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Impaired Avoidance Learning and Increased hsp70 mRNA Expression in Pentylenetetrazol-treated Zebrafish

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Abstract: The effects of pentylenetetrazol (PTZ), a GABA receptor antagonist, were studied on passive avoidance learning and expression of heat shock protein 70 (hsp70), neuroglobin, and fatty acid binding protein-7 (fabp-7) genes. Zebrafish were trained to stay in a dark compartment to avoid a weight dropping in an acryl shuttle box with a central sliding door. In two training sessions of 2 h interval, each consisting of 3 trials, the crossing time was significantly increased from 43.2±14.4 s to 149.3±38.5 s in the first training session and remained 116.1±36.0 s in the first trial of the second training session in the control. In zebrafish treated with PTZ before the first training session, the crossing time was significantly increased neither in the first nor in the second training session. However, the increased crossing time was maintained in the second training session when 10 mM PTZ was treated three times for 10 min at 30 min intervals between the first and second training session. Quantitative real-time PCR showed that expression level of hsp70 mRNA increased two to eight fold over that of control in the brain at 0-24 h after termination of PTZ treatment. No change in expression of neuroglobin and fabp-7 mRNA was shown in PTZ-treated zebrafish. Our studies suggest that PTZ impairs learning ability in avoidance response and also modifies expression of genes related to the neuroprotection.

Key words: pentylenetetrazol, zebrafish, learning, heat shock protein 70

INTRODUCTION

Several studies have shown that seizures are generated by various convulsants in developing and adult zebrafish. Blockade of GABAergic inhibitory synaptic transmission by pentylenetetrazol (PTZ) causes seizure-like discharges in the tectum of larvae (Baraban et al., 2005). Characteristic seizure behaviors such as whirlpool swimming were documented in the PTZ-treated larvae (Tiedeken and Ramsdell, 2007). Electrically induced neural activity was also transformed into the bursting activity in the isolated telencephalon of adult zebrafish (Kim et al., 2004). Based on these studies, the zebrafish has been used as a model to screen potential anticonvulsant drugs and to test the seizure liability of new drug candidates (Berghmans et al., 2007; Winter et al., 2008).

Yet, modification of gene expression during PTZ-induced seizures has not been reported except the up-regulation of *c-fos* expression in the larvae brain of zebrafish (Baraban et al., 2005). Expression levels of a number of genes are altered in the rat brain experiencing seizures, one of which is heat shock proteins gene expression. For example, kainic acid, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor agonist, has been shown to increase expression of hsp70 in the rat hippocampus (Krueger et al., 1999), and the expression appeared to correlate with seizure severity. Overall survival during convulsant PTZ-induced kindling was increased in mice overexpressing hsp70, which is thought to exert protective effects on the pathology of increased neuronal excitation in mice (Ammon-Treiber et al., 2007).

Seizures are also associated with behavioral abnormality; seizures impair cognitive performance in learning and memory (Dubé et al., 2009; Gröticke et al., 2008; Mortazavi et al., 2005; Szyndler et al., 2006). Animal models of seizures may help us understand mechanisms underlying the association between seizure and behavioral abnormalities (Gröticke et al., 2008). Recently, the zebrafish has been widely used as a model to study functions of the brain and mechanisms of neurological disorder. Behavioral studies have shown that the zebrafish is capable of learning a number of tasks such as spatial alternation and active avoidance conditioning (Pradel et al., 1999; Pradel et al.,

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2000; Swain et al., 2004; Xu et al., 2007). In this study, using avoidance response test and quantitative real-time PCR we aimed to study whether seizure activity changes the brain function and expression pattern of various genes including the hsp70 gene in the zebrafish brain.

MATERIALS AND METHODS

Animals

Adult zebrafish, purchased from a local fish shop, were maintained at 28.0°C with a 14 h light-10 h dark cycle in a container equipped with a continuous filtration and aeration system (Zebrafish AutoSystem, Genomic Design, Seoul, Korea). Zebrafish were fed twice a day with flake food and *Artemia nauplia*.

Zebrafish avoidance response test

An acryl shuttle box (28.5 cm long×10 cm wide×13.5 cm high) was subdivided into two compartments by a sliding door 5 cm in diameter. This chamber was made of black acryl, except for the upper part. One compartment remained dark; the other had a transparent window 3 cm in diameter at opposite side of the door so that a signal of flash light was allowed to pass through the door (Fig. 1).

A zebrafish was placed in the dark compartment. After 3 min of acclimation, the flash light turned on and the door was opened. The time for zebrafish to cross the door was measured from the door opening to the moment that the fish swam through the door. For a shock, a weight of small stone was dropped in front of zebrafish at 3 s after the fish crossed the door. Then, zebrafish were slightly pushed back to the dark compartment and the light turned off. With 3 min interval, the process was repeated two more times. Each crossing was named a trial and a training session was composed of 3 trials. In all experiments, the crossing time was measured up to 300 s. Zebrafish with crossing time longer than 300 s in the first trial were not further experimented. To induce seizure, zebrafish were placed three times, its duration being 10 min followed by 30 min recovery, in aquarium water containing 10 mM pentylenetetrazol (PTZ, Sigma-Aldrich, St. Louis, MO, USA.), a GABA receptor antagonist. The data were analyzed by Friedman repeated measures analysis of variance on ranks followed by Tukey multiple comparison test to determine significant differences of crossing times in a training session. The differences between the third trial of the first training session and the first trial of the second training session were compared using Wilcoxon rank sum test.

Measurement of swimming behavior

Swimming behavior of the zebrafish was monitored using a CCD camera and EthoVision 2.1 software (Nodulus, Wageningen, the Netherlands); the fish's position was collected 30 times per second. From the stored x, y coordinates, two behavioral parameters were quantitatively calculated, such as velocity (cm/seconds) and turn angle (degrees). Velocity is the distance moved divided by the duration observed; turn angle indicates a change of movement direction. These data were presented as mean ± S.E.M. and the significance of the data was evaluated using Student's *t*-test.

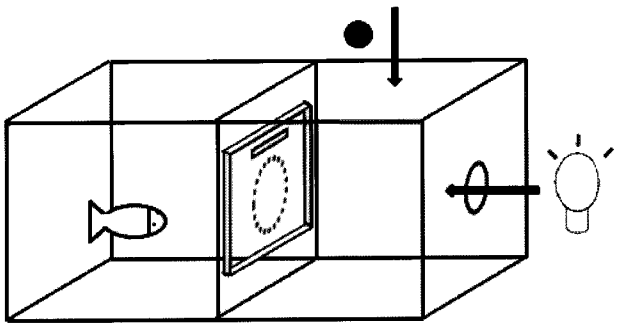

Fig. 1. Schematic drawing of an avoidance training apparatus. A black acryl shuttle box (28.5 cm long×10 cm wide×13.5 cm high) was used for all experiments. The light passed through the door from the right compartment to the left compartment. When zebrafish moved into the right compartment, a weight of small stone (●) was dropped in front of zebrafish.

In control group, zebrafish were placed in a container filled with normal aquarium water on the illuminating box. After acclimation of 5 min, the light was turned on and zebrafish were allowed to swim freely for 10 min. In PTZ-treated group, 1 mL of 1 M PTZ stock solution was added to make a 10 mM PTZ working solution.

Quantitative real-time PCR

At 0, 6, 12, and 24 h after termination of PTZ treatment, fish brains were removed and flash-frozen on dry ice. For each experiment, the brains were obtained from 8 to 10 zebrafish. Total RNA was extracted from 40 mg brain tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then further purified using RNeasy Kit (Qiagen, Hilden, Germany). Reverse transcription was performed from 3 μg of total RNA using SuperScript II (Invitrogen).

Quantitative real-time RT-PCR (qRT-PCR) was performed with ABI 7300 Real Time PCR System (Applied Biosystem, Foster City, CA, USA). The primer sequences were as follows: for β-actin: forward, 5'-ATG GAT GAG GAA ATC GCT GCC-3' and reverse, 5'-CTC CCT GAT GTC TGG GTC GTC-3', for fabp-7: forward, 5'-TTG ACA GCC AGA ACT TCG AC-3' and reverse, 5'-CAC CAC CAT CCA TCA TTG AC-3', for ngb: forward, 5'-AAG GTG ATG TTA GTG ATT GAT GCA G-3' and reverse, 5'-GGA CTC TCC AAC CAA AGC GAA-3', for hsp70: forward, 5'-GCA GGC CGC CAT CCT CAT-3' and reverse, 5'-GTA CTC CTC TTT ATC TGC CAG-3'.

PCR amplification was performed in triplicate in a 25 μL

final volume using SYBR Green I. The qRT-PCR protocol used an initial denaturation step at 94°C for 5 min followed by 28 cycles of 94°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec, and 72°C for 5 min. Relative quantification for each probe was performed by comparative C_T method based on manufacturer's instruction. The significance of the data was evaluated using Student's *t*-test.

RESULTS

Passive avoidance learning is impaired by PTZ-induced seizure

Avoidance responses were assessed by measuring the time for zebrafish to cross the sliding door in the middle of the shuttle box from the dark compartment to the lighted compartment. First, when no shock was delivered, the crossing times were 23.5±10.9 s, 40.9±13.4 s, and 24.7±9.4 s in three consecutive trials of the first training session, and 43.2±13.5 s, 42.4±14.6 s, and 43.9±11.1 s in the second training session, carried out 2 h after the first training session. No difference was shown among trials, indicating that learning did not occur without shock (Fig. 2A; Friedman's ANOVA, $P>0.05$, n=10). After 24 h recovery, the same zebrafish were tested again for avoidance response with shock delivered 3 s after crossing the door. Crossing times were 43.2±14.4 s, 86.7±29.9 s, and 149.3±38.5 s in the first training session and 116.1±36.0 s, 162.3±41.6 s, and 198.2±39.1 s in the second training session (Fig. 2B). The crossing time was increased along with trials in the first training session (Friedman's ANOVA, $P=0.012$; post hoc Tukey test, trial 1×trial 3, $P=0.009$, n=10), significantly longer in the third trial than the first trial. Also, despite a 2 h interval between the two training sessions, crossing time in the first trial of the second training session was decreased only slightly compared to that in the third trial of the first training session (Wilcoxon rank sum test, $P = 0.683$).

Next, to test whether pentylenetetrazol (PTZ), a GABA receptor antagonist, affected avoidance response in zebrafish, the effect of PTZ on the swimming pattern of zebrafish was studied. Distinct seizure-like behaviors could be classified into three stages in zebrafish treated with 10 mM PTZ for 10 min. At stage I, swimming activity was increased, and at stage II, an abrupt and rapid "whirlpool-like" circling movement was recorded. Finally, at stage III, a loss of posture with brief convulsions that were characterized by undirected vibratory movement was observed (Baraban et al., 2005) (Fig. 3A). To analyze locomotion activities, velocity (cm/s) and turn angle (degrees) were calculated for 10 min and compared in the control and PTZ-treated zebrafish using an EthoVision program (Noldus). Velocity was significantly increased in stage I and II (7.1±1.5 cm/s) of PTZ-treated zebrafish in comparison with the control

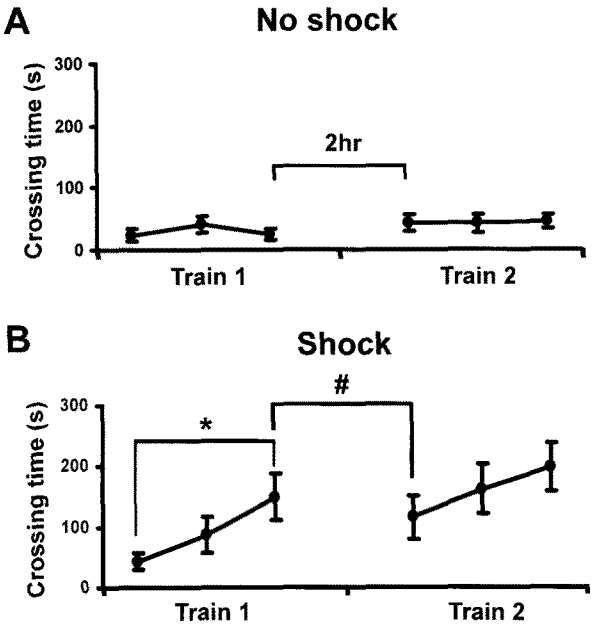

Fig. 2. Avoidance response of zebrafish. (A) No shock was delivered during experiment. Each training session consisted of three trials and 2 h of recovery was given between the first and second training sessions. (B) A shock was delivered immediately after the first and second trials in each training session. Crossing time was increased in the first training session and remained increased after 2 h interval. Each point represents the mean of crossing time±SEM for ten zebrafish. *; Difference of the first and third crossing times (Friedman's test followed by Tukey, $P<0.05$), #; difference of the third trial of the first training session and the first trial of the second training session (Wilcoxon rank sum test, $P>0.1$).

zebrafish (1.7±0.2 cm/s) (Fig. 3B, Student's *t*-test, $P< 0.05$). A characteristic increase in turn angle was shown in stage III (120.5±9.5 degrees), compared to the control (52.0±7.3 degrees) (Fig. 3C, $P<0.01$, n=4).

For the study of avoidance response, PTZ (10 mM) was treated three times of 10 min with 30 min interval in zebrafish. Following 30 min since the last PTZ treatment, the animals were tested in two training sessions of avoidance learning. Crossing times were 71.5±12.2 s, 51.3±15.3 s, and 68.1±15.7 s in the first training session and 30.2±10.9 s, 40.7±15.7 s, and 29.2±13.7 s in the second training session (Fig. 4A). No significant increase in crossing time was shown in either training session (Friedman's ANOVA, $P=0.265$ and 0.206 in the first and second training sessions, respectively, n=12). These results indicated that PTZ pre-treatment impaired acquisition of avoidance response. In the next experiments, PTZ was treated three times between the first and second training session, immediately following the first training session. Crossing times were 14.2±6.8 s, 33.4±10.2 s, and 85.7±24.0 s in the first training session and 93.9±33.0 s, 73.3±28.9 s, and 73.4±29.9 s in the second training session (Fig. 4B). The crossing time was significantly increased from the first to third trial in the first training session (Friedman's

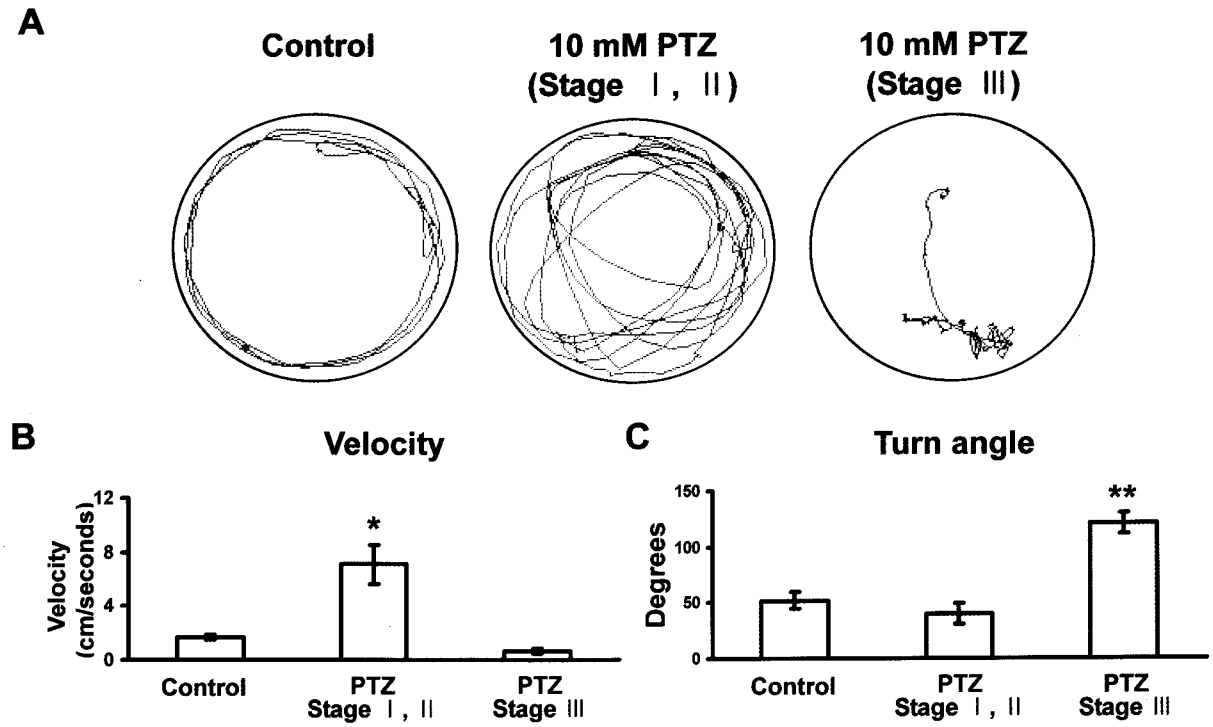

Fig. 3. Swimming patterns of PTZ-treated zebrafish. (A) Normal swimming traces were observed in the control (left). Abnormal swimming patterns were shown in 10 mM PTZ-treated zebrafish. In stages I and II, increased swimming activity was recorded, indicated by increased traces (middle). In stage III, brief convulsions were frequently shown (right). (B, C) Locomotion parameters such as velocity and turn angle were compared in control and PTZ-treated zebrafish. Velocity was increased in stages I and II, and turn angle was increased in stage III. Data are means±SEM and are subjected to Student's *t*-test. (*; $P<0.05$, **; $P<0.01$, n=4)

ANOVA, $P<0.001$; post hoc Tukey test, trial 1×trial 3, $P<0.001$, n=18). The increased crossing time was shown in the first trial of the second training, suggesting that zebrafish retained the learned avoidance response.

The effect of PTZ treatment appeared to last for three days. The normal acquisition of avoidance learning was shown in training sessions carried out three to seven days after PTZ treatment. The crossing times were 5.5±1.1 s and 105.2±33.4 s in the first and third trials at three days after PTZ treatment (Fig. 4C, Friedman test followed by Tukey, the first trial × the third trial, $P<0.05$, n=13). The crossing times also increased from 14.1±3.2 s to 72.0±16.1 s at four days after PTZ treatment, although the difference was not statistically significant ($P=0.2$, n=8). At seven days, they were 34.3±9.7 s and 119.2±28.2 s ($P<0.05$, n=13).

Hsp 70 mRNA expression increases after PTZ treatment

We next investigated whether behavioral changes observed in PTZ-treated zebrafish are accompanied by changes in gene expression. In this study, heat shock protein 70 (hsp70), neuroglobin (ngb), and fatty acid binding protein-7 (fabp-7) mRNA expression levels were measured in PTZ-treated zebrafish brains.

Ngb is an intracellular hemoprotein that provides protective effects under brain damages such as hypoxia and ischemia (Nienhaus and Nienhaus, 2007). Hypoxia-induced ngb has been reported to improve neuronal survival from brain injury (Sun et al., 2001). Fabp-7 is one of carrier proteins for fatty acid in neural tissues and has been proposed as a novel marker for detection of brain injury due to trauma or neurodegenerative diseases (Haunerland and Spener, 2004; Pelsers and Glatz, 2005). In addition, hsp70 is known to be induced following ischemia in the rats and protect the brain cells from oxidative stress and ischemia-like conditions in hsp70-overexpressed transgenic animals (Gaspary et al., 1995; Yenari et al., 1999). Thus, we hypothesized that the expression levels of ngb, fabp-7, and hsp70 mRNA might be altered in the adult zebrafish brain under seizure condition.

The expression of β-actin, a control gene, did not change after PTZ-induced seizure. We found that the level of hsp70 mRNA increased more than eight fold in PTZ-treated zebrafish brains compared to the control ($P<0.001$) (Fig. 5). Of particular interest, the increase in hsp70 mRNA appeared to be inversely correlated with the recovery time given to zebrafish after PTZ treatment. Hsp70 mRNA increased about six fold after 6 h of recovery, three fold after 12 h, and two fold after 24 h. After 48 h of recovery time, a 1.3 fold-increase in hsp70 mRNA expression was observed. The expression levels of fabp-7 and ngb mRNA were not significantly different in PTZ-treated zebrafish compared to the control.

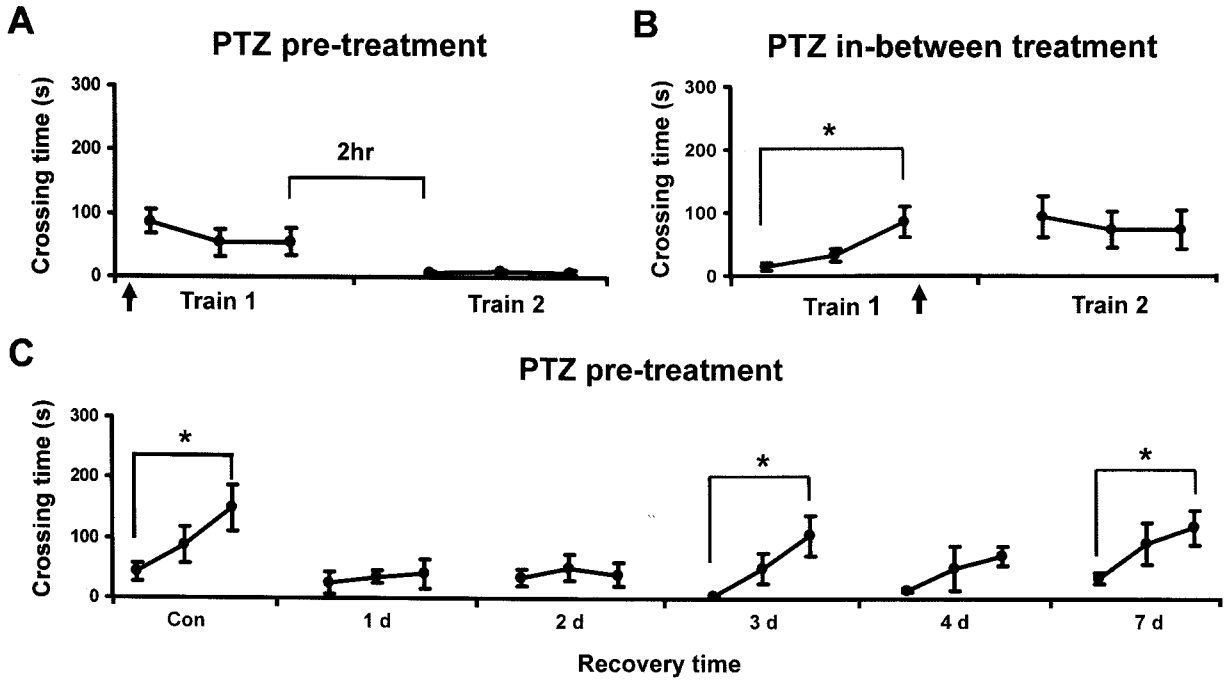

Fig. 4. Effects of PTZ-induced seizure on avoidance response of zebrafish. (A) PTZ (10 mM) was treated before the first trial of the first training session. No increase in crossing time was observed in two training sessions. (B) PTZ was treated between the first and second training sessions. Crossing time significantly increased in the first training session, but not in the second training session. (C) Increase in crossing time was shown 3-7 days after PTZ treatment. *; Difference of the first and third crossing times (Friedman's test followed by Tukey, $P<0.05$)

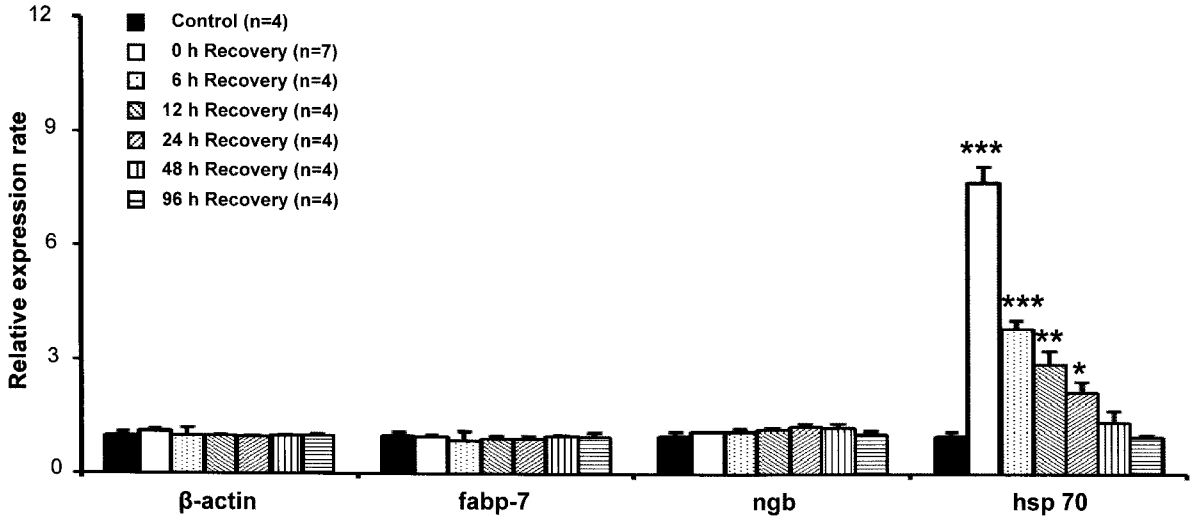

Fig. 5. Quantitative RT-PCR analysis of hsp70, fabp-7, and ngb mRNA in zebrafish brains after PTZ-induced seizure. No significant change in β-actin, fabp-7, and ngb mRNA expression was observed after PTZ-induced seizure, but expression levels of hsp70 mRNA increased in PTZ-treated zebrafish brains compared to the control. Values are means±SEM and are subjected to Student's *t*-test. (*; $P<0.05$, **; $P<0.01$, ***; $P<0.001$)

DISCUSSION

Learning and memory have been studied in several vertebrate models, such as birds, turtles and fish as well as mice and rats (Rodríguez et al., 2002). In this study, the effects of pentylentetrazol on learning ability were investigated by experimenting with the avoidance response of zebrafish. Our results demonstrated that zebrafish acquired avoidance response after being exposed to repeated warning stimuli; the fish then maintained avoidance behavior at least 2 h after terminating the training session, which was manifested by the increased crossing time with trials in the training session. Furthermore, seizure experiences caused by PTZ treatment impaired the acquisition of avoidance response.

PTZ, a common convulsant drug, induced spontaneous epileptiform activity in the hippocampal slices from the

rats. In *in vivo* studies of rats, PTZ-induced seizures impaired active avoidance learning and spatial learning in the Morris water maze test in rats (Omrani et al., 2007). Similarly, epileptiform activity was reported in the isolated telencephalon of the zebrafish when perfused with bicuculline, a $GABA_A$ receptor antagonist, -containing artificial cerebrospinal fluid (Kim et al., 2004). Telencephalon is thought to be mainly responsible for learning and memory in the teleost fish and analogous to the hippocampal and associated area of the mammalian brain (Lopez et al., 2000; Salas et al., 2006). Therefore, it is thought that the learning ability of zebrafish is altered by seizures in a manner similar to that of rats.

In parallel with the PTZ effect on avoidance response, the change of several mRNAs expression levels was studied in the zebrafish brain following PTZ treatment. Hsp70 and neuroglobin (ngb) were well known to have a neuroprotective function in neurodegenerative disorders. Our studies showed that the expression levels of hsp70 mRNA were increased in the zebrafish brain under seizure conditions. The expression of hsp70 mRNA was elevated 0, 6, 12, and 24 h after termination of PTZ treatment in 8, 6, 3, and 2 fold, respectively. Despite the elevated expression of hsp70 mRNA, the avoidance response was not learned in the range of 0 h to 48 h after PTZ treatment. Contradictory results on the role of heat shock protein on learning ability have been reported. One study suggests that the major inducible heat shock protein 72 has an anti-amnesic role in the scopolamine-injected rat (Hung et al., 2004). Hsp 72 was induced 16 h, but not 48 h, after heat treatment in brain areas including the hippocampus. When scopolamine was treated 30 min before training passive avoidance response, rats failed to learn passive avoidance response. However, in rats receiving heat treatment 16 h before training, no scopolamine-induced learning deficit was detected. Interestingly, the additional pretreatment of scopolamine at the time of heat treatment blocked the effect of the heat treatment on avoidance learning deficit. That is, heat shock protein seems to play a protective role in the brain when it is present before insults. In rats overexpressing hsp70, learning ability was impaired in active two-way avoidance learning, suggesting that the prolonged presence of hsp 70 is harmful to learning behavior (Ammon-Treiber et al., 2008). Since, in our study, learning ability was most severely impaired at the time of highest expression of hsp70 mRNAs and gradually recovered with reduced expression, hsp 70 is unlikely to be involved in ameliorating a learning deficit by seizure.

The expression levels of ngb were not significantly changed in PTZ-treated zebrafish brains, although ngb plays a protective role in the brain under hypoxia and oxidative stress, suggesting that hsp70 and ngb are differentially regulated in the adult zebrafish brain in the condition of PTZ treatment.

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REFERENCES

Ammon-Treiber S, Grecksch G, Angelidis C, Vezyraki P, Höllt V, and Becker A (2007) Pentylenetetrazol-kindling in mice overexpressing heat shock protein 70. *Naunyn Schmiedebergs Arch Pharmacol* 375: 115-121.

Baraban SC, Taylor MR, Castro PA, and Baier H (2005) Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. *Neuroscience* 131: 759-768.

Berghmans, S, Hunt, J, Roach A, and Goldsmith P (2007) Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. *Epilepsy Res* 75: 18-28.

Dubé CM, Zhou JL, Hamamura M, Zhao Q, Ring A, Abrahams J, McIntyre K, Nalcioglu O, Shatskih T, Baram TZ, and Holmes GL (2009) Cognitive dysfunction after experimental febrile seizures. *Exp Neurol* 215: 167-177.

Gaspary H, Graham SH, Sagar SM, and Sharp FR (1995) HSP70 heat shock protein induction following global ischemia in the rat. *Brain Res Mol Brain Res* 34: 327-332.

Gröticke I, Hoffmann K, and Löscher W (2008) Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainate. *Exp Neurol* 213: 71-83.

Haunerland NH and Spener F (2004) Fatty acid-binding proteins--insights from genetic manipulations. *Prog Lipid Res* 43: 328-349.

Hung CH, Lin MT, Liao JF, and Wang JJ (2004) Scopolamine-induced amnesia can be prevented by heat shock pretreatment in rats. *Neurosci Lett* 364: 63-66.

Kim YJ, Nam RH, Yoo YM, and Lee CJ (2004) Identification and functional evidence of GABAergic neurons in parts of the brain of adult zebrafish (*Danio rerio*). *Neurosci Lett* 355: 29-32.

Krueger AM, Armstrong JN, Plumier J, Robertson HA, and Currie RW (1999) Cell specific expression of Hsp70 in neurons and glia of the rat hippocampus after hyperthermia and kainic acid-induced seizure activity. *Brain Res Mol Brain Res* 71: 265-278.

Lopez JC, Broglio C, Rodriguez F, Thinus-Blanc C, and Salas C (2000) Reversal learning deficit in a spatial task but not in a cued one after telencephalic ablation in goldfish. *Behav Brain Res* 109: 91-98.

Nienhaus K and Nienhaus GU (2007) Searching for neuroglobin's role in the brain. *IUBMB Life* 59: 490-497.

Mortazavi F, Ericson M, Story D, Hulce VD, and Dunbar GL (2005) Spatial learning deficits and emotional impairments in pentylenetetrazole-kindled rats. *Epilepsy Behav* 7: 629-638.

Omrani A, Ghadami MR, Fathi N, Tahmasian M, Fathollahi Y, and Touhidi A (2007) Naloxone improves impairment of spatial performance induced by pentylenetetrazol kindling in

rats. *Neuroscience* 145: 824-831.

Pelsers MM and Glatz JF (2005) Detection of brain injury by fatty acid-binding proteins. *Clin Chem Lab Med* 43: 802-809.

Pradel G, Schachner M, and Schmidt R (1999) Inhibition of memory consolidation by antibodies against cell adhesion molecules after active avoidance conditioning in zebrafish. *J Neurobiol* 39: 197-206.

Pradel G, Schmidt R, and Schachner M (2000) Involvement of L1.1 in memory consolidation after active avoidance conditioning in zebrafish. *J Neurobiol* 43: 389-403.

Rodríguez F, López JC, Vargas JP, Broglio C, Gómez Y, and Salas C (2002) Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. *Brain Res Bull* 57: 499-503.

Salas C, Broglio C, Durán E, Gómez A, Ocaña FM, Jiménez-Moya F, and Rodríguez F (2006) Neuropsychology of learning and memory in teleost fish. *Zebrafish* 3: 157-171.

Sun Y, Jin K, Mao XO, Zhu Y, and Greenberg DA (2001) Neuroglobin is up-regulated by and protects neurons from hypoxic-ischemic injury. *Proc Natl Acad Sci (USA)* 98: 15306-15311.

Swain HA, Sigstad C, and Scalzo FM (2004) Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). *Neurotoxicol Teratol* 26: 725-729.

Szyndler J, Piechal A, Blecharz-Klin K, Skórzewska A, Maciejak P, Walkowiak J, Turzyńska D, Bidziński A, Płaźnik A, and Widy-Tyszkiewicz E (2006) Effect of kindled seizures on rat behavior in water Morris maze test and amino acid concentrations in brain structures. *Pharmacol Rep* 58: 75-82.

Tiedeken JA and Ramsdell JS (2007) Embryonic exposure to domoic acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. *Environ Health Perspect* 115: 1547-1552.

Winter MJ, Redfern WS, Hayfield AJ, Owen SF, Valentin JP, and Hutchinson TH (2008) Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. *J Pharmacol Toxicol Methods* 57: 176-187.

Xu X, Scott-Scheiern T, Kempker L, and Simons K (2007) Active avoidance conditioning in zebrafish (*Danio rerio*). *Neurobiol Learn Mem* 87: 72-77.

Yenari MA, Giffard RG, Sapolsky RM, and Steinberg GK (1999) The neuroprotective potential of heat shock protein 70 (HSP70). *Mol Med Today* 5: 525-531.

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