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Effects of 4-Aminopyridine on Chlorpromazine-induced Decrement of Muscle performance in Mice

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The interactions of 4-aminopyridine (4-AP) with chlorpromazine (CPZ) on rotarod performance, open field performance, and integral electromyography (IEMG) in mice were investigated. The rotarod performance of mice were inhibited by CPZ in a dose-dependent manner. CPZ also decreased the spontaneous motor activity (SMA) of the mice in the open field and attenuated IEMG, but not righting reflex. Catatonia was observed in higher concentration of CPZ (2.01 mg/kg) treatment mice. All the inhibition of CPZ (except SMA) were antagonized partly by 4-AP in this study. The site of action of 4-AP maybe include both central and peripheral synapses.

Keywords: 4-aminopyridine, chlorpromazine, rotarod, open field, IEMG

Our interest in 4-aminopyridine (4-AP) derives from the observation that the drug is a potent antagonist of the neuromuscular block caused by d-tubocurarine (1), botulinum toxin (2-3) and some antibitics (4). In addition, 4-AP increases directly evoked maximal twitches of isolated rat diaphragm (5), the cat tibialis anterior muscle (6), and chick biventer cervicis muscle (1). It also antagonizes the skeletal muscle relaxant effect of dantrolene sodium in the rat (5). The mechanism of action of 4-AP has been suggested that the blockade of the potassium channels could prolong the duration of the action potential in nerve terminals and thus increase the amount of acetylcholine release on evoked nerve (7-8). The present study has been carried out on rotarod performance, open field performance, and integral electromyography (IEMG). The aim of the present study is to investigate the action of 4-AP on muscle weakness induced by chlorpromazine (CPZ).

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MATERIALS AND METHODS

Adult, male mice (ICR strain) were obtained from the animal center of the National Taiwan University. A solid diet and tap water were provided ad libitum. These animals were used for experiments at the body weight of 25-35 g. Drugs used included chlorpromazine HCl (CPZ) (Sigma), and 4-aminopyridine (4-AP) (Sigma). Immediately before the administration the drugs were diluted with normal saline to 1.34, 1.68, 2.01 mg/kg of CPZ, and 0.8, 1.2, 2.4, 3.6 mg/kg of 4-AP. Both CPZ and 4-AP were given S.C.. Control animals were given the corresponding volumes of normal saline. Animals to be tested were fasted for 16 h prior to the test. The investigations of rotarod performance, open field performance, cataonia and IEMG were according to kuribara et al. (9) and Dyck (10), separately, and made some modification as below description.

Rotarod performance: A rotarod treadmill for mice (Ugo basile, Italy) was used. A plastic rod, 3 cm in diameter, 30 cm long with nonslippery surface and 15 cm over the base was used. This rod is divided into 5 equal sections by 6 discs, thus enabling 5 mice to walk on the rod at the same time. In the study, the speed of 28 r.p.m. only was used. After the administration of the drug, the performance time was measured at 0, 5, 15, 30, 35, 45, 60, 90, 120 min. The time each animal remained on the rod was recorded in seconds. Up to 360 seconds, after which time all remaining animals were removed. For the assay of each one dose, 6-9 mice were used. The training of mice by rotarod test was given the day before test, animals who performed on the rod for more than 360 sec were used for assaying the drug effects. The motor performance (% activity) was presented by the ratio of performance time (sec) in the rotarod/360 sec.

Open field performance: Tests for the effect of the drugs on spontaneous motor activity (SMA) were conducted by placing the animals in the open field, and recording their SMA of 3 min before any drug treatment, during 30-33 min after CPZ (2.01 mg/kg) treatment, and 5-8 min after 4-AP (3.6 mg/kg) administration. 4-AP was given at 35 min after CPZ administration. The righting reflex and explorating behavior also were observed.

Catatonia: A stainless steel bar, 15 mm in diameter was hung 30 cm over the base. The animals hung from the horizontal part by forelimbs, and after conforming that the gasp was good, they was left hanging, Catatonia of CPZ (2.01 mg/kg) treatment mice were observed, and the antagonism effect of 4-AP (1.2 mg/kg) against catatonia were tested. Integral electromyograph (IEMG): Tests for the effect of the drugs on IEMG were conducted by fixing the animals in the board, then inserted two needle electrodes in the rectus femoris muscle of left hindlimb, the distance of two record electrodes was 0.5 cm. The reference electrode was inserted into the triceps muscle of left forelimb. Directstimulation (30 Voltes) to the right hindlimb was applied by pressing manual switch. The effects of the drugs on EMG were recorded with a bioelectrical amplifier (San-Ei, japan) on computer (Data-Q), The amount of IEMG voltage of ten point data moving

average were calculated with MathCAD.

Results are expressed as means ± standard error of the means. Mean values were compared using one way analysis of variance (ANOVA) and Student's t test for statistical analysis. Values of < 0.05 were regarded as significant.

RESULTS

Chlorpromazine exhibited rotarod performance depression with a dose-dependent manner (Fig. 1), and the maximum effect was produced at about 30 min after CPZ administration. But normal saline and any concentration of 4-AP in this study didn't significantly depress rotarod performance during observing period. 4-AP (1.2, 2.4, 3.6 mg/kg) could partly recover rotarod performance that was depressed by CPZ (Fig. 2 and Fig. 3).

Mice at 30 min after CPZ 2.01 (mg/kg) administration hanged from a thin bar by means of their forelimbs, the animals exhibited' rigidility, and complete immobility with loss of responsiveness until fell from the bar within 5 sec. This catatonia state was relieved by 4-AP (1.2, 2.4, 3.6 mg/kg), and the animals could cling to the bar more than 30 sec within 5 min after 4-AP administration.

Table 1 shows CPZ (2.01 mg/kg) could extremely depress the SMA and exploring behavior in the open field, but not righting reflex. CPZ only decreased some degrees of IEMG (Table 1 and Fig. 4), it appears CPZ didn't induce extremely skeletal muscle relaxation. 4-AP didn't antagonize the depression of CPZ on SMA and exploring behavior, but could potentiate IEMG (Table 1 and Fig. 4).

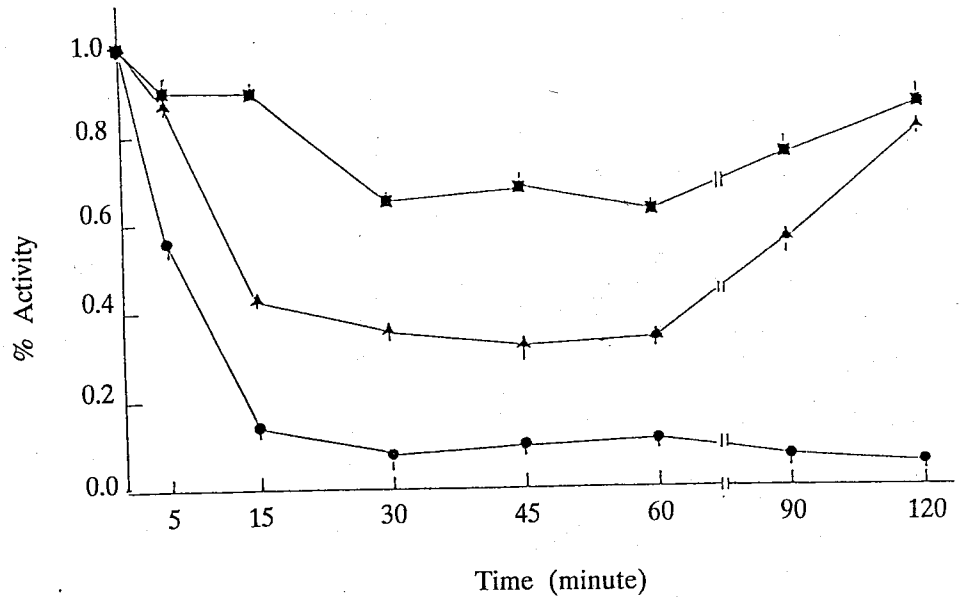

Fig 1. Temporal change of motor performance after CPZ administration in the rotarod through 120 min period. Motor performance (% activity) was presented by the ratio of performance time (sec) in the rotarod / 360 sec.
■ - ■, CPZ 1.34 mg/kg; ▲ - ▲, CPZ 1.68 mg/kg; ● - ●, CPZ 2.01 mg/kg.

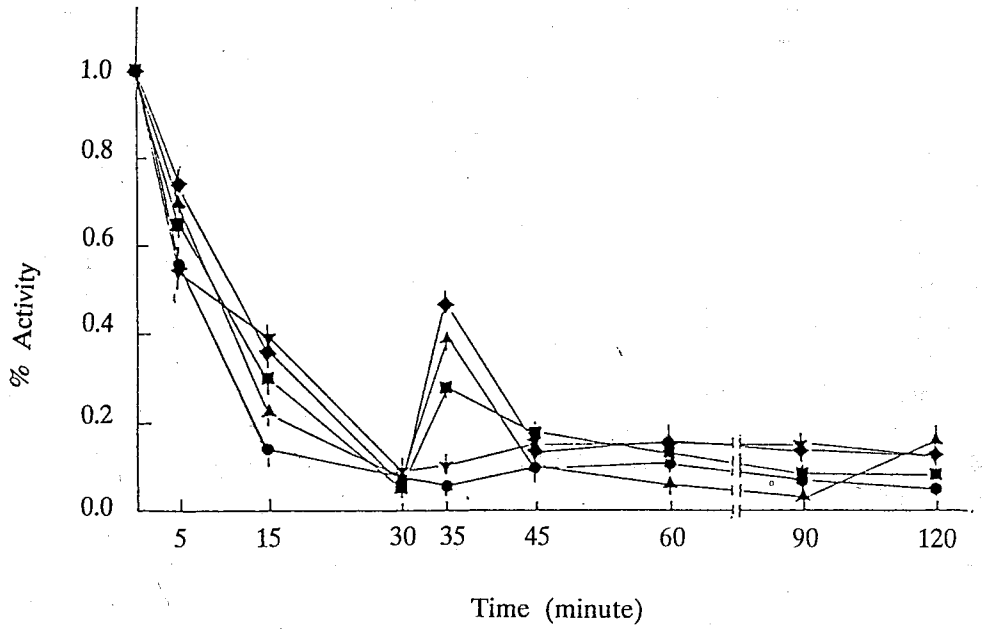

Fig 2. Effects of 4-AP on CPZ-induced rotarod decrement of muscle performance in mice. 4-AP was given at 30 min after CPZ (2.01 mg/kg) administration. Motor performance (% activity) was presented by the ratio of performance time (sec) in the rotarod / 360 sec.
● - ●, CPZ saline; ▼ - ▼, CPZ + 4-AP (0.8 mg/kg); ◆ - ◆, CPZ + 4-AP (1.2 mg/kg); ▲ - ▲, CPZ + 4-AP (2.4 mg/kg); ■ - ■, CPZ + 4-AP (3.6 mg/kg).

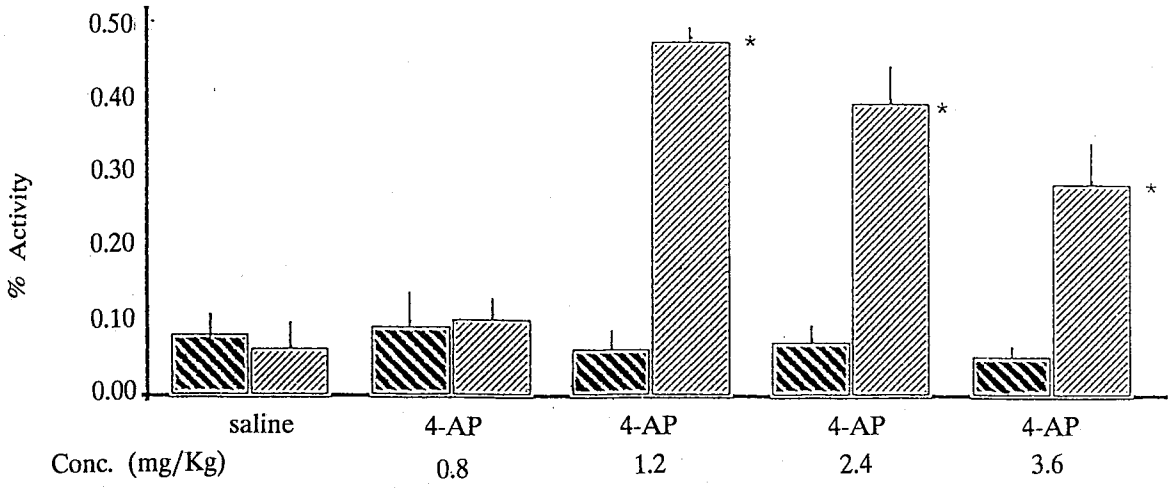

Fig 3. A comparison of variety concentration of 4-AP on CPZ (2.01 mg/kg) pretreament mice at 30 min and 35 min after CPZ admininstration. Motor performance (% activity) was presented by the ratio of performance time (sec) in the rotarod / 360 sec.
▨, CPZ alone at 30 min after CPZ administration
▨, CPZ + 4-AP or saline at 35 min after CPZ administration
* $P < 0.005$.

Table 1. Effects of chlorpromazine (CPZ) and 4-aminopyridine (4-AP) in mice

Treatment	Drug free	CPZ[2] alone	CPZ + saline	CPZ + 4-AP[2]
SMA (divisions/3 min)	105.5± 7.0 (6)[1]	6.7±3.5* (6)	6.0±1.7 (6)	0.8±0.6 (6)
Exploring behavior (numbers/3 min)	23.5± 5.6 (6)	0.3±0.3* (6)	0 (6)	0 (6)
IEMG (mv/sec)	114.6±10.8 (9)	90.6±3.9* (9)	91.6±4.7 (9)	118.6±8.9** (9)
Catatonia	− (6)	+ (6)	+ (6)	− (6)
Righting reflex	+ (6)	+ (6)	+ (6)	+ (6)

1. Numbers of animal in parentheses
2. The concentrations of CPZ and 4-AP are 2.01 mg/kg and 1.2 mg/kg separately
The datas of CPZ alone and CPZ+4-AP on SMA and exploring behavior were recorded at 30-33 min and 40-43 min, and 4-AP was given at 35 min after CPZ administration
* Differs significantly ($P<0.005$) from the drug free group.
** Differs significantly ($P<0.005$) from the CPZ alone group.

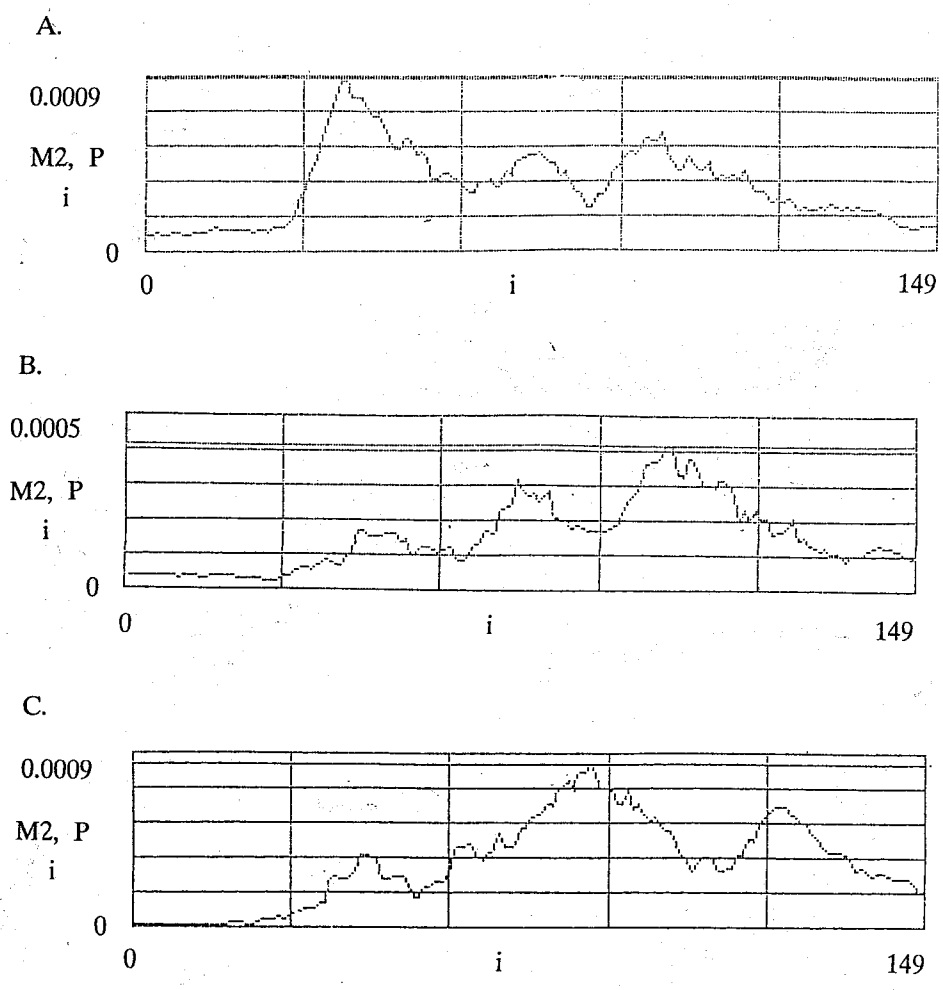

Fig 4. Effects of CPZ and 4-AP on IEMG. A. drug free B. CPZ (2.01 mg/kg) alone C. CPZ (2.01 mg/kg) + 4-AP (1.2 mg/kg).
4-AP was given at 30 min in CPZ pretreatment mice. B and C were recorded separately at 30 min and 35 min after CPZ administration.

DISCUSSION

When a mouse is repeatedly placed on a rod which is rotating at a constant speed, the animal gradually learns to walk on it, adapting itself to the rotation speed. After ingestion of inhibitory agents, however, the animal easily falls from the rod. This procedure is called "rotarod test" and was first introduced by Dunham and Miya for assaying the drug effects on the forced motor activity. The open field is used to observe the spontaneous motor activity (awareness of environment). In this study, CPZ decreased both rotarod performance and spontaneous motor activity. It appears CPZ decreased both the motor coordination and general motor activity. CPZ exerts a relatively selective action on reticular activating system, limbic system, hypothalamus, thalamus, and basal banglia (11), So the decrement of muscle performance and induced catatonia by CPZ is thought to be due to blockade of the function of neurotransmitters of these central site, at least in part to blockade of cholinergic activity (12). On the other hand, CPZ didn't induce hypnosis and extremely muscle relaxation so it didn't depress righting reflex.

The cholinergic activity in hippocampus, cortex, and striatum correlates with locomotor activity (13-15) and arousal behavior (16-17), and the striatum contains intrinsic cholinergic interneurons (18-19). Since 4-AP acts on the evoked nerve terminals to increase neurotransmitter release in both central and peripheral synapses (20-22). No doubt, 4-AP could augment motor muscle performance. Since the increment action of 4-AP is use dependently (7), and the site of depression of CPZ may be multiple, So 4-AP could partly recovery rotarod performance and IEMG, but not SMA and exploring behavior. obviously, The major site of action of 4-AP is at peripheral motor synapses to facilitate acetylcholine releas, but couldn't exclude the possibility of central effect.

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4-Aminopyridine對抗Chlorpromazine降低小白鼠運動活性之研究

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Chlorpromazine（CPZ）可降低小白鼠在rotarod之運動活性和在Open field中之自發性運動活性。Rotarod之運動活性為一種被迫性運動，當動物協調運動之能力被抑制或肌肉鬆弛時便容易由rotarod中掉下來。而動物在Open field中之自發性運動，則被視為一種探索環境的行為，當動物之清醒性受抑制時，自發性運動會降低。CPZ因可作用在腦邊緣系，網狀活化系統、視丘、下視丘和基底神經節而降低小白鼠協調運動之能力和抑制其清醒性而使其運動活性和綜合肌動作電位降低。由於膽素導性系統和運動活性息息相關，而4-AP不只在末梢運動神經促使乙醯膽鹼釋放，其亦可增加中樞神經傳遞物之釋放，如乙醯膽鹼，新腎上腺素和麩胺酸等。所以在CPZ存在下，4-AP能部份恢復小白鼠之rotarod運動能力，增加綜合肌動作電位之積分值，以及解除肌僵直等作用，相信主要是促使末梢運動神經釋放乙醯膽鹼而來，但不排除有中樞作用之可能。由於CPZ之作用位置相當複雜，且神經活性增加時，4-AP之作用才會顯現，所以4-AP無法對抗CPZ降低自發性運動和探險行為之作用。

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