Effect of R-Phenylpropyladenosine and Methylxanthines on Memory and Learning Performance in Goldfish

Hossein Hosseinzadeh*[1] and Mashallah Mehrabi[2]

1- Correspondence author: Department of Pharmacodynamy and Toxicology, Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, P.O. Box: 1365-91775, Mashhad, I. R. Iran, Fax: 98 5118823251, E-mail: hosseinzadehh@mums.ac.ir

2-Department of Pharmacodynamy and Toxicology, Faculty of Pharmacy, Mashhad University of Medical Sciences, POBox 91775-1365, Mashhad. I.R.Iran.

Summary

The aim of this study was to investigate the effects of adenosine receptor antagonists (caffeine theophylline) and agonists (R-Phenylisopropyladenosine=R-PIA) on learning and memory performance in goldfish (*Carassius auratus*). Twelve groups of fish were trained for seven days to find food in one out of two compartments (T-maze) until discrimination was achieved. On the last training day, they were injected intraperitoneally 30 min before the training session. Twenty-four hours later, the time spent to find the food was recorded. Reversal training was done for four consecutive days after this post-injection test and time spent to find the food was recorded again. In a first set, caffeine (20 and 40 mg/kg) or physostigmine (5 mg/kg) or vehicle were injected intraperitoneally. In a second set, theophylline (20 and 40 mg/kg) or physostigmine (5 mg/kg) or vehicle was injected intraperitoneally. In a third set, R-PIA (1 and 2 mg/kg) or physostigmine (5 mg/kg) or vehicle were injected intraperitoneally. The results indicated that the groups with caffeine, theophylline and physostigmine needed less time to find the food than control fish and the group with R-PIA needed more time to find the food than the reference control fish. It is suggested that the non-selective adenosine receptor antagonists. (caffeine and theophylline) can enhance memory and learning ability in goldfish but the adenosine receptor A1 agonist (R-PIA) reduced these effects.

Key words: adenosine, caffeine, theophylline, R-Phenylisopropyladenosine, R-PIA, learning, memory, goldfish, *Carassius auratus*

Introduction

Methylxanthines such as caffeine and theophylline have diverse pharmacological activities such as blocking adenosine receptors [1-4], inhibit phosphodiesterases [5] and other enzymes including 5´-nucleotidase and alkaline phosphatase [6]. They also cause the release of calcium from intracellular stores [7-8]. There are some reports that methylxanthines chaneged the inhibitory effect of antimicrobial agents [9-10].

Theophylline stimulates the central nervous system and has positive inotropic and chronotropic effects on the heart as well as diuretic, smooth muscle relaxant and contractive effects on striated muscle [11]. Caffeine is also a psychostimulant with three main mechanisms of action of on the central nervous system i.e. mobilization of intracellular calcium and inhibition of specific phosphodiesterases and blocking of adenosine receptors [12]. It was shown that methylxanthines have anti-inflammatory effects in mice [13]

There are a variety of studies that methylxanthines can improve cognitive performance. Caffeine consumed in adulthood may prevent recognition memory decline with aging [14]. Some of the selective adenosine A_1 antagonists exhibited anti-amnesic activities at several doses [15]. In contrary, the selective activation of a presumably central population of A1 receptors with N6-cyclopentyladenosine (CPA), may impair retention performance and influence information processing [16]. In another study A(2A) but not A(1) adenosine receptors are involved in memory retention and consolidation [17]. However, in contrast to the mentioned research some studies failed to show these effects. Theophylline induced memory/learning disabilities and hyperactivity in infants and young children [18]. It was shown that a psychoactive dose of caffeine does not impair or enhance memory in human [19].

Caffeine improves both motivation and cognitive performance in complex learning tasks (olfactory and visual learning) in the honey bee (*Apis mellifera*) [20]. As honeybee model may be useful in explaining caffeine-related behavioral changes, it is possible to use fish for showing cognition effects as well. Thus, in this study the effects of adenosine receptor antagonists (caffeine theophylline) and A1 agonists (R-Phenylisopropyladenosine=R-PIA) were evaluated on learning and memory performance in goldfish.

Materials and Methods

Animal

The experiments were done in goldfish, with 4-6 cm body length and a body weight of 1.5-2.5 g. The fish were purchased from a single source. They were maintained in a 40 liter aquarium, 10-15 fish per aquarium, under light conditions in continuously filtered and aerated water between 23-26 ºC. They were fed with fish flakes and, during the experiment, with dried compact cube food.

Appartus

A rectangular, three-compartment aquarium (50 × 10 ×16 for total length, width and height, respectively) was used as maze. A starting chamber (8 × 10 ×16) with a sliding door that was connected to the median of the aquarium. Two compartments at either end of the aquarium were, one white and the other black.

Procedure

The fish were kept in a 40 liter tank for at least one month prior to the experiment. After this period, the fish were food-deprived for 48 h, and put in the start chamber of the experiment aquarium for 30 Sec. A dried cube food was placed on the back corner of the black compartment, and the sliding door was removed and the time spent by each fish to find the food was recorded. A time limit of 10 min was allowed for each fish. This procedure (training) was repeated for 7 days. After each training session, animals received fish flaked for 1 min and then they were returned to the maintenance aquarium.

On the last training day, the fish were injected with agents or normal saline and put in the test aquarium 30 min after treatment. After the fish found the food and ate, they were removed and returned to the maintenance aquarium. On the next day (test) the procedure was repeated without drug treatment. For the following four days, the procedure was repeated with the compact cube food attached to the back corner of the white compartment (reversal training) [21].

The maximum non-fatal dose and acute toxicity

Different doses of the extracts were injected to the separated groups of four. After 48 h, the highest dose that did not induce any mortality was considered as the maximum non-fatal dose. LD50 values and the corresponding confidence limits (CL) were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4).

Statistical analysis

The data were expressed as mean values ± S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer.

Results

The maximum non-fatal doses of physostigmine, theophylline and caffeine were 7.5 g/kg, 80 mg/kg and 60 g/kg, respectively.

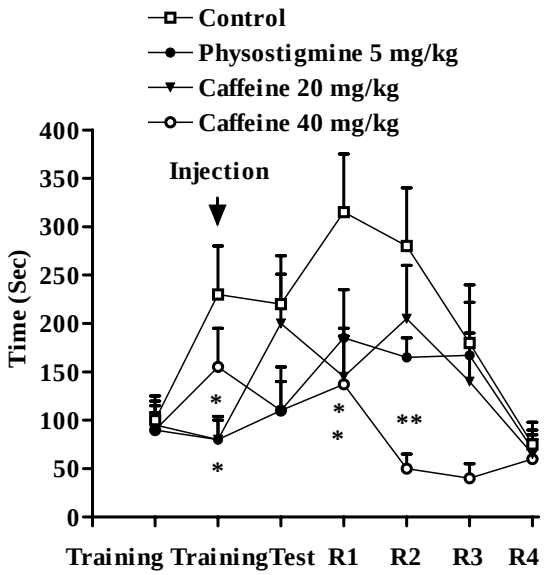

Figure 1. Effect of caffeine on learning task in goldfish. Data was reported as mean + SEM for the last two training days and for the test and reversal (R) days. *P<0.05, **P<0.01 *vs* control, n=18, Tukey-Kramer test.

LD50 values of physostigmine, theophylline and caffeine were 27.48 mg/kg (CL: 12.01, 62.84), 146.94 mg/kg (CL: 103.04, 209.05) and 112.61 mg/kg (CL: 82.29, 154.10), respectively. The LD50 value of R-PIA was not evaluated.

Physostigmine, 5 mg/kg, decreased time to find the food in training or reversal day (Figure 1, 2 and 3). Caffeine, 20 mg/kg, decreased time to find the food in the second day of training and the first reversal day. At the 40 mg/kg, it reduced this time in the first and the second reversal day

(Figure 1). Theophylline, 20 and 40 mg/kg, also decreased time to find the food in the test day and the third reversal day (Figure 2).
R-PIA, 1 and 2 mg/kg, increased the time to find the food in training day and reversal day (Figure 3).

Discussion

The results indicate that adenosine receptor antagonists, theophylline and caffeine, improve learning capacity while A1 receptor agonist, R-PIA, impairs.

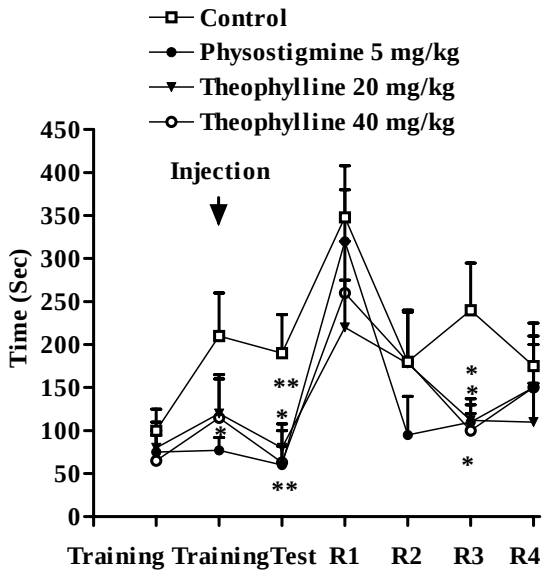

Figure 2. Effect of theophylline on learning task in goldfish. Data was reported as mean + SEM for the last two training days and for the test and reversal (R) days. *P<0.05, **P<0.01 *vs* control, n=18, Tukey-Kramer test.

The present data are consistent with those studies that methylxanthines facilitate memory and learning performance [15, 17]. It was shown that the mechanism of caffeine improving the working memory in rats is related to blocking the adenosine A1 receptors of nerve centre and increasing the release of acetycholine in the nerve center [22]. In this study A1 selective agonist (R-PIA) showed inhibitory effect on appetitively motivated learning task. This implies that adenosine A1 receptor has a role on cognition behaviors. This study also showed that goldfish like honeybee model [20] may be useful in explaining learning-related behavioral changes.

The results demonstrated that theophylline and caffeine treated fish needed less time to find food in the reversal learning test. This effect was also seen with H1 receptor blocker chlorpheniramine [23] that means fish when were existing with a new task, they could learn the new food location faster than those that did not receive the drug.

Present data demonstrate that methylxanthines facilitate learning performance via at least A1 adenosine receptor blockade.

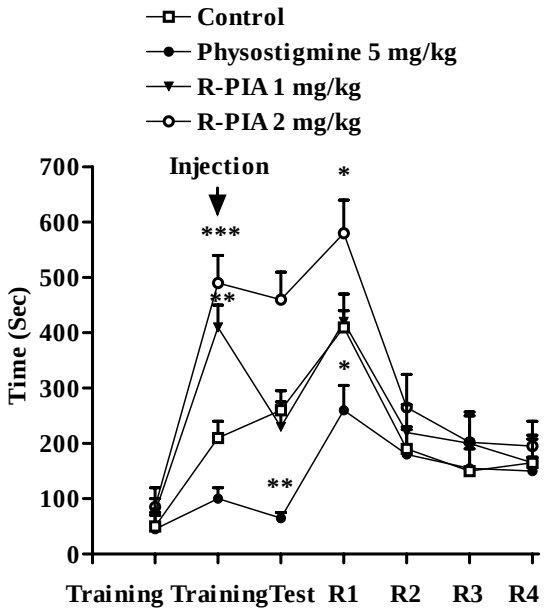

Figure 3. Effect of R-Phenylisopropyladenosine (R-PIA) on learning task in goldfish. Data was reported as mean + SEM for the last two training days and for the test and reversal (R) days. *P<0.05, **P<0.01 ***P<0.001 *vs* control, n=18, Tukey-Kramer test.

References

1. Daly JW, Bruns RF, Snyder SH. Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci 1981; 28: 2083-2097.
2. Fredholm BB, Persson CGA. Xanthine dervatives as adenosine receptor antagonists. Eur J Pharmacol 1982; 81: 673-76.
3. Fredholm BB, Chen J-F, Masino SA, Vaugeois J-M. Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs.Ann Rev Pharmacol Toxicol 2005; 45:385-412.
4. Hosseinzadeh H, Stone TW. (1996). Adenosine in the central nervous system. Med J Isl Rep Iran 9: 361-368.
5. Butcher RW, Therland, EW. Adenosine 3 ',5 '-phosphate in biological materials. J Biol Chem 1962; 237: 1244-1250.
6. Fredholm BB, Hedqvist P, Vernet L. Effect of theophylline and other drugs on rabbit renal nucleotide phosphodiesterase, 5 '-nucleotidase and adenosine deaminase. Biochem Pharmacol 1978; 27: 2845-2850.
7. Lee HC. Potentiation of calcium and caffeine-induced calcium release by cyclic ADP-ribose. J Biol Chem 1993; 268: 647-56.
8. Tanaka Y, Tashjian Jr AH. Functional identification and quantitation of three intracellular calcium pools in OH4C1 cells: evidence that the caffeine-responsive pool is coupled to a thapsigargin-

resistant, ATP-dependent process. Biochemistry 1993; 32: 12062-12073.
9. Hosseinzadeh H, Fazly Bazzaz BS, Sadati MM. In vitro evaluation of methylxanthines and some antibiotics: interaction against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Iran Biomed J.2006; 10: 163-167.
10. Hosseinzadeh H, Fazly Bazzaz BS, Ahmadzadeh S. In vitro evaluation of methylxanthines and some antifungal drugs: interaction against Candida albicans . Pharmac Pharmacol Lett 2001; 11: 34-36.
11. David S. Cooling Theophylline toxicity J Emerg Med 1993; 11: 415-425.
12. Nehlig A, Daval J-L, Debry G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Rev 1992; 17:139-169.
13.Hosseinzadeh H, Dehghan R. Anti-inflammatory effects of methylxanthines in some models of acute and chronic inflammation in mice and rats. Pharmac Pharmacol Lett 1999; 9: 18-19.
14. Costa MS, Botton PH, Mioranzza S, Souza DO, Porciúncula LO. Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tirosine kinase receptor (TrkB) content in mice. Neuroscience 2008; 153: 1071-1078.
15. Suzuki F, Shimada J, Shiozaki S, Ichikawa S, Ishii A, Nakamura J, Nonaka H, Kobayashi H, Fuse E. Adenosine A1 antagonists. 3. Structure-activity relationships on amelioration against scopolamine- or N6-((R)-phenylisopropyl)adenosine-induced cognitive disturbance. J Med Chem 1993; 36: 2508-2518
16. Normile HJ, Barraco RA. N6-cyclopentyladenosine impairs passive avoidance retention by selective action at A1 receptors. Brain Res Bull 1991; 27:101-104.
17. Kopf SR, Melani A, Pedata F, Pepeu G. Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. Psychopharmacology (Berl). 1999; 146: 214-219.
18. Hirose M, Yokoyama H, Iinuma K.Theophylline impairs memory/learning in developing mice Brain Develop 2004; 26: 448-452.
19. Herz RS. Caffeine effects on mood and memory. Behav Res Ther 1999; 37: 869-879.
20. Si A, Zhang S-W, Maleszka R. Effects of caffeine on olfactory and visual learning in the honey bee (Apis mellifera). Pharmacol Biochem Behav 2005; 82: 664-672.
21. Mattioli R, Santangelo EM, Costa AC, Vasconcelos L. Substance P facilitates memory in goldfish in an appetitively motivated learning task. Behav Brain Res 1997; 85: 117-120.
22. Shao B-X. Effect and mechanism of caffeine on improving the working memory in rats. Chin Pharm J 2006; 41: 512-514.
23. Spieler RE, Nelson CA, Huston JP, Mattioli R. Post-trial administration of H1 histamine receptor blocker improves appetitive reversal learning and memory in goldfish, Carassius auratus. Neurosci Lett 1999; 277: 5-8.