

**SHORT COMMUNICATION**

**A FUNCTIONAL INTERACTION BETWEEN GABA AND 5-HT IN INHIBITING PICROTOXIN-INDUCED MYOCLONUS IN RATS**

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**Abstract:** Pretreatment with 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, antagonised while pretreatment with p-chlorophenylalanine (PCPA), a 5-HT depletor, potentiated the myoclonus induced by picrotoxin, a GABA antagonist. Pretreatment with aminooxyacetic acid (AOAA), a GABA transaminase inhibitor, antagonised picrotoxin-induced myoclonus. The combined effect of the least protective doses of AOAA and 5-HTP was greater than the sum of their individual inhibitory effects on picrotoxin-induced myoclonus. Further, AOAA failed to inhibit picrotoxin-induced myoclonus in PCPA pretreated rats. These findings suggest that the central 5-HT-ergic system exerts a facilitatory influence on the GABA-ergic system and thus it is involved in the antimyoclonic action of GABA.

**Key words:** GABA      5HT      picrotoxin      AOAA      5HTP      myoclonus

**INTRODUCTION**

Clinical findings reveal that malfunctioning of gamma aminobutyric acid (GABA) and 5-hydroxytryptaminergic (5-HT) mechanisms is casually related to myoclonic syndrome and agents which enhance the activities of these neuronal systems are therapeutically effective (1, 2, 3, 4, 5, 6). However, it is yet to be investigated whether a functional interaction exists between these neurotransmitters in alleviating myoclonic movements. The present study evaluates the relationship between GABA and 5-HT in inhibiting experimentally-induced myoclonus in rats. Since jerky movements induced by the GABA antagonist, picrotoxin (7) are reminiscent of human myoclonic syndrome (8), this model has been chosen for the present study. In order to investigate the interaction, the individual antimyoclonic effects of a GABA degradation inhibitor, aminooxyacetic acid (AOAA) and the 5-HT precursor, 5-hydroxy-

tryptophan (5-HTP), which are known to inhibit myoclonic convulsions by elevating the brain levels of GABA (9) and 5-HT (10) respectively, were compared with that produced by them concomitantly. The effect of AOAA was then tested in rats pre-treated with p-chlorophenylalanine (PCPA), a 5-HT depletor (11).

**METHODS**

Adult Wistar strain male albino rats weighing 150-200 g were used. Picrotoxin, AOAA, 5-HTP and PCPA (Sigma, U.S.A.) were dissolved in distilled water. Gentle heating was required to dissolve 5-HTP. All solutions were freshly prepared and injected (ip) in a volume of 0.2 ml/100 g. Control rats received the vehicle at corresponding time interval. All experiments were conducted on fresh groups of rats at a room temperature of 30-35°C.

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In the first set of experiments, groups of rats were treated with graded doses of AOAA and 6 hr later challenged with the myoclonic dose of picrotoxin (3 mg/kg). Picrotoxin was administered 6 h after AOAA as a correlation between a rise in synaptosomal GABA content and an inhibition of convulsive responses has been reported to occur 6 hr after AOAA administration (9).

In the second set, groups of rats received graded doses of 5-HTP 30 min prior to picrotoxin (3 mg/kg).

In the third set, the animals received the minimum effective dose of AOAA (2 mg/kg) followed 5.5 hr later by the minimum effective dose of 5-HTP (25 mg/kg). Thirty min after 5-HTP treat-

ment they were challenged with picrotoxin (3 mg/kg).

In the fourth set, the animals received PCPA (100 mg/kg daily for 3 days). Twenty four hr after the third injection of PCPA, the animals were challenged with picrotoxin (3 mg/kg).

In the fifth set, 24 hr after the third injection of PCPA the animals were treated with a protective dose of AOAA (4 mg/kg) and 6 h later were challenged with picrotoxin (3 mg/kg).

Picrotoxin challenged animals were caged singly and observed for 1 hr. The onset of the first jerky movement (myoclonic latency) was recorded in each rat. Myoclonus appeared intermittently

TABLE I : The individual (A) and combined (B) effects of AOAA and 5-HTP and the effect of AOAA in PCPA pretreated (C) rats against picrotoxin-induced myoclonus. Rats received AOAA and 5-HTP 6 h and 30 min prior to picrotoxin (3 mg/kg), respectively. PCPA (100 mg/kg) was injected daily for 3 days. Values are mean  $\pm$  SEM of animals in a group (n = 6) responding to picrotoxin. SEM was not determined if the number is less than 3. Figures in parenthesis indicate the number not responding to picrotoxin.

Treatment (mg/kg)	Myoclonic Latency (min) (Mean $\pm$ SEM)	Myoclonic score (Mean $\pm$ SEM)		
		Min after picrotoxin		
		10-20	30-40	50-60
A. Control	9.8 $\pm$ 0.8	3.2 $\pm$ 0.4	3.0 $\pm$ 0.3	2.4 $\pm$ 0.2
AOAA				
2.0	11.3 $\pm$ 1.0	2.3 $\pm$ 1.0*	2.3 $\pm$ 0.1*	1.1 $\pm$ 0.1*
4.0	13.1 $\pm$ 1.1+	(1) 1.5 $\pm$ 0.5*	1.6 $\pm$ 0.5*	0.7 $\pm$ 0.3*
8.0	21.3 $\pm$ 1.0++	(3) 0.5 $\pm$ 0.2**	0.9 $\pm$ 0.3**	0.0**
Control	9.6 $\pm$ 0.5	3.0 $\pm$ 0.3	3.2 $\pm$ 0.1	2.4 $\pm$ 0.2
5-HTP				
25	14.2 $\pm$ 2.0+	2.7 $\pm$ 0.3	2.8 $\pm$ 0.2	1.8 $\pm$ 0.2*
50	18.2 $\pm$ 1.0+	(1) 1.6 $\pm$ 0.2**	2.2 $\pm$ 0.2*	1.1 $\pm$ 0.2**
100	25.5 $\pm$ 1.1++	(2) 0.4 $\pm$ 0.2**	0.5 $\pm$ 0.1**	0.0**
B. AOAA				
2.0				
+ 5-HTP	26.0 (4)	0.6	0.6	0.0
25				
C. PCPA				
PCPA	5.8 $\pm$ 0.2+	3.5 $\pm$ 0.3	3.2 $\pm$ 0.2	2.8 $\pm$ 0.1
+ AOAA	6.5 $\pm$ 0.5+	3.1 $\pm$ 0.2**	3.0 $\pm$ 0.3 <sup>†</sup>	2.6 $\pm$ 0.2**
4.0				

+P<0.05, ++ P<0.01 compared to control value (t-test)

\*P<0.05, \*\* P<0.01 compared to control value (Mann-Whitney U-test)

†P<0.05, \*\*P<0.01 compared to that produced by the same dose of AOAA alone (Mann-Whitney U-test).

every 5-10 min. Each animal was, therefore, observed for 10 min at 10 min intervals (10-20, 30-40 and 50-60 min) and the intensity of myoclonic movements was scored as described by Slater and Dickinson (12); no jerking = 0, weak occasional jerking = 1, mild intermittent jerking of head and forelimbs = 2, pronounced jerking of head and forelimbs = 3 and a short period of myoclonic convulsions = 4. The number of rats not responding to picrotoxin (total protection during the test period) was also recorded.

Student's t-test was used to analyze the latency data. Scoring data was analyzed using the Mann-Whitney U-test.

## RESULTS

Both AOAA and 5-HTP were effective in a dose-dependent manner against picrotoxin-induced myoclonus (Table I A). Concurrent administration of them in their least protective doses resulted in a powerful inhibition of myoclonic movements. This combination afforded total protection to 4 rats in the group (n = 6) and markedly inhibited myoclonic scorings of the other rats (Table I B). The data presented in Table I C show that PCPA pretreatment has shortened myoclonic latency indicating a potentiation of picrotoxin-induced

myoclonus. An effective dose of AOAA (4 mg/kg) failed to protect these animals. All of them responded to picrotoxin with a shortening of the myoclonic latency and an enhancement of myoclonic scorings than that recorded in animals treated with the same dose of AOAA alone.

## DISCUSSION

In the present study, drugs which influence the central 5-HT-ergic mechanism have been found to affect the myoclonus induced by the GABA antagonist, picrotoxin. 5-HTP, a precursor of 5-HT, antagonized while PCPA, a 5-HT depletor, potentiated picrotoxin-induced myoclonus. Further, the combined effect of the least protective doses of AOAA and 5-HTP was greater than the sum of their individual inhibitory effects on picrotoxin-induced myoclonus in 5-HT depleted rats. Taken together, our findings suggest that the central 5-HT-ergic system exerts a facilitatory influence on the GABA-ergic system and is involved in the antimyoclonic action of GABA. Supportive to our contention are the recent immunohisto-chemical, radiographic and immunocytochemical studies which have demonstrated a co-occurrence of GABA and 5-HT in central neurons (13, 14) and the report stating that GABA mediated synaptic action is facilitated by endogenously released 5-HT (15).

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According to picrotoxin (total protection during the test period) was also recorded.

Statistical test was used to analyze the latency data. Scoring data was analyzed using the Mann-Whitney U-test.

RESULTS

Both AOOA and 5-HTP were effective in a dose-dependent manner against picrotoxin-induced myoclonus (Table I A). Concurrent administration of them in their least protective doses resulted in a powerful inhibition of myoclonic movements. This combination afforded total protection to 4 rats in the group (n = 6) and markedly inhibited myoclonic scores of the other rats (Table I B). The data presented in Table I C show that PCPA pretreatment has shortened myoclonic latency indicating a potentiation of picrotoxin-induced

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