

## 20. EFFECT OF PYRIDOSTIGMINE (PYR) AND NON PENETRATIVE TRAUMATIC BRAIN INJURY ON NEUROMUSCULAR PERFORMANCE IN THE RAT

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### INTRODUCTION

Pyridostigmine, a reversible carbamate acetylcholinesterase, has been recommended as a pretreatment in warfare nerve agent intoxication. It is used by the military to obtain 20-30% whole blood acetylcholinesterase (AChE) inhibition to enhance the effectiveness of the standard therapeutic regimen jase (ChE) inhibitions, when administered in symptom producing doses, may cause rhabdomyonecrosis [3]. Morphological alterations of the neuromuscular junction have been demonstrated in diaphragm, soleus and extensor digitorum longus muscles of rats given PYR either acutely or subacutely [4-6]. There have been relatively few studies documenting the functional effects of PYR on the neuromuscular junction. Whether PYR exert an adverse on neuromuscular activities with closed head injury has not been documented.

Closed head injury is associated with high mortality and morbidity. It has been reported that survivors of closed head injury may develop cognitive dysfunctions and retarded neuromuscular performance. Whether it may exert an adverse effect on neuromuscular activities in the survivors with closed head injury has not been studied.

The present study was designed to investigate the effect of PYR on neuromuscular performance in rats subjected to closed head injury [7].

### MATERIALS AND METHODS

Male Sprague rats (300+5g) were pretreated with normal saline or PYR (7 mg/kg body weight, intraperitoneally), daily for 3 days. They were subjected to (a) rotametric strength test (b) forelimb grip test, 3 h after the last injection. They were anesthetized with a Clinical Research Center cocktail (0.3 ml per 100 g body weight) and were subjected to a closed head injury of weight 300 g height 1.5m. They were subjected to the rotametric test and grip strength tests after 16 or 40 h.

*Rotametric test* - A rotametric device (Columbus Instruments Rotamex 4/8 system, Ohio, USA) was used to examine the ability of the animal to coordinate itself while being placed on a rotating rod with the rotational speed of 6 rpm (start speed) and 20 rpm (end speed) for a period of 120 seconds. An internal micro-controller was used to detect the time when a subject fell from the rod. The average reading (in seconds) for two successive trials was taken from each animal.

*Grip-strength test* - The fore limb grip-strength was measured using a grip-strength meter (Columbus Instruments, Ohio, USA). The animal was placed on the electronic digital force gauge that measures the peak force exerted on it by the action of the animal. While drawing along a straight line leading away from the sensor, the animal was released at some point and the maximum force attained was stored on the display. The highest reading (in Newton) of three successive trials was taken from each animal.

*Induction of head injury* - The scalp of the anesthetized animal was shaved, and a helmet was cemented to the calvaria with a thin layer of dental acrylic. The animal was then placed in a prone position on a foam bed, with the lower end of the Plexiglas tube positioned directly above the helmet. The injury was delivered by dropping 300 g of weight from 1.5 m.

The weight-drop device consists of a column of brass weights falling freely by gravity onto a metallic helmet fixed to the skull vertex of the rat by dental acrylic. The brass weights, each about 50 g, are threaded so that they can be connected to produce a falling weight ranging from 50 to 500 g. From a designated height, the weight falls through the vertical section of a transparent Plexiglas tube held in place with a ring stand. The helmet consists of a stainless steel disc, 10 mm in diameter and 3 mm thick. The contact side of the disc is grooved concentrically to accept acrylic and firm the contact. After release of the weight, the Plexiglas frame is removed rapidly to prevent a second impact.

### RESULTS AND DISCUSSION

*Rotametric score* - Saline-pretreated rats were associated with a rotametric score of 114±4 sec. With sham operation, the score was 117±3 and >120 seconds at 16 and 40 h, respectively. When subjected to closed head injury, the score was 98±13 and > 120 sec at 16 and 40 h, respectively. Pyridostigmine pre-treatment, however, reduced the rotametric score to 94±8. With sham-operation, the rotametric score was 83±2 and 87±2 at 16 and 40 h, respectively. With closed head injury, the rotametric score was 105±12 and 119±1 at 16 and 40 h, respectively.

*Fore limb grip-strength score* - Saline pre-treated rats obtained a grip-strength score of  $8.3 \pm 0.3$ . With sham operation, the grip-strength score was  $8.2 \pm 0.7$  and  $7.7 \pm 0.9$  at 16 and 40 h, respectively. In contrast, closed head injury reduced the grip-strength score to  $6.9 \pm 0.5$  and  $7.3 \pm 1.0$  at 16 and 40 h, respectively. Pyridostigmine administration was associated with a grip-strength score of  $6.3 \pm 0.3$  ( $p < 0.05$  vs. saline-treated group). With sham-operation, the grip-strength score was  $6.2 \pm 1.1$  and  $7.3 \pm 0.9$  at 16 and 40 h, respectively. With closed head injury, the score was  $5.8 \pm 0.6$  and  $6.8 \pm 1.9$  at 16 and 40 h, respectively.

The results showed that PYR administration caused a significant decrease in rotametric and forelimb grip-strength scores in rats. However, no statistically significant decrement in performances could be evidence after 16 and 40 h. These observations suggested that PYR may have a short term deleterious effect on the neuromuscular performance in rats. Closed head injury caused a numerical decrease in the rotametric and grip-strength scores, but statistically significant decrement was not observed. The results showed that there was no synergistic effect between PYR administration and closed head injury on neuromuscular performance in rats. The significance of these observations remains to be clarified and the present findings need to be substantiated in humans.

## **SUMMARY**

The effect of pyridostigmine (PYR) and closed head injury on neuromuscular performance in the rat is described. The rats were pretreated with either saline or PYR (7 mg/kg body weight, IP) daily for 3 days before being subjected to rotametric and grip-strength tests. They were then subjected to closed head injury and underwent the tests. PYR administration caused a significant short-term decrement in rotametric and grip-strength performances suggesting that PYR may exert a short-term deleterious effect on the neuromuscular performance in the rat. Closed head injury caused a numerical decrease in the rotametric and grip-strength scores but the effect was not statistically significant. There was no synergistic effect between PYR and closed head injury on the neuromuscular performance in the rat.

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## **KEYWORDS**

Rotametry, grip-strength, acetylcholinesterase inhibition

## FIGURES

Figure 1. Effect of pyridostigmine and closed head injury on rotametric performance in the rat.

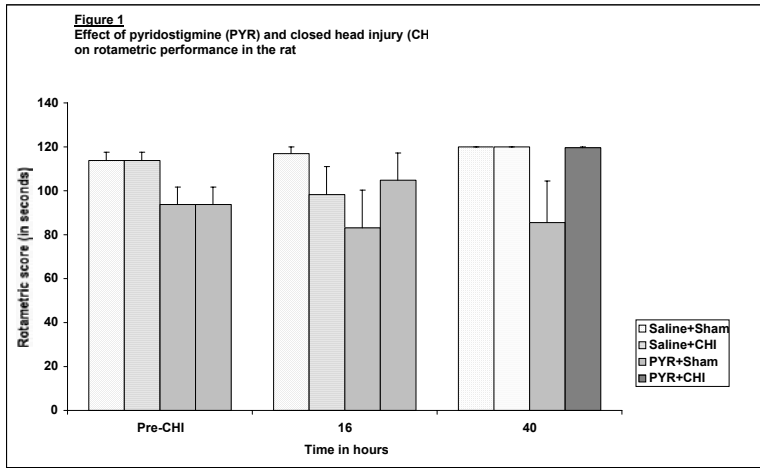


Figure 2. Effect of pyridostigmine and closed head injury on grip-strength performance in the rat.

