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RESEARCH ARTICLE

Investigating the Effects of Rivastigmine and Ketamine on Brine Shrimp Morphology, Mortality, and Acetylcholinesterase Activity

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Abstract: Brine shrimp (Artemia salina) has emerged as a valuable alternative animal model for preclinical pharmacological research due to its cost-effectiveness, ease of maintenance, and sensitivity to a wide range of toxins. This study aimed to investigate the effects of rivastigmine, an acetylcholinesterase inhibitor, and ketamine, an NMDA receptor blocker, on the morphology, mortality, and acetylcholinesterase (AChE) activity of brine shrimp and their egg homogenates. Brine shrimp eggs were hatched in 5% and 2% NaCl solutions, and the live shrimps were exposed to various concentrations of rivastigmine and ketamine for 24 hours. Morphological changes and mortality were assessed using a binocular microscope. Additionally, the effects of these drugs on AChE activity in brine shrimp egg homogenates were evaluated using Ellman's method. The results revealed that 5% NaCl solution yielded higher hatchability (>90%) compared to 2% NaCl. No significant morphological abnormalities or mortality were observed in shrimps treated with different doses of rivastigmine and ketamine. Rivastigmine (100-800 µg/ml) significantly increased AChE activity in egg homogenates, confirming the presence of AChE in brine shrimp. However, ketamine did not show any significant effect on AChE activity. These findings suggest that brine shrimp and their egg homogenates can serve as a valuable model for studying the effects of neuroactive compounds on cholinergic systems and provide insights into the potential use of this model in neuropharmacological research.

Keywords: Brine shrimp; Acetylcholinesterase; Rivastigmine; Ketamine; Neuropharmacology.

1. Introduction

Brine shrimp (Artemia salina) has gained increasing attention as an alternative animal model in preclinical pharmacological research due to its numerous advantages, including cost-effectiveness, ease of maintenance, short life cycle, and sensitivity to a wide range of toxins and environmental changes [1-3]. Brine shrimp shares a significant portion of its core metabolic pathways with higher vertebrates, allowing for some degree of extrapolation of findings to mammalian systems [4]. Moreover, the lack of a complex nervous system in brine shrimp reduces ethical concerns associated with animal testing compared to using mammals [5].

Acetylcholinesterase (AChE) is a key enzyme in the cholinergic nervous system, responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) [6]. Dysregulation of cholinergic signaling and alterations in AChE activity have been implicated in various neurological disorders, such as Alzheimer's disease (AD) [7]. Rivastigmine, a reversible AChE inhibitor, is commonly used in the treatment of AD, while ketamine, an NMDA receptor blocker, has been investigated for its potential antidepressant and analgesic effects [8,9].

Despite the growing interest in using brine shrimp as an alternative model, limited research has been conducted on the effects of neuroactive compounds on their morphology, mortality, and cholinergic system. This study aimed to investigate the effects of rivastigmine and ketamine on brine shrimp morphology, mortality, and AChE activity in both live shrimp and their egg homogenates. The findings of this study could provide valuable insights into the potential use of brine shrimp as a model for neuropharmacological research and contribute to the development of novel therapeutic strategies targeting the cholinergic system.

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2. Materials and Methods

2.1. Chemicals and Reagents

Sodium chloride (NaCl), rivastigmine, ketamine, Tris-HCl, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetylthiocholine iodide (ATCI) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Merck (Darmstadt, Germany). All other chemicals and reagents used were of analytical grade.

2.2. Brine Shrimp Hatching and Maintenance

Brine shrimp eggs were obtained from a local aquarium store and hatched in two different NaCl concentrations: 5% and 2%. The hatching solutions were prepared by dissolving the appropriate amount of NaCl in distilled water and adjusting the pH to 7.5-8.5. The eggs were added to the hatching solutions and incubated at 28°C under constant aeration using an aquarium pump and illumination with a 60W light. After 24 hours, the number of hatched nauplii (stage II) was counted, and the hatchability percentage was calculated [10].

2.3. Acute Exposure to Rivastigmine and Ketamine

Live brine shrimp nauplii (stage II) were exposed to various concentrations of rivastigmine and ketamine (1-80 µg/ml) for 24 hours at room temperature. Each treatment group consisted of 25 nauplii in 100 µl of the respective drug solution, and the experiments were performed in triplicate. After the exposure period, the number of dead nauplii in each group was counted using a binocular microscope (Olympus SZ61, Japan) [11].

2.4. Morphological Analysis

Brine shrimp nauplii from the control and drug-treated groups (n=5 per group) were fixed on glass slides and observed under a binocular microscope (Olympus SZ61, Japan) at 40× magnification. Images were captured using the Toupview imaging software system, and morphological characteristics, such as the appearance of the head, foregut, midgut, and hindgut, were evaluated [12].

2.5. Preparation of Brine Shrimp Egg Homogenates

Unhatched brine shrimp eggs (1 g) were soaked in 1 ml of distilled water for 1 hour and then homogenized in 1 ml of Tris-HCl buffer (50 mM, pH 7.4) using a mortar and pestle. The homogenate was centrifuged at 1500 rpm for 5 minutes, and the supernatant was collected for further analysis [13].

2.6. Protein Estimation

The protein concentration in the brine shrimp egg homogenates was determined using the Bradford method [14], with BSA as the standard. The absorbance was measured at 595 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan).

2.7. Acetylcholinesterase (AChE) Activity Assay

AChE activity in the brine shrimp egg homogenates was measured using Ellman's method [15]. The reaction mixture contained 100 µl of egg homogenate, 800 µl of Tris-HCl buffer (50 mM, pH 7.4), and 100 µl of DTNB (0.5 mM). The mixture was incubated at 37°C for 5 minutes, followed by the addition of 50 µl of ATCI (1 mM). The change in absorbance was recorded at 412 nm for 5 minutes at 30-second intervals using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan).

2.8. Effect of Rivastigmine and Ketamine on AChE Activity

The effect of rivastigmine and ketamine on AChE activity in brine shrimp egg homogenates was evaluated by incubating 100 µl of the homogenate with various concentrations of the drugs (100-800 µg/ml) for 30 minutes at 37°C. The AChE activity was then measured using Ellman's method, as described in section 2.7.

2.9. Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (version 8.0). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the means between groups. A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Brine Shrimp Hatchability

The hatchability of brine shrimp eggs in 5% and 2% NaCl solutions was assessed to determine the optimal hatching conditions. 5% NaCl solution yielded a significantly higher hatchability percentage (>90%) compared to the 2% NaCl solution. This finding is consistent with previous reports that suggest that higher salinity favors the hatching of brine shrimp eggs [16]. The optimal hatching conditions ensure a sufficient number of nauplii for subsequent experiments and minimize variability in the results.

3.2. Acute Exposure to Rivastigmine and Ketamine

The effects of rivastigmine and ketamine on brine shrimp mortality were evaluated following acute exposure to various concentrations of the drugs (1-80 µg/ml) for 24 hours. Neither rivastigmine nor ketamine caused significant mortality in brine shrimp nauplii at the tested concentrations. This observation suggests that brine shrimp nauplii can tolerate a wide range of concentrations of these neuroactive compounds, making them a suitable model for studying the effects of these drugs on morphology and AChE activity [17].

3.3. Morphological Analysis

The morphological characteristics of brine shrimp nauplii exposed to rivastigmine and ketamine were examined under a binocular microscope to assess any potential drug-induced alterations. As shown in Figure 1, no significant morphological abnormalities were observed in the head, foregut, midgut, or hindgut regions of the nauplii treated with either drug compared to the control group. This finding indicates that acute exposure to rivastigmine and ketamine does not induce overt morphological changes in brine shrimp nauplii, further supporting their use as a model for neuropharmacological studies [18].

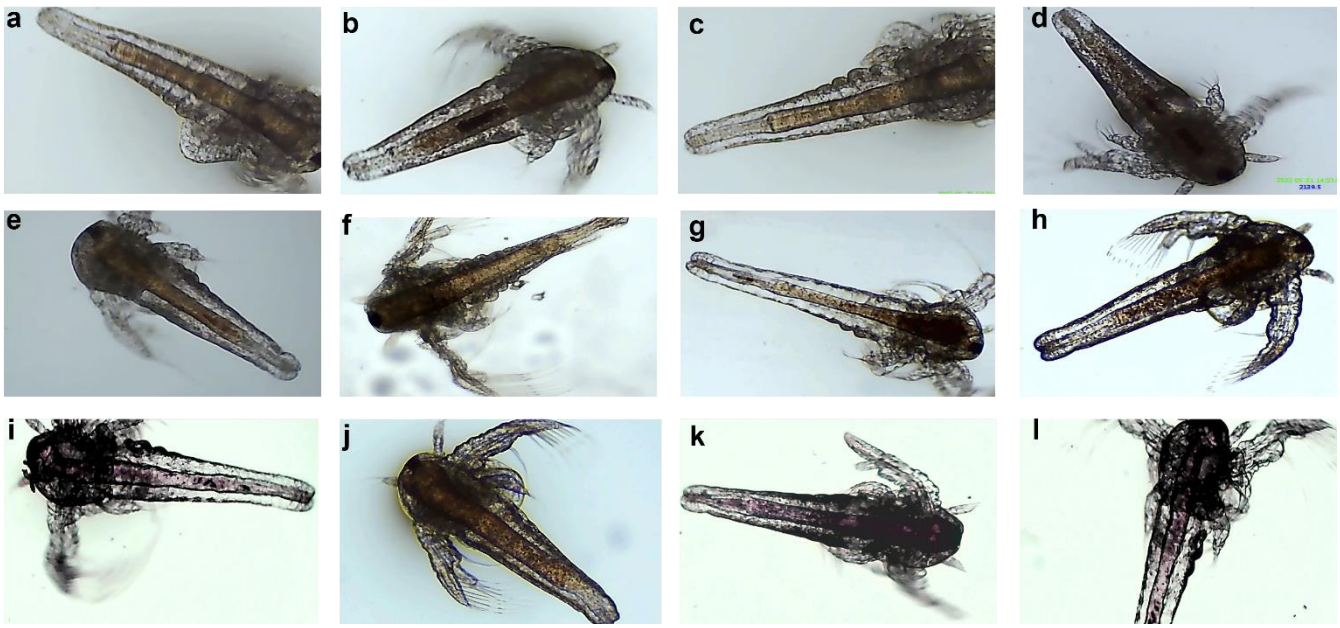

Figure 1. Brine shrimp morphology upon acute exposure with a. Control b. Rivastigmine (5 µg/ml) c. Rivastigmine (10 µg/ml) d. Rivastigmine (20 µg/ml) e. Rivastigmine (40 µg/ml) f. Rivastigmine (80 µg/ml) g. Ketamine (5 µg/ml) h. Ketamine (10 µg/ml) i. Ketamine (20 µg/ml) j. Ketamine (40 µg/ml) l. Ketamine (80 µg/ml)

3.4. Protein Estimation in Brine Shrimp Egg Homogenates

The protein concentration in brine shrimp egg homogenates was determined to ensure that sufficient protein was available for the AChE activity assay. As presented in Table 1, the protein concentration increased with increasing volumes of egg homogenate, ranging from 28 µg/ml in 125 µl to 94 µg/ml in 2 ml of homogenate. These results demonstrate that brine shrimp eggs contain a significant amount of protein, which can be utilized for enzymatic assays [19].

Table 1. Protein concentration (µg/ml) from egg homogenate

S. No	Concentration of egg homogenates (µl)	Absorbance at 548 nm (Mean ± SEM)*	Calculated Protein concentration (µg/ml) (Mean ± SEM)*
1.	125	0.316 ± 0.012	27.9787234 ± 1.22
2.	250	0.457 ± 0.036	49.4072948 ± 1.47
3.	500	0.536 ± 0.023	61.4133739 ± 1.01
4.	1000	0.609 ± 0.031	72.5075988 ± 2.56
5	2000	0.752 ± 0.056	94.2401216 ± 2.84

*n= 3 observations

3.5. Effect of Rivastigmine on AChE Activity

The effect of rivastigmine on AChE activity in brine shrimp egg homogenates was investigated to assess the presence and functionality of the cholinergic system in this model. As illustrated in Figure 2, rivastigmine (100-800 µg/ml) significantly increased AChE activity in the egg homogenates compared to the control group. This finding confirms the presence of AChE in brine shrimp eggs and demonstrates the sensitivity of the enzyme to rivastigmine, a known AChE inhibitor [20]. The increase in AChE activity observed with rivastigmine treatment may be attributed to a compensatory mechanism in response to the inhibition of the enzyme [21].

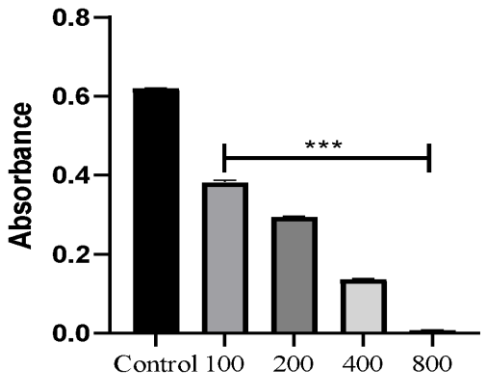

Figure 2. effect of various concentrations of Rivastigmine on brine shrimp egg homogenates using acetylthiocholine iodide as a substrate. There is a significant decrease in absorbance was observed between the control and rivastigmine-treated groups. One-way ANOVA of the data suggests that there was a significant [Df (4, 50) = 94240; p<0.0001] difference between changes in absorbance noted in the control and rivastigmine-treated group. Further Bartlett's test comparison among the treatment group suggests a significant [Df (4, 50) = 22.45; p< 0.005] decrease in rivastigmine treated group

3.6. Effect of Ketamine on AChE Activity

The effect of ketamine on AChE activity in brine shrimp egg homogenates was evaluated to determine whether this NMDA receptor blocker influences cholinergic signaling in this model. As shown in Figure 3, ketamine did not significantly alter AChE activity in the egg homogenates at the tested concentrations (100-800 µg/ml) compared to the control group. This observation suggests that ketamine, an NMDA receptor antagonist, does not directly modulate AChE activity in brine shrimp eggs [22]. The lack of effect of ketamine on AChE activity highlights the specificity of the cholinergic system in brine shrimp and its potential for studying compounds targeting this pathway

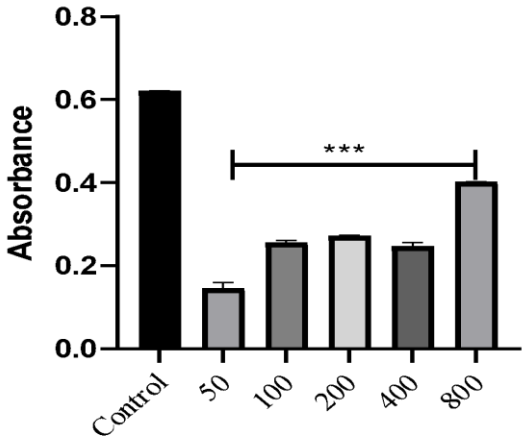

Figure 3. effect of various concentrations of Ketamine on brine shrimp eggs homogenates using acetylthiocholine iodide as a substrate. There is a significant decrease in absorbance was observed between the control and ketamine treated groups. One way ANOVA of the data suggests that there was a significant [Df (5, 60) = 6776; p<0.0001] difference between changes in absorbance noted in the control and ketamine-treated group. Further Bartlett's test comparison among the treatment group suggests a significant [Df (4, 50) = 2.966; p< 0.00186] decrease in ketamine treated group

4. Conclusion

This study demonstrates the suitability of brine shrimp (Artemia salina) as an alternative animal model for investigating the effects of neuroactive compounds on morphology, mortality, and cholinergic signaling. The optimal hatching conditions, tolerance to a wide range of drug concentrations, and the presence of a functional AChE enzyme in brine shrimp eggs support their use in neuropharmacological research. The differential effects of rivastigmine and ketamine on AChE activity in brine shrimp egg homogenates highlight the specificity of the cholinergic system in this model and its potential for studying compounds targeting this pathway. Further research is warranted to explore the utility of brine shrimp in screening novel neuroactive compounds and elucidating their mechanisms of action.

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