAME treatment[54]. This suggests an important role of NO in HKA activity that maintains proton gradient particularly acid/base balance in these fish. Hypoxia stress has been shown to induce a reduction in K^+ efflux and depolarization due to the inhibition of K^+ channels[55,56]. The decreased activity of HKA in the cardiac tissue of stressed fish may be due to a reduced K^+ efflux, as heart is known to be the major target of hypoxia in teleosts[57]. However in hepatic and cardiac tissues, the activity remained unaltered after SNP treatment in stressed condition. But in immersed fish, L-NAME-treatment increased its activity which indicates that NO depletion favors higher proton gradient which is typical to a stressed condition. This further points to the role of basal levels of NO on H^+ and K^+ efflux which are essential for maintaining H^+ or K^+ homeostasis in this tissue.

The inhibition of endogenous NO by L-NAME in immersion-stressed fish activates H^+ transport driven by H^+-ATPase both in hepatic and cardiac tissues. This observation supports the view that NO and its derivatives are essential for regulating proton gradient in these tissues. In addition, the higher availability of endogenous NO by SNP treatment in stressed fish lowers H^+-ATPase-driven H^+ transport in both hepatic and cardiac tissues of stressed fish, pointing to a major action of NO in lowering H^+-ATPase driven proton-gradient. This further indicates

that NO could play a recovery role in minimizing the H^+-ATPase-induced higher proton-gradient, a typical stress-induced ion status in stressed fish. Overall, these indications of modified response of proton transporters to NO in stressed condition reveal that NO has an essential role in H^+ transport function in cardiac and hepatic tissues of climbing perch.

5. Acknowledgments

We gratefully acknowledge the financial support from iCEIB project (Higher Education Department, Govt. of Kerala). Thanks are due to UGC-SAP-DRS II facility in the Department of Zoology of the University of Kerala. RG acknowledges iCEIB for Research Associateship and VSP acknowledges the Emeritus Scientist award of Kerala State Council of Science, Technology and Environment (KSCSTE) and iCEIB of the University of Kerala.

6. References

1. Wendelaar Bonga SE. The stress response in fish. Physiol Rev. 1997; 77:591-625. https://doi.org/10.1152/physrev.1997.77.3.591. PMid:9234959
2. Peter VS, Peter MCS. The interruption of thyroid and interrenal and the inter-hormonal interference in fish: Does it promote physiologic adaptation or maladaptation? Gen Comp Endocrinol. 2011; 174:249-258. https://doi.org/10.1016/j.ygcen.2011.09.018. PMid:22001502.
3. George N, Peter VS, Peter MCS. Physiologic implications of inter-hormonal interference in fish: Lessons from the interaction of adrenaline with cortisol and thyroid hormones in climbing perch (*Anabas testudineus* Bloch). Gen Comp Endocrinol. 2013; 181:122-129. https://doi.org/10.1016/j.ygcen.2012.11.002. PMid:23153652.
4. Maffia M, Acierno R, Rollo M, Rizzello A, Storelli C, Pellegrino D, Tota B. Ionic regulation in antarctic teleosts. Ital J Zool. 2000; 67:47-52. https://doi.org/10.1080/11250000009356355.
5. Nishi T, Forgac M. The vacuolar H^+-ATPases- nature's most versatile proton pumps. Nat Rev Mol Cell Biol. 2002; 3(2):94-103. https://doi.org/10.1038/nrm729. PMid:11836511.
6. Maxson ME, Grinstein S. The vacuolar type H^+ ATPase at a glance- more than a proton pump. J Cell Sci. 2014; 127(23):4987-4993. https://doi.org/10.1242/jcs.158550. PMid:25453113.
7. Perry SF, Fryer JN. Proton pumps in the fish gill and kidney. Fish Physiol Biochem. 1997; 17: 363-369. https://doi.org/10.1023/a:1007746217349.
8. Piermarini PM, Evans DH. Immunochemical analysis of the vacuolar proton-ATPase β-subunit in the gills of a euryhaline stingray *(Dasyatis sabina)*: Effects of salinity and relation to Na^+/K^+ATPase. J Exp Biol. 2001; 204:3251-3259.
9. Guffey S, Esbaugh A, Grosell M. Regulation of apical H^+ATPase activity and intestinal HCO_3 secretion in marine fish osmoregulation. Am J Physiol Regul Integr Comp Physiol. 2011; 301:R1682-R1691. https://doi.org/10.1152/ajpregu.00059.2011. PMid:21865541.
10. Shin JM, Munson K, Vagin O, Sachs G. The gastric HK-ATPase: structure, function and inhibition. Pflugers Arch. 2009; 457(3):609-622. https://doi.org/10.1007/s00424-008-0495-4. PMid:18536934 PMCid:PMC3079481.
11. Faller L, Jackson R, Malinowska D, Mukidjam E, Rabon E, Saccomani G, Sachs G, Smolka A. Mechanistic aspects of gastric H^+/K^+ATPase. Ann N Y Acad Sci. 1982; 402:146-163. https://doi.org/10.1111/j.1749-6632.1982.tb25738.x. PMid:6301328.
12. Sachs G, Chang HH, Rabon E, Schackman R, Lewin M, Saccomani G. A nonelectrogenic H^+ pump in plasma membranes of hog stomach. J Biol Chem. 1976; 251:7690-7698. https://doi.org/10.1016/S0021-9258(17)32907-32911.
13. Song I, Yamada T, Trent JM. Mapping of the gene coding the α-subunit of the human H^+/K^+ATPase to chromosome 19q13. 1 by fluorescent in situ hybridization. Genomics. 1992; 14:547-548. https://doi.org/10.1016/S0888-7543(05):80266-80266.
14. Shirihai O, Smith P, Hammar K, Dagan D. Microglia generate external proton and potassium ion gradients utilizing a member of the H^+/K^+ATPase family. Glia. 1998; 23:339-348. https://doi.org/10.1002/(SICI)1098-1136(199808)23:4<339::AID-GLIA6>3.0.CO;2-Y.
15. Choe KP, Verlander JW, Wingo CS, Evans DH. A putative H^+/K^+ATPase in the Atlantic stingray, Dasyatis sabina: primary sequence and expression in gills. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R981-R991. https://doi.org/10.1152/ajpregu.00513.2003. PMid:15217793.
16. Simi S, Peter VS, Peter MCS. Zymosan-induced immune challenge modifies the stress response of hypoxic air-breathing fish (Anabas testudineus Bloch): Evidence for reversed patterns of cortisol and thyroid hormone interaction, differential ion transport. Gen Comp Endocrinol. 2016; 251:94-108. https://doi.org/10.1016/j.ygcen.2016.11.009. PMid:27871800.
17. Peter MCS, Simi S. Hypoxia stress modifies Na^+/K^+ATPase, H^+/K^+ATPase, $Na^+/NH4^+$ ATPase and nkaα1

isoform expression in brain of immune-challenged air-breathing fish. J Exp Neurosci. 2017; 11:1-8. https://doi.org/10.1177/1179069517733732. PMid:29238219 PMCid:PMC5721975.
18. Johansen IB, Lunde IG, Rosjo H, Christensen G, Nilsson GE, Bakken M, Overli O. Cortisol response to stress is associated with myocardial remodeling in salmonid fishes. J Exp Biol. 2011; 214:1313-1321. https://doi.org/10.1242/jeb.053058. PMid:21430209.
19. Fago A, Jensen FB. Hypoxia tolerance, nitric oxide, and nitrite: Lessons from extreme animals. Physiology. 2015; 30:116-126. https://doi.org/10.1152/physiol.00051.2014. PMid:25729057.
20. Perry S, Kumai Y, Porteus CS, Tzaneva V, Kwong RWM. An emerging role for gasotransmitters in the control of breathing and ionic regulation in fish. J Comp Physiol B Biochem Syst Environ Physiol. 2016; 186:145-159. https://doi.org/10.1007/s00360-015-0949-x. PMid:26660653.
21. Garofalo F, Parisella ML, Amelio D, Tota B, Imbrogno S. Phospholamban S-nitrosylation modulates Starling response in fish heart. Proc R Soc B Biol Sci. 2009; 276:4043-4052. https://doi.org/10.1098/rspb.2009.1189. PMid:19726482 PMCid:PMC2825783.
22. Imbrogno S, Capria C, Tota B, Jensen FB. Nitric oxide improves the hemodynamic performance of the hypoxic goldfish (*Carassius auratus*) heart nitric oxide. Biol Chem. 2014; 42:24-31. https://doi.org/10.1016/j.niox.2014.08.012. PMid:25178168.
23. Filice M, Amelio D, Garofalo F, David S, Fucarino A, Jensen FB, Imbrogno S, Cerra MC. Angiotensin II dependent cardiac remodeling in the eel *Anguilla anguilla* involves the NOS/NO system. Nitric oxide. 2017; 65:50-59. https://doi.org/10.1016/j.niox.2017.02.007. PMid:28232085.
24. Danson EJ, Choate JK, Paterson DJ. Cardiac nitric oxide: Emerging role for nNOS in regulating physiological function. Pharmacol Ther. 2005; 106:57-74. https://doi.org/10.1016/j.pharmthera.2004.11.003. PMid:15781122
25. Andreakis N, Aniello SD, Albalat R, Patti FP, Procaccini G, Sordino P, Palumbo A, Garcia-ferna J. Evolution of the nitric oxide synthase family in metazoans. Mol Bio Evol. 2011; 28:163-179. https://doi.org/10.1093/molbev/msq179. PMid:20639231.
26. Fritsche R, Schwerte T, Pelster B. Nitric oxide and vascular reactivity in developing zebrafish, *Danio rerio*. Am J Physiol Regul Integr Comp Physiol. 2000; 279:R2200-R2207. https://doi.org/10.1152/ajpregu.2000.279.6.R2200. PMid:11080086
27. Cioni C, Bordieri L, De Vito L. Nitric oxide and neuromodulation in the caudal neurosecretory system of teleosts. Comp Biochem Physiol. - B Biochem Mol Biol. 2002; 132:57-68. https://doi.org/10.1016/S1096-4959(01)00532-2.
28. Tota B, Amelio D, Pellegrino D, Ip YK, Cerra MC. NO modulation of myocardial performance in fish hearts. Comp Biochem Physiol. - A Mol Integr Physiol. 2005; 142:164-177. https://doi.org/10.1016/j.cbpb.2005.04.019. PMid:15982912.
29. Garofalo F, Imbrogno S, Tota B, Amelio D. Morpho-functional characterization of the goldfish (*Carassius auratus* L.) heart. Comp Biochem Physiol. - A Mol Integr Physiol. 2012; 163:215-222. https://doi.org/10.1016/j.cbpa.2012.05.206. PMid:22705557.
30. Park KH, Kim KH, Choi MS, Choi SH, Yoon JM, Kim YG. Cyclooxygenase-derived products, rather than nitric oxide, are endothelium-derived relaxing factor(s) in the ventral aorta of carp (*Cyprinus carpio*). Comp Biochem Physiol. - A Mol Integr Physiol. 2000; 127:89-98. https://doi.org/10.1016/S0305-0491(00)00264-9.
31. Tipsmark CK, Madsen SS. Rapid modulation of Na^+/K^+-atpase activity in osmoregulatory tissues of a salmonid fish. J Exp Biol. 2001; 204(4):701-709.
32. Evans DH, Piermarini PM, Choe KP. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev. 2005; 85:97-177. https://doi.org/10.1152/physrev.00050.2003. PMid:15618479.
33. Ebbesson LOE, Tipsmark CK, Holmqvist B, Nilsen T, Andersson E, Stefansson SO, Madsen SS. Nitric oxide synthase in the gill of Atlantic salmon: colocalization with and inhibition of Na^+,K^+-ATPase. J Exp Biol. 2005; 208:1011-1017. https://doi.org/10.1242/jeb.01488. PMid:15767302.
34. Faught E, Vijayan MM. Mechanisms of cortisol action in fish hepatocytes . Comp Biochem Physiol. Part B. 2016; 199:136-145. https://doi.org/10.1016/j.cbpb.2016.06.012. PMid:27445122.
35. Iwakiri Y, Kim MY. Nitric oxide in liver diseases. Trends Pharmacol Sci. 2015; 36(8):524-536. PMid:26027855 PMCid:PMC4532625.
36. Carnovale C, Ronco MT. Role of nitric oxide in liver regeneration. Ann Hepatol. 2012; 11(5):636-647. https://doi.org/10.1016/S1665-2681(19)31436-X.
37. Ishimura N, Bronk SF, Gores GJ. Inducible nitric oxide synthase up-regulates Notch-1 in mouse cholangiocytes: Implications for carcinogenesis. Gastroenterology. 2005; 128:1354-1368. https://doi.org/10.1053/j.gastro.2005.01.055. PMid:15887117
38. Abu-Amara M, Yang SY, Seifalian A, Davidson B, Fuller B. The nitric oxide pathway - evidence and mechanisms for protection against liver ischaemia reperfusion injury.

Liver Int. 2012; 32:531-543. https://doi.org/10.1111/j.1478-3231.2012.02755.x. PMid:22316165

39. Peter MCS, Mini VS, Bindulekha DS, Peter VS. Short-term In situ effects of prolactin and insulin onion transport in liver and intestine of freshwater climbing perch (*Anabas testudineus* Bloch). J Endocrinol Reprod. 2014; 18(1):47-58.
40. Dubyak GR. Ion homeostasis, channels, and transporters: an update on cellular mechanisms. AJP Adv Physiol Educ. 2004; 28:143-154. https://doi.org/10.1152/advan.00046.2004. PMid:15545343.
41. Stankovic KM, Brown D, Alper SL, Adams JC. Localization of pH regulating proteins H^+ATPase and Cl^-/HCO_3^- exchanger in the guinea pig inner ear. Hearing Research. 1997; 114:21-34. https://doi.org/10.1016/S0378-5955(97)00072-5.
42. Seidelin M, Brauner CJ, Jensen FB, Madsen SS. Vacuolar-type H^+ATPase and Na^+/K^+ATPase expression in gills of atlantic salmon (*Salmo salar*) during isolated and combined exposure to hyperoxia and hypercapnia in fresh water. Zoo Sci. 2001; 18:1199-1205. https://doi.org/10.2108/zsj.18.1199. PMid:11911075
43. Wagner CA, Finberg KE, Breton S, Marshansky V, Brown D, Geibel JP. Renal vacuolar H^+ATPase. Physiol Rev. 2004; 84:1263-2004. https://doi.org/10.1152/physrev.00045.2003. PMid:15383652.
44. Poole-Wilson PA. Regulation of intracellular pH in the myocardium; Relevance to pathology. Mol Cell Biochem. 1989; 89:151-155. https://doi.org/10.1007/BF00220768 PMid:2682208.
45. William M, Vien J, Hamilton E, Garcia A, Bundgaard H, Clarke RJ, Rasmussen HH. The nitric oxide donor sodium nitroprusside stimulates the Na^+-K^+ pump in isolated rabbit cardiac myocytes. J Physiol. 2005; 565:815-825. https://doi.org/10.1113/jphysiol.2005.086447. PMid:15817632 PMCid:PMC1464570.
46. White CN, Hamilton EJ, Garcia A, Wang D, Chia KKM, Figtree GA, Rasmussen HH. Opposing effects of coupled and uncoupled NOS activity on the Na^+-K^+ pump in cardiac myocytes. Am J Physiol. - Cell Physiol. 2008; 294:572-579. https://doi.org/10.1152/ajpcell.00242.2007. PMid:18057120.
47. Gupta S, McArthur C, Grady C, Ruderman N. Stimulation of vascular Na^+/K^+-ATPase activity by nitric oxide: a cGMP-independent effect. Am J Physiol. 1994; 266:H2146-H2151. https://doi.org/10.1017/CBO9781107415324.004.
48. Vlkovičová J, Javorková V, Mézešová L, Pecháňová O, Vrbjar N. Regulatory role of nitric oxide on the cardiac Na,K-ATPase in hypertension. Physiol Res. 2008; 57(2):S15-S22. https://doi.org/10.16136/j.joel.2015.05.0903.
49. Muriel P, Sandoval G. Nitric oxide and peroxynitrite anion modulate liver plasma membrane fluidity and Na^+/K^+-ATPase activity. Nitric Oxide - Biol Chem. 2000; 4:333-342. https://doi.org/10.1006/niox.2000.0285. PMid:10944417.
50. Peter VS. Nitric oxide rectifies acid-base disturbance and modifies thyroid hormone activity during net confinement of air-breathing fish (*Anabas testudineus* Bloch). Gen Comp Endocrinol. 2013; 181:115-121. https://doi.org/10.1016/j.ygcen.2012.10.011. PMid:23153653.
51. Valles PG, Manucha WA. H^+ATPase activity on unilateral ureteral obstruction: Interaction of endogenous nitric oxide and angiotensin II. Kidney Int. 2000; 58:1641-1651. https://doi.org/10.1046/j.1523-1755.2000.00325.x. PMid:11012898
52. Gayathry R, Peter VS, Peter MCS. Nitric oxide drives mitochondrial energetics in heart and liver mitochondria of hypoxia-stressed climbing perch (*Anabas testudineus* Bloch). J Endocrinol Reprod. 2018; 22(1):1-14.
53. Alexandre H, Mathieu B, Charpentier C. Alteration in membrane fluidity and lipid composition, and modification of H^+ATPase activity in *Saccharomyces cerevisiae* caused by decanoic acid. Microbiology. 1996; 142(3):469-475. https://doi.org/10.1099/13500872-142-3-469. PMid:8868421.
54. Baratta VM, Norz V, Barahona MJ, Gisinger TM, Mulligan D, Geibel JP. Pencillin G induces H^+,K^+ATPase via a nitric oxide-dependent mechanism in the rat colonic crypt. Cell Physiol Biochem. 2020; 54:1132-1142. https://doi.org/10.33594/000000305. PMid:33175479.
55. BucklerKJ, Vaughan-Jones RD. Effects of mitochondrial uncouplers on intracellular calcium, pH and membrane potential in rat carotid body type I cells. J Physiol. 1998; 513: 819-833. https://doi.org/10.1111/j.1469-7793.1998.819ba.x. PMid:9824720 PMCid:PMC2231310.
56. WyattCN, Wright C, Bee D, Peers C. O-2-Sensitive K^+ currents in carotid-body chemoreceptor cells from normoxic and chronically hypoxic rats and their roles in hypoxic chemotransduction. Proc Natl Acad Sci USA. 1995; 92:295-299. https://doi.org/10.1073/pnas.92.1.295. PMid:7529413 PMCid:PMC42865.
57. Gattuso A, Garofalo F, Cerra MC, Imbrogno S. Hypoxia tolerance in teleosts: Implications of cardiac nitrosative signals. Front Physiol. 2018; 9:1-13. https://doi.org/10.3389/fphys.2018.00366. PMid:29706897 PMCid:PMC5906588.