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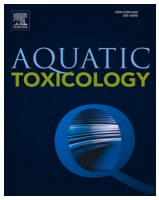

The acute neurotoxicity of inorganic mercury in *Mactra chinensis philippi*

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ABSTRACT

Inorganic mercury (IHg) is hazardous to marine organisms especially resulting in neurotoxicity, bivalves are sensitive to pollutants as "ocean sentinel", but data on the neurotoxicity of IHg in bivalves are sparse. So we chosed *M. chinensis philippi* with typical neural structures in bivalves to investigate the neurotoxicity of IHg, which could be helpful to understand the specificity of neural regulation and the response characteristics of bivalves. After acute exposed to IHg ($HgCl_2$) for 24 h, the metabolites of ganglion tissues in *M. chinensis philippi* were evaluated using ^{1}H-nuclear magnetic resonance based metabolomics; Ca^{2+}, neurotransmitters (nitric oxide, glutamate, acetylcholine) and related enzymes (calcineurin, nitric oxide synthase and acetylcholinesterase) were measured using biochemical detection. Compared to the control group, the levels of the nitric oxide (81.04 ± 12.84 μmol/g prot) and acetylcholine (30.93 ± 12.57 μg/mg prot) in *M. chinensis philippi* of IHg-treated were decreased, while glutamate (2.11 ± 0.61 mmol/L) increased significantly; the activity of nitric oxide synthase (679.34 ± 135.33 U/mg prot) was increased, while acetylcholinesterase (1.39 ± 0.44 U/mg prot) decreased significantly, and the activity of calcineurin (0.52 ± 0.02 U/mg prot) had a statistically insignificant increasing tendency. The concentration of Ca^{2+} (0.92 ± 0.46 mmol/g prot) in the IHg-treated group was significantly higher than that in the control group. OPLS-DA was performed to reveal the difference in metabolites between the control and IHg-challenged groups, the metabolites of glucose, glutamine, inosine, succinate, glutamate, homarine, and alanine were sensitive to IHg, subsequently metabolic pathways that were affected including glucose metabolism, glutamine metabolism, nucleotide metabolism, Krebs cycle, amino acid metabolism and osmotic regulation. In our study, IHg interfered with metabolites in *M. chinensis philippi*, thus the corresponding metabolic pathways were changed, which influenced the neurotransmitters subsequently. Furthermore, Ca^{2+}overload affected the synthesis or degradation of the neurotransmitters, and then the altered neurotransmitters involved in changes in metabolic pathways again. Overall, we hypothesized that the neurotoxic effects of IHg on bivalve were in close contact with metabolism, neurotransmitters, related enzymes and Ca^{2+}, which could be effective neurotoxic biomarkers for marine environmental quality assessment, and also provide effective data for the study of the regulatory mechanism of the nervous system in response to IHg in bivalves.

1. Introduction

Recently, the toxicity of inorganic mercury (IHg) has attracted more and more attention (Vaidya, 2023) (Revathi and Jagadeesan, 2022). IHg can cause reproductive dysfunction (Jahan et al., 2019), spleen inflammation (Fan et al., 2020), kidney necroptosis (Chu et al., 2022), immunological system damages (Ahmad and Mahmood, 2019) and nervous system injury (Cardoso et al., 2017). The nervous system is the key target for IHg toxicity (Biswas et al., 2018). IHg can induce metabolic disorders (Wang et al., 2015), alter the cell apoptosis markers TNF-α, IL-6, and IL-1β (Xia et al., 2021) and the autophagy markers LC3I, LC3II, and Beclin1 (El Asar et al., 2019), and lead to cell cycle alterations in proliferation (Sudo et al., 2019). IHg can also cause a neurodegenerative process which gradually results in motor function

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deficits (Bittencourt et al., 2022).

In marine environments, IHg can accumulate in the brain tissue of marine organisms (Polak-Juszczak, 2018) (Minet et al., 2022), and subsequently lead to symptoms of neurological toxicity (Pereira et al., 2016). Bivalves are deemed "sentinel" for the pollution environment due to their camp fixation living, wide distribution, quick accumulation and strong tolerance to trace elements ions (Mandich, 2018). *Crassostrea gigas* (Metian et al., 2020) and *Mytilus edulis* (Amachree et al., 2014) are widely used as bioindicators of IHg pollution, but the toxicity mainly focus on gill, digestive gland and muscle, and the neurotoxicity is less studied which need further study. The study of the response characteristics of ganglion to environmental stress is helpful to understand the specificity of neural regulation and has important significance for understanding the behavior characteristics of bivalves (Yurchenko et al., 2019) (Ojima et al., 2022). As an important bivalve, *Mactra chinensis philippi* are essential components of marine ecosystems, sensitive to environmental change and widely distributed in the marine areas of China (Guo et al., 2013) (Choi and Nam, 2015) (Zhang et al., 2016) (Dai et al., 2023). In contrast to other bivalves, the ganglion of *M. chinensis philippi* can be directly separated, contains three typical pairs of ganglia (cerebral, visceral and pedal ganglia) which is of great significance to fully reveal the neurotoxicity of IHg on bivalves.

As part of "omics" technologies, metabolomics has been used to understand the interactions between marine organisms and their environment (Bayona et al., 2022) (Xie et al., 2023). Changes in the metabolites and related neurotransmitters can monitor brain injury state, and the release of neurotransmitters is regulated by calcium ions (Wang et al., 2019) (Wojnicz et al., 2016) (Lai et al., 2022). It follows that metabolites, neurotransmitters and calcium ions have a direct connection in the functioning of the nervous system. Several studies have used metabolomics to investigate the effects of IHg on nervous system (Lin et al., 2021) (Xie et al., 2023), but insight into the associations between metabolites, neurotransmitters, and calcium ions in bivalves is still unkown, further study is required to establish a clear link between the nervous system damage and molecular alterations caused by exposure to IHg.

In our study, the nervous system of *M. chinensis philippi* were tested after acute exposure to IHg ($HgCl_2$), and we're trying to answer two questionsons: Firstly, what is the neurotoxicity of IHg to *M. chinensis philippi* by using metabolomics and biochemical detection techniques, and secondly, what is the relationship between metabolites, Ca^{2+}, neurotransmitters (nitric oxide, glutamic acid, acetylcholine) and their related enzymes (calcineurin, nitric oxide synthase and acetylcholinesterase). Through our experiments, the neurotoxic effects of IHg on bivalve were revealed from the perspective of metabolism and neurotransmitters, providing effective data for the study of the regulatory mechanism of the nervous system in response to environmental stimuli of IHg. And furthermore, *M. chinensis philippi* were intended to be a good material for neurotoxicity studies in bivalves.

2. Materials and methods

2.1. Experimental animals and exposure conditions

M. chinensis philippi (shell length of 2.7–3.3 cm) were obtained from a local Hongli Aquatic Products Market in Yantai, China. The clams were put in plastic boxes (The length, width and height were 60, 40 and 12 cm respectively), the volume of sea water in the tanks was 9 L, and fed with *Chlorella vulgaris Beij* (2 % tissue dry weight daily) for approximately 7 days before the toxicity experiment. The adaptive culture conditions were continuously aerated, 32 practical salinity units (psu) were used in sea water, the temperature was 25 °C and photoperiod conditions were in natural (Liu et al., 2011).

After adaptive culture, the clams were randomly divided into control and IHg -exposed groups. The concentration of IHg ($HgCl_2$) was 3 μg L^{-1} and 30 μg L^{-1} for metabolomics experiments and 30 μg L^{-1} for measurement of neurotransmitters, enzyme activities and calcium ion content, each group contained 15 individuals. The concentration of $HgCl_2$ was based on mercury levels reported in seawater (Gworek et al., 2016). After exposure for 24 h, the nervous system of *M. chinensis philippi* (a pair of cerebral ganglia, visceral ganglia and pedal ganglia) were separated using eye tweezers and scissors (Carroll and Catapane, 2007). The cerebral ganglia, visceral ganglia and the pedal ganglia were respectively located at the posterior edge of the anterior obturator muscle, the anterior edge of the posterior obturator muscle, and the central part of the foot. After sampling, the ganglia were frozen in liquid nitrogen and then stored in an ultralow temperature freezer for subsequent experiments.

2.2. Extraction of ganglion metabolites in M. chinensis philippi and NMR analysis

The metabolites in *M. chinensis philippi* ganglion tissues ($n = 15$) were extracted by the protocol according to Wu (Wu et al., 2008). Briefly, 100 mg (wet weight) ganglion tissue was extracted in methanol (4 mL g^{-1}), water (5.25 mL g^{-1}) and chloroform (2 mL g^{-1}). The polar layer was dried and then suspended in 0.6 mL phosphate buffer (100 mM Na_2HPO_4 and NaH_2PO_4, 0.5 mM TSP, pH = 7.0). After vortexing thoroughly, the mixture was centrifuged (3000 g, 5 min, 4 °C), the supernatant (0.55 mL) was transferred into a glass tube (5 mm in diameter) for NMR analysis. Metabolites (0.55 mL) were analyzed on a Bruker AV 500 NMR spectrometer (500.18 MHz, 25 °C) as described previously (Liu et al., 2011). All spectra were analyzed manually using TopSpin (version 2.1, Bruker), TSP was rectified at 0.0 ppm, and the water spectra between 4.70 and 5.20 ppm were excluded. Metabolites were recognized using chemical shifts (Fan, 1996) and quantified by using Chenomx (Evaluation Version, Chenomx Inc., Edmonton, Alberta, Canada).

2.3. Measurement of neurotransmitters, enzyme activities and content of calcium ion

The content of neurotransmitters (nitric oxide, glutamate, and acetylcholine), the activity of enzymes (nitric oxide synthase, acetylcholinesterase and calcineurin), and the concentration of Ca^{2+} were all assessed according to the instructions for the test kits (Nanjing Jiancheng Bioengineering Institute, the kit reference of nitric oxide, glutamate, acetylcholine, nitric oxide synthase, acetylcholinesterase, calcineurin and Ca^{2+} was 20,161,119, 20,160,704, 20,160,329, 20,161,119, 20,160,704, 20,161,119, 20,161,117 respectively). The concentration of the ganglion homogenate was 10 %, and the Coomassie brilliant blue method was used to quantify the proteins. The absorbances of nitric oxide, glutamate, acetylcholine, nitric oxide synthase, acetylcholinesterase, calcineurin, and Ca^{2+}were measured at wavelengths of 550, 595, 550, 530, 412, 636 and 610 nm respectively by a Multiskan Spectrum microplate spectrophotometer (Infinite M200, Tecan).

2.4. Determination of mercury in M. chinensis philippi ganglion tissues

The ganglion tissues of *M. chinensis philippi* ($n = 3$) were rinsed in ultrapure water, dried at 80 °C in the oven to constant weights and accurately weighed. About 10 mg dried ganglion tissue was digested in 1 mL nitric acid (70%) by microwave digestion system (CEM, MAR5), and the program was heating at 10 min to 200 °C and holding at 200 °C for 10 min at 200 °C for 15 min. After completely digested, the sample was diluted with ultrapure water to 10 mL for the quantification of Hg using ICP-MS (Agilent 7500i, Agilent Technologies Co. Ltd, USA). Mercury recovery was in the range of 90–110%. Radio-frequency power was 1.5 kW, sampling depth was 10 mm, and the number of repetitions was five.

2.5. Statistical analysis

All the data were expressed as means ± standard deviations. The concentrations of metabolites were tested using normal distribution (Ryan-Joiner's test) and homogeneity of variances (Bartlett's test). SPSS 15.0 software with Tukey's test was used to compare the data between the control and Hg-challenged groups. $p < 0.05$ was considered statistically significant. Orthogonal partial least-squares-discriminant analysis (OPLS-DA) was performed to reveal the difference in metabolites between the control and Hg-challenged groups. OPLS-DA is a supervised statistical method of discriminant analysis, the differences between the groups can be seen from the direction of the horizontal coordinate; and the differences within the groups can be seen from the ordinate (Zhao et al., 2020). The R^2 value and difference between R^2 and Q^2 were used to evaluate the possibility of overfitted models (Feng et al., 2013) (Feng et al., 2011).

3. Results and discussion

3.1. ^1H NMR spectrum of ganglion extracts in M. chinensis philippi

Fig. 1 shows typical ^1H NMR spectra of ganglion extracts of *M. chinensis philippi* from the control and Hg-treated (3 μg L^{-1} and 30 μg L^{-1}) groups. The spectral peaks were assigned with chemical shifts in horizontal axis and the left parts were enlarged by 20 times. All the NMR spectra were generalized log transformed (glog) with a transformation parameter $\lambda = 2.0 \times 10^{-8}$ to stabilize the variance across the spectral bins and to increase the weightings of the less intense peaks. 37 metabolites were obtained totally in Fig. 1, and there were two unknown metabolites among these in 1.10 ppm and 5.41 ppm of chemical shift.

Metabolic pathway which these 37 metabolites took part in were searched according to KEGG website (https://www.kegg.jp/), they were mainly included valine, leucine and isoleucine biosynthesis (isoleucine, leucine, valine, and 3-hydroxyisobutyrate); glycolysis (lactate, α-glucose, and β-glucose); glycine, serine and threonine metabolism (threonine, glycine, homarine, serine, and dimethylglycine); alanine, aspartate and glutamate metabolism (alanine, β-alanine, and aspartate); arginine biosynthesis (arginine); citrate cycle (acetate, fumarate, and succinate); tyrosine metabolism (tyrosine); de novo purine biosynthesis (glutamine); leucine degradation (acetoacetate); taurine and hypotaurine metabolism (hypotaurine); malonate semialdehyde pathway (malonate); cholinergic synapse (choline); glutamatergic synapse (glutamate); glycerophospholipid metabolism (phosphocholine); betaine biosynthesis (betaine); taurine and hypotaurine metabolism (taurine); adenine ribonucleotide biosynthesis (ATP); histidine metabolism (histidine); and phenylalanine metabolism (phenylalanine).

Among these metabolites, isoleucine, leucine and valine have been shown to reduce the toxic effects on the central nervous system (Berry et al., 1990). Glycine and taurine are inhibitory neuromodulatory amino acids (Aliyev and Aliyev, 2005) (O'Donnell et al., 2016), while glutamate and aspartate are excitatory neurotransmitters (Gusev et al., 2000). These amino acids are neuroprotective agents indicatd that they could counter the toxic effects of IHg poisons within a certain range. When choline is used as a methyl donor, it is converted to betaine which is typical intracellular osmolytes (Böckmann et al., 2022) (O'Connor et al., 2022) (Willingham et al., 2023). Lactate has been shown to serve as an alternative energy substrate in the central nervous system (Schmid et al., 2008). In brief, these metabolites may play key roles in detoxification and regulation of osmotic balance in *M. chinensis philippi*, and was of great significance in elucidating the mechanism of the bivalves response to aquatic IHg stress.

3.2. Metabolic changes in M. chinensis philippi under IHg treatment

Fig. 2 shows OPLS-DA results derived from ^1H NMR spectra of the ganglion extracts from control and IHg treatments (3 μg L^{-1} and 30 μg

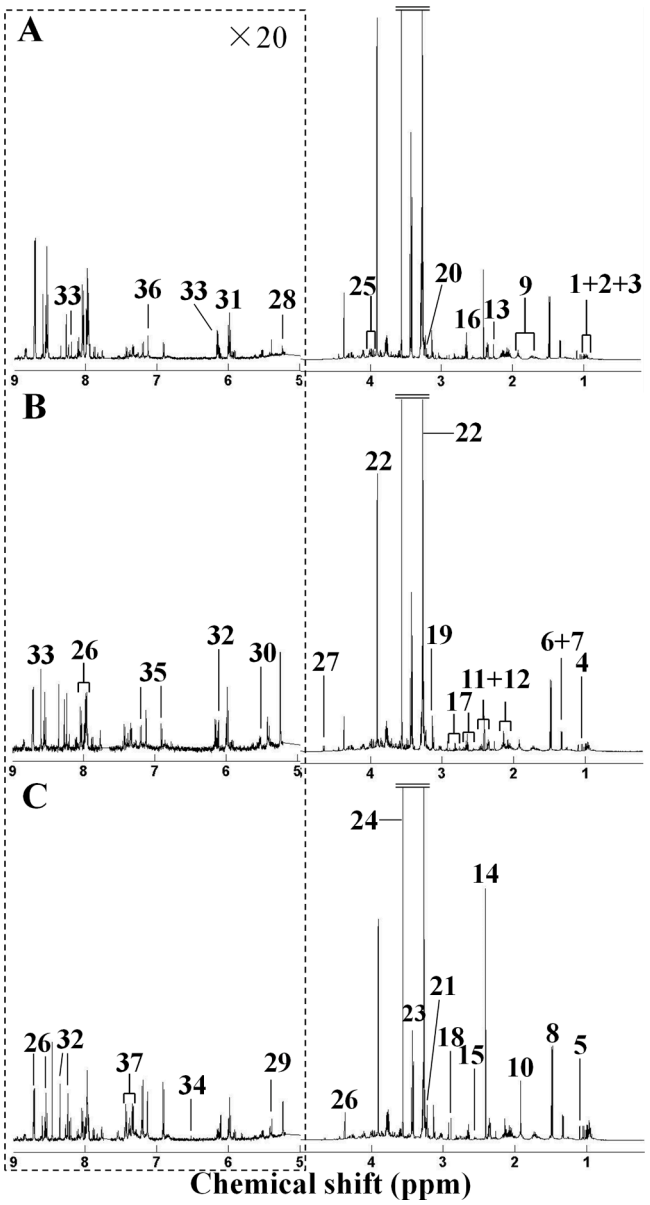

Fig. 1. Typical ^1H NMR spectra of tissue extracts of *M. chinensis philippi* from the control (A), 3 μg L^{-1} IHg- (B) and 30 μg L-1 (C) IHg-treated groups. All the NMR spectra were generalized log transformed (glog) with a transformation parameter $\lambda = 2.0 \times 10^{-8}$ to stabilize the variance across the spectral bins and to increase the weightings of the less intense peaks. **Keys:** (1) Isoleucine, (2) Leucine, (3) Valine, (4) 3-Hydroxyisobutyrate, (5) unknown 1 (1.10 ppm), (6) Lactate, (7) Threonine, (8) Alanine, (9) Arginine, (10) Acetate, (11) Glutamate, (12) Glutamine, (13) Acetoacetate, (14) Succinate, (15) β-Alanine, (16) Hypotaurine, (17) Aspartate, (18) Dimethylglycine, (19) Malonate, (20) Choline, (21) Phosphocholine, (22) Betaine, (23) Taurine, (24) Glycine, (25) Serine, (26) Homarine, (27) β-Glucose, (28) α-Glucose, (29) Unknown 2 (5.41 ppm), (30) Unknown 3 (5.54 ppm), (31) Unknown 4 (5.98 ppm), (32) Inosine, (33) ATP, (34) Fumarate, (35) Tyrosine, (36) Histidine and (37) Phenylalanine.

L^{-1} $HgCl_2$) in *M. chinensis philippi*. OPLS-DA results from the analysis of NMR spectral data showed clear separations between control and IHg-exposed groups. Peaks in the positive direction indicate metabolites that are more abundant in IHg treatments, and metabolites that are more abundant in control group are presented as peaks in the negative direction. In *M. chinensis philippi* of the IHg 3 μg/L-treated group, glucose, glutamine and inosine increased; succinate, homarine, alanine, and glutamate decreased. Fewer metabolites were altered in the 30 μg/L IHg-treated group than in the 3 μg/L IHg group. Glutamate was decreased,

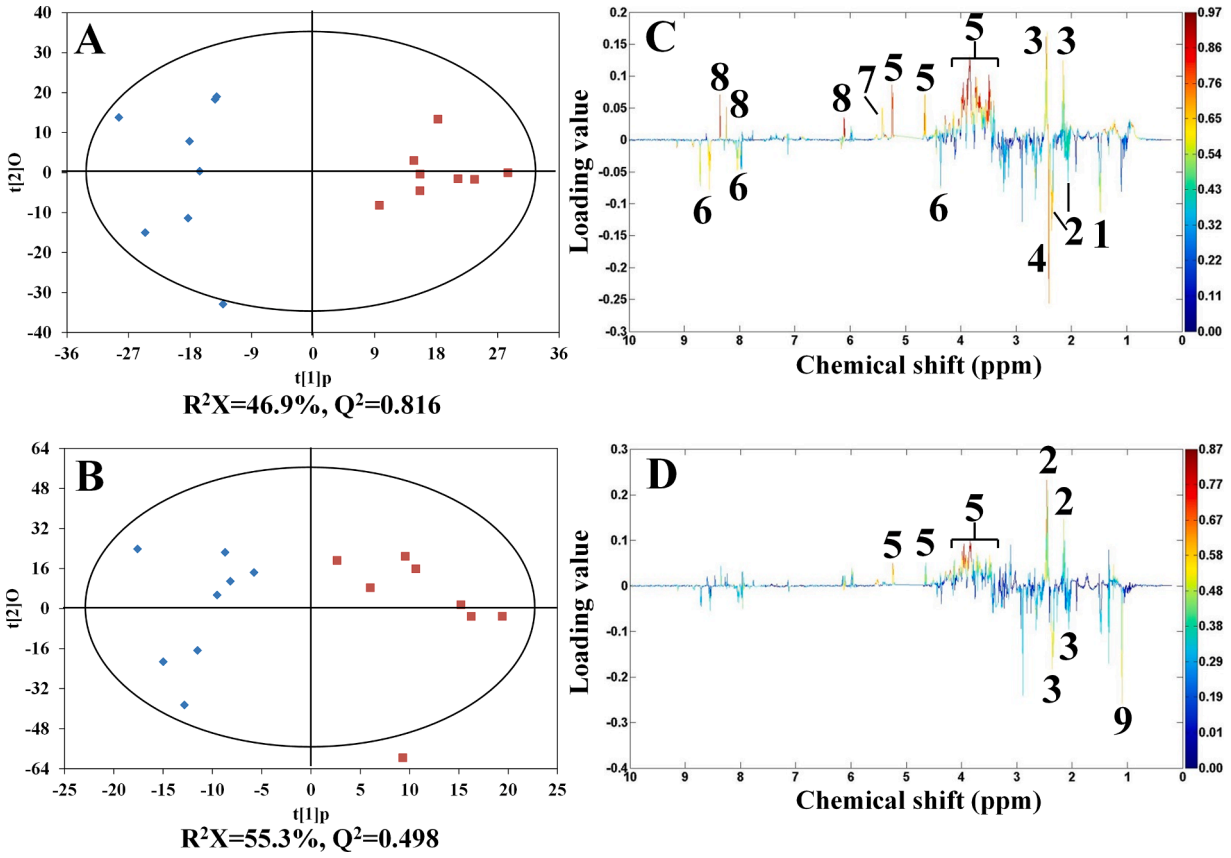

Fig. 2. OPLS-DA scores derived from ^{1}H NMR spectra of the tissue extracts from *M. chinensis philippi* from control (◆) and IHg treatments (■), (A) control Vs. 3 μg L^{-1} IHg treatment and (C) control Vs. 30 μg L^{-1} IHg treatment and corresponding coefficient plots (B) and (D). The color map shows the significance of metabolite variations between the two classes (control and Hg treatment). Keys: (1) alanine, (2) glutamate, (3) glutamine, (4) succinate, (5) glucose, (6) homarine, (7) unknown 1, (8) inosine and (9) unknown 2. Altered metabolites related to Hg treatments: In IHg 3 μg L^{-1} group decreased metabolites included alanine, glutamate, succinate and homarine; Increased metabolites included glutamine, glucose, unknown 1 and inosine. In IHg 30 μg/L group decreased metabolites included glutamate and unknown 2; Increased metabolites included glutamine and glucose.

glutamine and glucose were increased, and the three metabolites showed the same changes in the 3 μg/L and 30 μg/L IHg -treated groups.

It has been reported that mercury disturbs glycolysis in mice which is major pathway of glucose metabolism (Ramírez-Bajo et al., 2014) (Ahmad and Mahmood, 2019), and inhibits glutamine metabolism (Passerini and Welbourne, 1979). Our experiment also proved that IHg exerted some influence on the two metabolites of glucose and glutamine in *M. chinensis philippi*, so it affected two metabolic pathways of glycolysis and glutamine metabolism. Glutamate not only participates in amino acid metabolism but also is a typical neurotransmitter in glutamatergic synapses, the glutamate-glutamine cycle plays a key role in glutamate homeostasis, ensuring that ideal neurophysiological activity is maintained (Gras et al., 2006). The changed metabolites of glutamate and glutamine indicated that IHg disrupted the glutamate-glutamine balance in *M. chinensis philippi*.

Biochemical analysis showed $HgCl_2$ exposure affects proteins and nucleic acids of the zebrafish larvae (Abu Bakar et al., 2017), inosine is a posttranscriptional modification (Dutta et al., 2022), the increase in inosine in our study proved that IHg disturbed nucleotide metabolism. Succinate, which is involved in the Krebs cycle, participates in the body's response to the environment in a marine bivalve *Crassostrea gigas* (Murphy and O'Neill, 2018) (Adzigbli et al., 2022), the altered succinate in our study also verified its role in the stress response of IHg. Both homarine and alanine are osmolytes in aquatic animals (Lenky et al., 2012) (Abe et al., 2005), in our study the two decreasing osmolytes showed that IHg caused osmotic regulation disorder in *M. chinensis philippi*.

In brief, from OPLS-DA statistical analysis, we could find seven metabolites (glucose, glutamine, inosine, succinate, glutamate, homarine, and alanine) markering of brain injury induced by IHg in *M. chinensis philippi*. Correspondingly, the metabolic pathways related to these metabolites included glucose metabolism, glutamine metabolism, nucleotide metabolism, Krebs cycle, amino acid metabolism and osmotic regulation. The change trend of the two metabolites glutamate and glutamine was opposite at the two doses of our experiment, the specific reason remains to be further studied.

3.3. Effects of IHg on neurotransmitters and related enzymes in *M. chinensis philippi*

Table 1 shows the contents of neurotransmitters (nitric oxide, glutamic acid and acetylcholine) and the activity of related enzymes (nitric oxide synthase and acetylcholinesterase) in the ganglion of *M. chinensis philippi* after 24 h of exposure to 30 μg/L IHg. Compared to the control group, the levels of the neurotransmitters nitric oxide (NO) and

Table 1
The content of neurotransmitters and the activity of related enzymes in the ganglion of *M. chinensis philippi* after 24 h exposed to 30 μg/L IHg.

	NO (μmol/g prot)	Glu (mmol/L)	ACh (μg/mg prot)	NOS (U/mg prot)	AChE (U/mg prot)
Control	115.17 ±22.67	1.37 ±0.53	47.79 ±11.90	394.7 ± 190.58	3.12±0.85
IHg	81.04 ±12.84*	2.11 ±0.61*	30.93 ±12.57*	679.34 ±135.33*	1.39±0.44 *

* $p < 0.05$.

acetylcholine (ACh) were decreased, while glutamate (Glu) increased significantly; and the activity of nitric oxide synthase (NOS) was increased, while acetylcholinesterase (AChE) decreased significantly after exposure to IHg. The three neurotransmitters NO, Glu-and ACh, which are key signals in the nervous system, were significantly changed in IHg-treated group compared to the control group of *M. chinensis philippi*.

Neurotransmitters are important messenger molecules to stress pollutants (Siblerud et al., 2019), and monitoring changes in neurotransmitters and related enzymes can provide insight into the neurotoxicity of IHg. NO participates in the protection of mollusc nerve cells from environmental stress (Kotsiuba, 2008), NOS catalyzes the formation of NO and involves in the neurotransmission processes (Annikova et al., 2000) (Saini et al., 2021). The data obtained in our study showed that decreased NO resulting in a compensatory increase in NOS in the IHg stress group of *M. chinensis philippi*. ACh is an important neurotransmitter released from the presynaptic membrane, AChE catalyzes its degradation (Schena and Johnsson, 2014). In the IHg-treated group ACh and AChE were both decreased, which could indicate that IHg inhibited the synthesis and degradation of ACh. Glutamate belongs to a excitatory neurotransmitter and hyper-glutamatergic state causes damage to the body (Gusev et al., 2000) (Szumlinski et al., 2017). Excessive glutamate after IHg stress in our study demonstated that IHg could cause glutamic excitotoxic effects. Changes in these three neurotransmitters and related enzymes suggested that IHg affect the nervous system of *M. chinensis philippi* by disturbing the synthesis or degradation of neurotransmitters, or by causing excitotoxic effects of amino acids.

3.4. Effects of IHg on the concentration of Ca^{2+} and activity of calcineurin in M. chinensis philippi

Table 2 shows the concentration of $Ca^{2+}$and the activity of calcineurin (CaN) in the ganglion of *M. chinensis philippi* after 24 h of exposure to IHg. The concentration of Ca^{2+} in the IHg-treated group was significantly higher than that in the control group, and the activity of CaN was not statistically significant but showed an increasing tendency.

CaN is a calmodulin-dependent protein involved in a variety of signal transduction pathways regulated by Ca^{2+} (Rusnak and Mertz, 2000) (Mehta et al., 2014). High Ca^{2+} content has harmful effects to bodies (Marcusson et al., 1997) (Severi et al., 2014), and the binding of ACh and Glu-to their corresponding receptors also requires the participation of Ca^{2+} (Wu et al., 2010) (Qiu et al., 2020). Recent research shows that Ca^{2+} signaling pathway are enriched in brain injury induced by mercury in Common Carp (Zhang et al., 2023).The excess of Ca^{2+} after IHg stress in our study indicated that IHg may cause Ca^{2+} overload which could induce damage to the nervous system in *M. chinensis philippi*.

3.5. Accumulation of mercury in M. chinensis philippi

The Hg concentrations in the *M. chinensis philippi* ganglion of control, 3 μg/L and 30 μg/L IHg-treated groups were 0.0841 ± 0.0285, 0.2067 ± 0.0849 and 0.1000 ± 0.0609 μg/g respectively. The Hg concentrations in both 3 μg/L and 30 μg/L were higher than that of control group, and 3 μg/L IHg-treated group was significantly higher than the control ($p < 0.05$).

Filter-feeding organisms accumulate and respond to toxic agents efficiently (Parisi et al., 2021), and mercury of inorganic state can accumulate in brain tissue and cause neurologic symptoms (Hansen et al., 1989) (Yasutake et al., 2010). In our experiment, mercury accumulation in the 3 μg/L IHg treatment group was higher than that in the 30 μg/L group. This phenomenon could result from the hormesis which is low-dose stimulation and high-dose inhibition of chemicals (Nagpal and Yuan, 2023). When the content of mercury was low, it didn't exceed the tolerance range of the body and the body didn't start the detoxification mechanism in *M. chinensis philippi*, thus easy to accumulate mercury. However, when the content increase continually, the body starts to detoxify mercury, and the accumulation of mercury in the body decreased instead. This was also why we chose a stress concentration of 30 μg/L of IHg when detecting the neurotransmitters, related enzymes and Ca^{2+} in *M. chinensis philippi*.

Table 2
The concentration of Ca^{2+} and the activity of calcineurin (CaN) in the ganglion of *M. chinensis philippi* after 24 h exposed to 30 μg/L IHg.

	Ca^{2+} (mmol/g prot)	CaN (U/mg prot)
Control	0.59±0.24	0.43±0.04
IHg	0.92±0.46*	0.52±0.02

* $p < 0.05$.

3.6. The integrated effects of IHg on metabolism, neurotransmitters, enzymes and Ca^{2+}

Fig. 3 shows the integrated map of pathways in metabolites, neurotransmitters, related enzymes and Ca^{2+} in the IHg-treated groups of ganglion in *M. chinensis philippi*. As we can see from the figure, IHg influenced metabolic pathways of *M. chinensis philippi*, including glucose metabolism, glutamine metabolism, nucleotide metabolism, Krebs cycle, amino acid metabolism, osmotic regulation and reactive oxygen species (ROS). These metabolic pathways were connected with neurotransmitters of ACh, Glu-and NO to their corresponding receptors or enzymes. Overloaded Ca^{2+} participates in the binding of ACh to acetylcholine receptors and Glu-to N-methyl-d-aspartate receptors, and also participates in the synthesis of NO. Through our experiments, the neurotoxic effects of IHg on *M. chinensis philippi* were revealed from the perspective of metabolism, neurotransmitters, related enzymes and Ca^{2+}, which could provide effective data for the study of the regulatory mechanism of the nervous system in response to environmental stimuli in bivalves.

4. Conclusions

In this study, the acute effects (24 h) of IHg ($HgCl_2$) on ganglion tissue of *M. chinensis philippi* were investigated using metabolomics, Ca^{2+}, neurotransmitters and related enzymes. The metabolites (glucose, glutamine, inosine, succinate, glutamate, homarine, and alanine), neurotransmitters (NO, Glu, and ACh), enzymes (NOS, AChE and CaN) and Ca^{2+} in *M. chinensis philippi* were sensitive to IHg, which could be effective neurotoxic biomarkers for marine environmental quality assessment. IHg interfered with metabolites in *M. chinensis philippi*, thus the corresponding metabolic pathways were changed, which influenced the neurotransmitters. Furthermore, Ca^{2+}overload affected the synthesis or degradation of the neurotransmitters, and then the altered neurotransmitters involved in changes in metabolic pathways again. *M. chinensis philippi* showed an effective response to IHg, which could be used as an ideal biological indicator to evaluate the neurotoxicity of marine pollutants.

Overall, we hypothesized that the neurotoxic effects of IHg on bivalve were in close contact with metabolism, neurotransmitters, related enzymes and Ca^{2+}, which could provide effective data for the study of the regulatory mechanism of the nervous system in response to IHg in bivalves. Our research is limited, digging deeper into the mechanism of bivalve nervous system response to the environment will have more important significance for revealing the adaptive characteristics of marine organisms.

Compliance with ethical standards

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

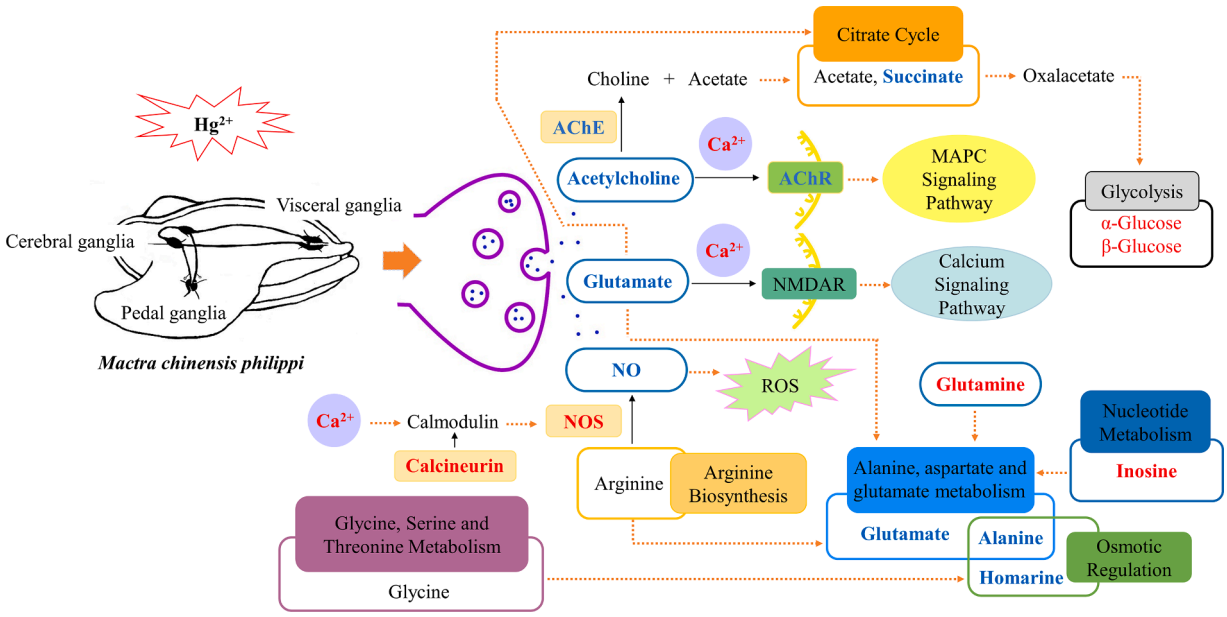

Fig. 3. Integrated map of pathways in the IHg-treated group of ganglion in *M. chinensis philippi*. Red indicates an increase in the substances and blue indicates a decrease.

CRediT authorship contribution statement

Bangguo Ma: Writing – original draft. **Xiaoli Zhao:** Investigation. **Xiaoning Zhang:** Data curation. **Bowen Yang:** Formal analysis. **Zimin Cai:** Methodology. **Zihan Xing:** Methodology. **Mingzhe Xu:** Formal analysis. **Liuya Mi:** Methodology. **Jianning Zhang:** Project administration. **Lei Wang:** Supervision. **Yancui Zhao:** Supervision. **Xiaoli Liu:** Writing – review & editing, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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