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RESEARCH PAPER

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The Effect of Waterborne Mercury and Nickel on the ATPases and AChE Activities in the Brain of Freshwater Fish (*Oreochromis niloticus*) depending on the Ca^{2+} Concentrations

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Abstract

In this study, freshwater fish *Oreochromis niloticus* were exposed to acute (3 days) and chronic (30 days) Hg^{2+} and Ni^{2+} in differing Ca^{2+} levels (15, 30 and 90 mg Ca^{2+}/L). In the acute duration 30 µM Ni^{2+} and 0.3 µM Hg^{2+}, in the chronic duration 10 µM Ni^{2+} and 0.03 µM Hg^{2+} were tested. At the end of the exposures, activities of ATPase (Total-ATPase, Na^{+}/K^{+}-ATPase and Mg^{2+}-ATPase) and AChE were measured in the brain. There were changes upon Hg^{2+} and Ni^{2+} effects depending on the Ca^{2+} concentration though low dependence on Ca^{2+} exposure alone. Decreased activities were recorded after acute metal exposures even at high Ca^{2+} concentration. There were variable alterations in ATPase activities whereas AChE activity was mostly altered by Hg^{2+} exposure. ATPase and AChE activities were changed in both durations due to metal type and Ca^{2+} concentration differences. However, ATPase responses were more affected. Changes in the activities demonstrated the sensitivity of these vital enzymes towards environmental factors. Therefore, there is a potential using of these enzymes as biomarkers on the basis of multi-biomarker attitude in environmental toxicology studies.

Introduction

Aquatic biota can be affected by metals caused by several anthropogenic activities leading several dysfunctions in key mechanisms of fish metabolism. Among the metals mercury is one of the most toxic metals leading to hematological, osmoregulatory and histopathological changes and also reproductive toxicity as a non-essential metal (Sorensen, 1991; Richetti, et al., 2011). Ambient levels of Hg^{2+} (USEPA standard) was given as 0.19 µg/L for freshwaters (USEPA, 1980) and 96 h LC50 values were as 0.22 mg/L in *Oreochromis niloticus* fingerlings (Ishikawa, Ranzani-Paiva, &

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Lombardi, 2007). On the other hand Ni^{2+} is a common metal in most surface waters including both natural and anthropogenic sources and also known as an essential trace metal for normal growth in living organisms. Ni^{2+} excess or deficiency can cause a decrease in the aquatic vertebrate overall fitness (Pane, Richards, & Wood, 2003). Threshold limits have been established for Ni^{2+} as 70 µg/L according to the WHO (2008) and the concentration of Ni^{2+} can reach up to 2.5 mg/L in contaminated freshwaters (Eisler, 1998). LC50 value of 36 mg/L nickel chloride was found for *O. niloticus* to evaluate the Ni^{2+} toxicity (Abou Hadeed, Ibrahim, El-Sharkawy, Saleh Sakr, & Abd El-Hamed, 2008). Physiological function and tissue alterations can be counted dependent upon the Ni^{2+} toxicity (Canli, 1995; Topal et al., 2017). Previous data also confirmed that mercury LC50 values were lower than nickel LC50 values (Brown & Dalton, 1972; Speher et al., 1982).

Physico-chemical properties (salinity, hardness, pH, etc.) affect metal bioavailability by altering the metal uptake in fish (Jorgensen, 2010; Wood, Farrel, & Brauner, 2012). Calcium known as the divalent ion responsible for the freshwater hardness is one of the most significant abiotic factors. It was demonstrated that Ca^{2+} can affect metal toxicity which its reduction is dependent upon the Ca^{2+} concentration increase in media as it affects bioavailability of metals (Saglam, Atli, & Canli, 2013; Brix, Schlekat, & Garman, 2017). A negative relationship between increasing water hardness (Ca^{2+} and Mg^{2+}) and toxicity of metals to aquatic organisms has been studied for a several divalent metals (Wood, 2012).

Maintenance of the osmotic concentrations of the ions and extracellular fluids are the vital functions of the osmoregulation system. Membrane bound ATPase enzymes have significant roles in osmotic balance, ion regulation and membrane permeability. Na^+/K^+-ATPase (EC 3.6.3.9) transporting the Na^+ and K^+ through cell membranes and Mg^{2+}-ATPase (EC 3.6.3.2) responsible for the transepithelial Mg^{2+} regulation are found to be altered by the exposures of different toxic compounds (Sancho, Fernandez-Vega, Ferrando, & Andreu-Moliner, 2003; Parvez, Sayeed, & Raisuddin, 2006; Atli & Canli, 2011). Measurement of ATPase activities can be useful as an early sign for the osmoregulatory system damage due their variable responses in several metal exposure in aquatic medium with different water hardness (Grosell, Nielsen, & Bianchini, 2002; Monteiro, Mancera, Fontainhas-Fernandes, & Sousa, 2005; Atli & Canli 2011; Saglam et al., 2013). Acetylcholinesterase (AChE, EC 3.1.1.7) functioning in the neurotransmitter acetylcholine hydrolysis is important component of the neuronal communication. AChE is known to be abundant for brain tissue and therefore AChE response can be used for the xenobiotic diagnosing particularly for neurotoxicity determining in the aquatic environment (Üner, Sevgiler, & Piner, 2010; Richetti et al. 2011; Silva, Assis, Oliveira, Carvalho, & Bezerra, 2013). In addition, metals are also effective since they cause significant changes in AChE activities in fish as indicated in the literature (Romani et al., 2003; Jebali et al., 2006; Attig et al., 2010). There are more detailed knowledge of the acute toxic mechanisms of several waterborne trace metals such as silver, cadmium and copper than nickel (Brix et al., 2017).

It has gain significance to highlight the Ca^{2+} effect on metal toxicity consequences on osmoregulatory responses and neurotoxicity in fish. In this purpose, the main objective of current study was to evaluate the alteration in ATPase and AChE activities in the means of osmo- and neuroregulatory effects of Hg^{2+} and Ni^{2+} in different Ca^{2+} levels in the brain of Nile tilapia, *O. niloticus*.

Materials and Methods

One-year-old *O. niloticus* (Perciformes: Cichlidae) were obtained from fish culturing pools of Çukurova University and transferred to the laboratory, where they

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were acclimatized (20± 1°C) and illuminated for 12 h with fluorescent lamps (daylight 65/80 W) for one month. The experiments were carried out in glass aquariums sized 40x40x100 cm that contained 120 L Nestle water (Nestle Pure Life, Turkey). All experiments were conducted by the Nestle Water containing relatively low Ca^{2+} levels (15 mg Ca^{2+}/L) and used as normal (base) control. The aquaria were aerated with air stones attached to an air compressor to saturate with oxygen (7.03 ± 0.81 mg O_2/L). The pH, conductivity, total hardness and alkalinity levels of the Nestle water were 6.83 ± 0.01, 77 µS/cm, 105.4 ± 20.8 mg $CaCO_3$/L and 76.2 ± 13.0 mg $CaCO_3$/L, respectively. No contamination in this water was stated based on the Nestle quality control document (Nestle, Turkey). Fish size and weight were measured as 15.4 ± 1.33 cm and 57.5 ± 16.4 g, respectively and they did not differ significantly (P > 0.05) among different exposure groups.

For the experimental design, control-Ca and metal+Ca groups were used for acute (3 d) and chronic (30 d) durations. A total of six fish were used for each experimental group. Control-Ca groups had different Ca^{2+} levels as 15 (low), 30 (medium) and 90 mg Ca^{2+}/L (high), respectively. Calcium levels of this water were increased by adding $CaCl_2.2H_2O$. Calgon Titration Method was used to measure the exposure media Ca^{2+} levels and they were found within 5% of the nominal target Ca^{2+} levels. Total hardness levels of the Ca^{2+} groups were found as 105.4 ± 20.8 (L-Ca), 194.0 ± 12.3 (M-Ca) and 318.0 ± 13.0 mg $CaCO_3$/L (H-Ca), respectively. The pH values were in the range of 6.83–6.73 ± 0.01 in the groups. Metal+Ca groups were exposed to Hg^{2+} ($HgCl_2$) and Ni^{2+} ($NiCl_2.6H_2O$) at three Ca^{2+} media (low, medium and high). Acute (0.3 µM) and chronic (0.03 µM) Hg^{2+} exposed groups were mentioned as Hg+L-Ca, Hg+M-Ca and Hg+H-Ca, respectively. Acute (30 µM) and chronic (10 µM) Ni^{2+} exposed groups were mentioned as Ni+L-Ca, Ni+M-Ca and Ni+H-Ca, respectively (Table 1). Fish were fed (2% of their weight) with Pinar Sazan commercial fish food (Izmir, Turkey). The aquaria of experimental groups were cleaned every 2 days after the feeding to eliminate the food remains and also to minimize metal loss in the medium (Atli & Canli, 2007).

At the end of the exposure durations, fish were killed by transaction of the spinal cord based on the Çukurova University Ethic Committee decision. Brain tissues were dissected out with sterile equipment and stored (Revco Ultima II, Newsbreak, UK) at −80°C until the analysis.

Enzyme Analysis

Tissues were homogenized in ice-cold buffer (20 mM Tris–HCl, 0.25 M Sucrose and 1 mM EDTA, pH 7.7) with a ratio of 1/10 (v/w) at 9500 rpm for 1-2 min. Homogenates were centrifuged at 13 000 g for 20 min (4°C). The supernatants were used for enzymatic activity and protein level measurements.

ATPase activity measurement was performed by using the incubation media (pH 7.7) contained 40 mM Tris–HCl, 120 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, and 1 mM for ouabain. The reaction was started by the addition of 3 mM Na_2ATP as a substrate and incubated for 30 min at 37°C. Inorganic phosphate measurement at 390 nm was used for the ATPase activity determination (Atkinson, Gatemby, & Lowe, 1973). KH_2PO_4 was used as a standard. Na^+/K^+-ATPase activity was calculated by using the activity differences between the presence (Mg^{2+}-ATPase activity) and absence (Total-ATPase activity) of the ouabain. ATPase activities were calculated as µmol Pi/mg prot./min. The characterization of the ATPase activities in *O. niloticus* was presented in our laboratory previously (Atli & Canli, 2011).

AChE specific activity was measured according to Ellman, Courtney, Andres and Featherstone (1961)

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based on the absorbance increase at 412 nm for 1 min. The incubation media contained 0.1 M sodium-phosphate buffer (pH 8.0), 0.01 M DTNB and 8.52 mM ethopropazine (BChE inhibitor). Reaction was started by 0.015 M acetylthiocholine (substrate) addition. AChE activity was calculated as U/mg prot. by using an extinction coefficient 13.6 mM^{-1} cm^{-1}. Total protein levels were determined based on the method of Lowry, Rosebrough, Farra and Randall (1951) and bovine serum albumin used as a standard. Assays were performed in triplicate. All chemicals were obtained from Sigma (Germany) or Merck (Germany).

Statistics

Statistical analysis were performed by using SPSS statistical package program (SPSS 13, Chicago, IL, USA) and given as mean and standard error (N = 6). Kolmogorov–Smirnov normality test was used to control the homogeneity of variance among different exposure groups. One-way ANOVA followed by Duncans' test and T-test (P< 0.05) was used to compare the groups individually in acute and chronic exposures. In addition the control-Ca groups (L-, M- and H-Ca groups) and metal-Ca^{2+}groups were compared individually for determination of the Ca^{2+} effect alone and Ca^{2+} effect on metal toxicity, respectively.

Results

In acute exposure, there were no alteration in the enzyme activities of the control groups in differing Ca^{2+} concentrations except Mg^{2+}-ATPase activities which increased in H-Ca group in contrast to L-Ca group (P<0.05). The trend of the changes were observed as decreases in metal exposed groups summarized in Table 2. Total-ATPase activities were decreased in M-Ca and H-Ca groups with Ca concentration dependent in contrast to L-Ca groups after acute Hg^{2+} and Ni^{2+} exposures. When comparing with their own Ca concentrations; Total-ATPase activities increased in L-Ca groups though they decreased in H-Ca groups after both metal exposures (P<0.05) (Fig. 1). Mg^{2+}-ATPase activities decreased in Hg+H-Ca and Ni+H-Ca groups compared to Hg+L-Ca and Ni+L-Ca groups, respectively. Mg^{2+}-ATPase activities increased in Hg+L-Ca, Ni+L-Ca and Ni+M-Ca groups though decreased in Hg+H-Ca group in contrast to their alone own Ca^{2+} environments (P<0.05) (Fig. 2). Na^{+}/K^{+}-ATPase activity decreased after both acute Hg^{2+} and Ni^{2+} exposures in M-Ca and H-Ca groups when compared to Hg and Ni+L-Ca groups, respectively. Na^{+}/K^{+}-ATPase activity increased in Hg+L-Ca group though Ni+M-Ca and Ni+H-Ca group activities decreased in contrast to their own Ca^{2+} concentrations (P<0.05) (Fig. 3). AChE activity during acute durations decreased in Hg+M-Ca group in contrast to Hg+L-Ca group. Increases were found in AChE activity in L- and H-Ca groups after Hg exposures when compared to their own alone Ca^{2+} media (P<0.05) (Fig. 4).

In chronic exposure, the ATPase and AChE activities did not change among the studied Ca^{2+} concentrations in control groups (P>0.05). The altered activities were found lower in this duration than acute exposure groups and both increases and decreases were observed after metal exposures in differing Ca^{2+} conditions (Table 3). Total-ATPase activity increased after Hg+M-Ca exposure in contrast to Hg+L-Ca group. All activities of Total-ATPase decreased after Hg^{2+} exposure in all Ca^{2+} environments when comparing to their own control Ca^{2+} concentrations (P<0.05) (Fig. 1). Mg^{2+}-ATPase activity increases were recorded in Ni+M-Ca and Ni+H-Ca groups in contrast to Ni+L-Ca group. However its activity decreased after Hg+M-Ca and Hg+H-Ca exposures when compared to M-Ca and H-Ca exposures (P<0.05) (Fig. 2). Na^{+}/K^{+}-ATPase activity increased only in Hg+M-Ca compared to Hg+L-Ca group. Its activity decreased after Hg+L-Ca, Ni+L-Ca and Ni+M-Ca exposures in contrast to their own Ca^{2+}

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environments (P<0.05) (Fig. 3). AChE activity decreased only in L-Ca groups after Hg^{2+} and Ni^{2+} exposures compared to their own Ca^{2+} alone environment (P<0.05) (Fig. 4).

Discussion

Data showed variable responses of ATPase and AChE activities dependent upon the metal species, their concentrations and durations in different Ca^{2+} media. In addition unchanged enzyme activities were also recorded mostly in control groups with different Ca^{2+} media (Table 2 and 3). It is interesting to observe the decreased enzyme activities upon acute metal exposures even at high Ca^{2+} concentration for several cases. However, they became to return to their control values after chronic exposure at high Ca^{2+} media. According to this, acute and chronic exposures seemed to be differed in the opposite way from a whole point of view based on the activity responses also associated with metal concentration differences. Metal effects were still observed, particulary at L-Ca group in contrast to their Ca^{2+} alone group.

ATPase Activities

ATPase activities were more affected in acute exposure than chronic duration and this could be also related with higher concentrations of mercury and nickel. One can conclude that Ca^{2+} increase might cause a reduction in metal toxicity due to the less alteration in the activities when compared to their same Ca^{2+} environment during chronic exposure period. It could be occurred in a linked with Ca^{2+} protection by decreasing the metal bioavailability. In general, after acute duration, ATPase activities decreased at higher Ca^{2+} media according to both L-Ca group and own Ca^{2+} alone media comparisons. However they mostly increased after metal exposures in contrast to their own Ca^{2+} media. Nevertheless in chronic duration, less affected ATPase activity trend was as decreases in contrast to own Ca^{2+} group. Metals, particularly Hg^{2+} significantly decreased the ATPase activities when compared to their own Ca^{2+} media due to its high toxicity. Data presented its activity change as mostly in decreases could be in relation to disruption of ion concentrations after Hg^{2+} and Ni^{2+} exposures at different Ca^{2+} environment. This decrease could be also associated with metal binding directly to the enzyme structure leading to a dysfunction in its activity (Dogan, Atli, & Canli, 2015). However different pattern was observed in Mg^{2+}-ATPase activity as both increases and decreases in relation to metal and Ca^{2+} concentration differences. Its role is known to transport the Mg^{2+} which is essential for the cellular membrane integrity and permeability. The increases could be associated with the increased ion concentrations and other compensation mechanisms acting to maintain the fish metabolism under stress.

It was obvious that both Ca^{2+} and also metal (Hg^{2+} and Ni^{2+}) effects disturbance the ATPase activity as a result of imbalanced ion-based osmoregulation. One of the most effective factors, Ca^{2+} can compete with metal ions for uptake in freshwater organisms is known to be able to reduce the metal bioavailability and toxicity consequently (Heath, 1995). Inverse relationship was observed between toxicity of metals and water hardness as Ca^{2+} ions in *O. niloticus* (Hollis, McGeer, McDonald, & Wood, 2000; Franklin, Glover, Nicol, & Wood, 2005; Saglam et al., 2013). Nevertheless, Ca^{2+} protection was appeared to be occurred particularly at chronic duration when compared to acute period. This could be linked with the high metal concentrations used for acute exposure. On this basis, it can be concluded that protective role of the Ca^{2+} can be varied/altered according to the other criteria such as metal concentration and exposure duration which was also supported with this and ours previous studies

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(Canli, Atli, & Canli, 2016). Our previous data also emphasized the variable ATPase responses depending upon the metal type, concentration, exposure duration, tissue differences and also physicochemical properties of aquatic environment (Atli & Canli, 2007; Atli & Canli, 2011; Saglam et al., 2013). In addition ion concentrations in both tissues and the fish serum significantly altered by metal exposures after acute and chronic durations (Oner et al., 2008; Atli & Canli, 2011; Saglam et al., 2013). Enhanced ATPase activities might be occurred by increased enzyme turnover rates and/or enzyme molecule numbers. In addition to this, possible changes in the levels of ions which are essential in membrane stability and permeability, followed by osmotic gradient changes may also cause alterations in ATPase activities.

Inhibition in brain Na^+/K^+-ATPase activity after Hg^{2+} exposure were also demonstrated in its ability to interfere with metal binding sites on ATPase molecule which may lead to an alteration in enzyme configuration (Verma, Jain, & Tonk, 1983; Sastry & Sharma, 1980; Omotayo, Rocha, Ibukun, & Kade, 2011). Reduced effect of Ag^+ exposure due to increased water hardness was observed in ion levels and Na^+/K^+-ATPase activity in the gill of rainbow trout (Morgan, Guadagnolo, Grosell, & Wood, 2005). Although lower sensitivity of Mg^{2+}-ATPase than Na^+/K^+-ATPase activity found in some of our earlier data (Atli & Canli, 2011; 2013) against metal toxicity, they showed equivalent responses in the present study. This different endpoint might be explained by tissue differences. The brain utilizing approximately 20% total oxygen demand of the metabolism has a vital role in neuro-endocrine functions. Also it can be very sensitive to oxidative damage due to containing high amounts of unsaturated lipids. Decreased Na^+/K^+-ATPase activity with altered antioxidant enzyme activities in fish exposed to metals were observed (Atli & Canli, 2007; Maiti, Saha, & Paul, 2010). Increased ATPase activity in order to supply the energy demand was also supported by previous data (Sastry & Sharma, 1980; Saglam et al., 2013). In addition, this study pointed out that the duration effect belonging to the different metal concentrations was not the only factor lead to activity changes. Calcium was also found as a significant component responsible for these variations.

AChE Activity

AChE activity showed both increases and decreases based on the metal, concentration, duration and the Ca^{2+} media. Significant alterations in AChE activities were observed in acute duration as variations after only Hg^{2+} exposure. In chronic duration as decreases in low Ca^{2+} conditions were found after both metal exposures in contrast to their own Ca^{2+} concentrations. Alterations in AChE activity by Hg^{2+} exposure could point out its anticholinergic effect. AChE represented minor changes compared to ATPase activities in the current data. This may be related to the fact that the enzyme is less sensitive to these metals than ATPase responses. Brain tissue is endowed with AChE that functions in acetylcholine hydrolyze into choline and acetic acid at the cholinergic synapses. The AChE increases at different Ca^{2+} media after Hg^{2+} exposure in acute duration could be linked with de novo synthesis of the enzyme. There were several studies demonstrating the increased AChE activity after metal exposures despite AChE activity decreases shown literature in general. Increased brain AChE activities were demonstrated in several fish species after metal exposures (Gill, Tewari, & Pande, 1991; Romani et al., 2003; Jebali et al., 2006). Variability of AChE activity responses against metal toxicity dependent with the duration differences in several aquatic organisms were also recorded in the literature (Aziz, Amin, & Shakoori, 1993; Reddy & Venugopal, 1993; Attig et al., 2010). On the other hand one of the major roles of Ca^{2+} is to act

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as a significant intracellular second messenger of central nervous system and Ca^{2+} has been also found to involve in the release of neurotransmitter (Reddy, Jinna, Uzodinma, & Desaiah, 1988). Based on this information, there is a question of whether the increase in AChE activity is caused by also this role of calcium in relation with the different Ca^{2+} concentrations used in this study.

On the other hand, decreased AChE activity pattern showed similarity with the Na^{+}/K^{+}-ATPase activity. Possible reasons of the AChE activity decline might be associated with metal binding to the enzyme structure leading to compromised catalytic activity and/or increased lipid peroxidation which can indirectly effects its activity (Pretto et al., 2014). Acetylcholine accumulation can be occurred due to the AChE inhibition which in turn can impact the physiological mechanisms such as swimming, behavioral and survival of fish as a result of prevention of nerve and muscle fiber communication (Uner et al., 2010; Katuli, Massarsky, Hadadi, & Pourmehran, 2014). Decreased brain AChE activity after Ni^{2+} treatment for 21 days in rainbow trout (Topal et al., 2015) showed similarity with this study only after chronic Ni^{2+} exposure at low Ca^{2+} media. In addition, there were no alterations in its activity at higher Ca^{2+} concentrations. This result might be related with Ca^{2+} protection against metal toxicity in chronic duration. Inhibitions were demonstrated in fish *Pomatoschistus microps* (Vieira, Gravato, Soares, Morgado, & Guilhermino, 2009) and *Danio reiro* (Zhen e al., 2014) AChE activities against acute mercury exposures. De la Torre, Salibian, and Ferrari (2000) demonstrated unaltered brain AChE activity, inhibition in Na^{+}/K^{+}-ATPase and activation in Mg^{2+}-ATPase activities in the gill of *Cyprinus carpio* exposed to Cd^{2+} and they commented that AChE was seemed to be not a suitable marker in relation to Cd^{2+} toxicity. Na^{+}/K^{+}-ATPase and AChE activities inhibited in different fish species exposed to metals as a result of metal interference with iono- and neuro-regulation underlying their usage as an early biomarker concept in contaminated waters (Katuli et al., 2014; Leitemperger et al., 2016).

As a result it can be concluded that both osmo- and neuro-regulation were affected by metal exposures in different concentrations and durations with increased Ca^{2+} media. It is interesting to observe the decreased enzyme activities after acute metal exposures even at high Ca^{2+} concentration. And also higher metal effects were remarkable at low Ca^{2+} groups in both durations in contrast to their control group. Therefore, AChE and ATPase activity changes should be taken into account while evaluating the toxicity response of the brain. However, osmotic and ionic disruption can be pronounced as higher in the means of ATPase activity changes than AChE activity in tilapia after metal stress. Nevertheless, to measure the multiple biomarker responses simultaneously has gain significance for showing correspondence to aquatic stress conditions. Therefore, approach of specific and sensitive multi-biomarker usage and also validity of different biomarker integration are seemed to be essential to evaluate the osmo- and neuro-toxicity mechanisms under metal stress with different Ca^{2+} levels.

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Table 1. The scheme of the exprimental group design in differing Ca^{2+} concentrations for acute and chronic duration

	Acute (3 days)			**Chronic (30 days)**		
	Control	**Hg**	**Ni**	**Control**	**Hg**	**Ni**
Low-Ca (15 mg Ca^{2+}/L)	L-Ca	0.3 µM Hg+L-Ca	30 µM Ni+L-Ca	L-Ca	0.03 µM Hg+L-Ca	10 µM Ni+L-Ca
Medium-Ca (30 mg Ca^{2+}/L)	M-Ca	0.3 µM Hg+M-Ca	30 µM Ni+M-Ca	M-Ca	0.03 µM Hg+M-Ca	10 µM Ni+M-Ca
High-Ca (90 mg Ca^{2+}/L)	H-Ca	0.3 µM Hg+H-Ca	30 µM Ni+H-Ca	H-Ca	0.03 µM Hg+H-Ca	10 µM Ni+H-Ca

Table 2. A summary of significant changes (P<0.05) of ATPase and AChE activities in the brain of *O. niloticus* exposed to acute mercury and nickel exposures in differing Ca^{2+} concentrations. Significant increases and decreases (P<0.05) were represented as "↑" and "↓", respectively while "-" indicated the insignificant data (P>0.05)

Enzyme	**Group**	**Acute**					
		In contrast to L-Ca group			**In contrast to own Ca alone media**		
		L-Ca	**M-Ca**	**H-Ca**	**L-Ca**	**M-Ca**	**H-Ca**
Total-ATPase	Control	52.8±2.69	57.4±1.08	57.7±2.07			
	Hg	61.0±0.54	57.7±1.19 ↓	50.9±0.75 ↓	↑	-	↓
	Ni	62.6±0.98	56.3±1.88 ↓	51.7±0.43 ↓	↑	-	↓
Mg^{2+}-ATPase	Control	28.1±1.94	32.0±0.77	34.0±2.07 ↑			
	Hg	33.1±0.92	31.4±0.94	28.8±0.75 ↓	↑	-	↓
	Ni	42.7±0.92	39.5±1.30	34.5±0.43 ↓	↑	↑	-
Na^{+}/K^{+}-ATPase	Control	22.3±1.50	24.5±1.07	24.9±0.89			
	Hg	26.4±0.98	23.3±0.53 ↓	22.8±0.88 ↓	↑	-	-
	Ni	19.9±0.66	15.9±0.86 ↓	17.1±0.52 ↓	-	↓	↓
AChE	Control	0.083±0.003	0.081±0.004	0.076±0.001			
	Hg	0.090±0.002	0.074±0.004 ↓	0.087±0.006	↑	-	↑
	Ni	0.077±0.002	0.071±0.004	0.068±0.001	-	-	-

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Table 3. A summary of significant changes (P<0.05) of ATPase and AChE activities in the brain of *O. niloticus* exposed to chronic mercury and nickel exposures in differing Ca^{2+} concentrations. Significant increases and decreases (P<0.05) were represented as "↑" and "↓", respectively while "-" indicated the insignificant data (P>0.05)

Enzyme	Group	Chronic					
		In contrast to L-Ca group			In contrast to own Ca alone media		
		L-Ca	M-Ca	H-Ca	L-Ca	M-Ca	H-Ca
Total-ATPase	Control	62.7±4.23	65.1±2.62	63.1±2.64			
	Hg	48.7±1.37	55.4±0.90 ↑	52.5±1.59	↓	↓	↓
	Ni	57.0±1.70	61.7±2.05	58.7±1.11	-	-	-
Mg^{2+}-ATPase	Control	34.8±2.87	40.5±2.05	41.9±1.81			
	Hg	30.2±1.62	32.9±1.01	31.8±1.42	-	↓	↓
	Ni	39.8±0.66	43.4±0.80 ↑	43.4±0.99 ↑	-	-	-
Na^{+}/K^{+}-ATPase	Control	23.3±1.28	23.6±1.02	18.8±2.29			
	Hg	18.5±1.32	22.1±1.00 ↑	20.7±0.62	↓	-	-
	Ni	17.2±1.16	17.2±1.14	17.2±0.68	↓	↓	-
AChE	Control	0.096±0.003	0.077±0.002	0.080±0.001			
	Hg	0.077±0.002	0.080±0.002	0.082±0.003	↓	-	-
	Ni	0.080±0.001	0.085±0.004	0.079±0.004	↓	-	-

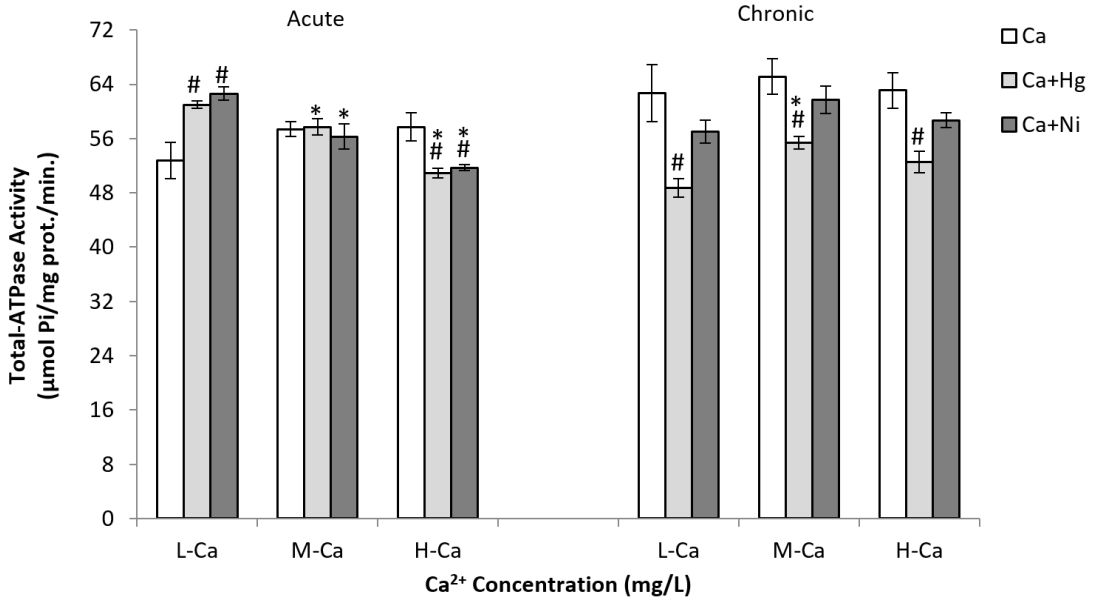

Figure 1. Total-ATPase activities in the brain of *O. niloticus* exposed to acute and chronic Hg^{2+} and Ni^{2+} in differing Ca^{2+} concentrations. Data (N=6) are given as mean ± standard error. Statistical significances (P<0.05) were indicated as "*" between L-Ca group and M-Ca (30 mg Ca^{2+}/L) and H-Ca (90 mg Ca^{2+}/L) groups, while "#" was used to indicate the significant (P<0.05) changes between metal+Ca groups (Metal+L-Ca, Metal+M-Ca and Metal+H-Ca) and their own control-Ca groups (L-Ca, M-Ca and H-Ca).

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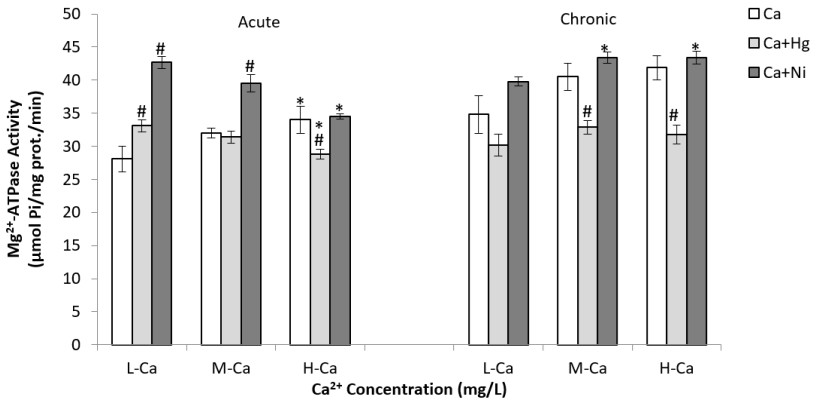

Figure 2. Mg^{2+}-ATPase activities in the brain of *O. niloticus* exposed to acute and chronic Hg^{2+} and Ni^{2+} in differing Ca^{2+} concentrations. See Fig. 1 for details.

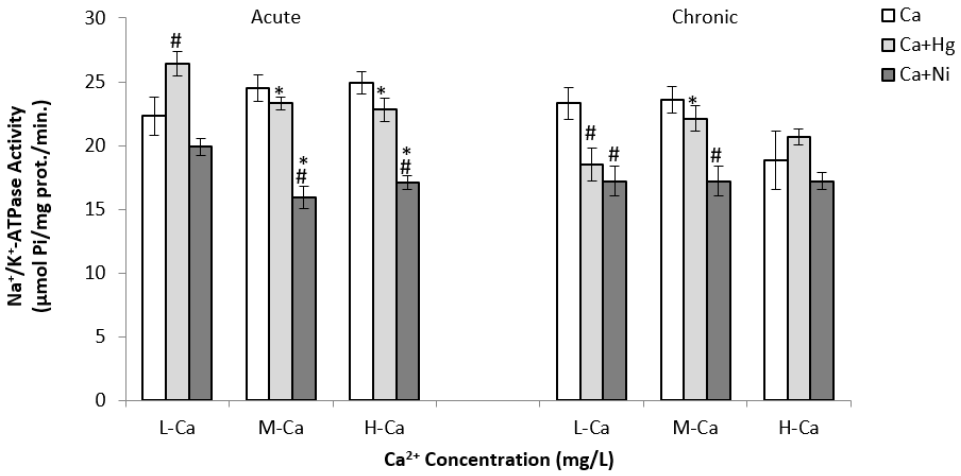

Figure 3. Na^{+}/K^{+}-ATPase activities in the brain of *O. niloticus* exposed to acute and chronic Hg^{2+} and Ni^{2+} in differing Ca^{2+} concentrations. See Fig. 1 for details.

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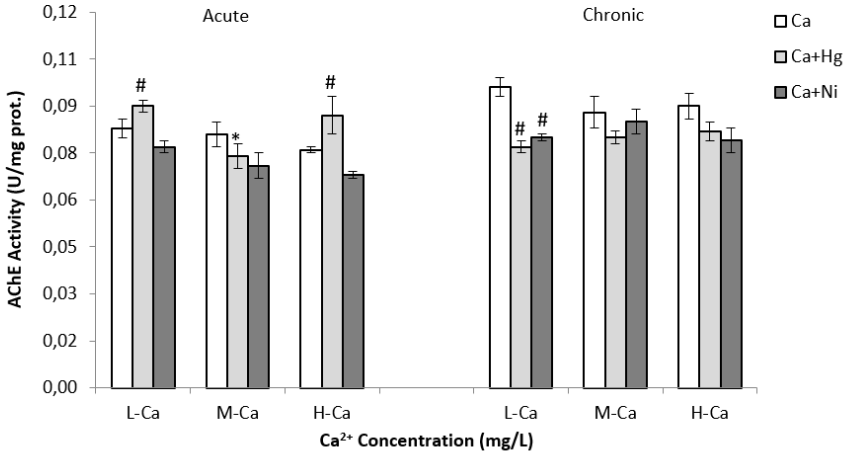

Figure 4. AChE activities in the brain of *O. niloticus* exposed to acute and chronic Hg^{2+} and Ni^{2+} in differing Ca^{2+} concentrations. See Fig. 1 for details.