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SHORT COMMUNICATION

THE ANTIMYOCLONIC ACTION OF CLONAZEPAM THROUGH A GABA—INDEPENDENT MECHANISM

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Abstract : The present study investigates whether clonazepam exerts its antimyoclonic action through a GABA independent mechanism. We have studied the antimyoclonic effect of clonazepam and compared it with that of aminooxyacetic acid (AOAA), a GABA transaminase inhibitor, against myoclonus induced by picrotoxin, a GABA receptor antagonist and allylglycine, a drug which inhibits synthesis and release of GABA. We have also investigated the effect of clonazepam against picrotoxin-induced myoclonus in rats pretreated with either AOAA or submyoclonic dose of allylgylycine.

Clonazepam pretreatment inhibited both picrotoxin and allylglycine-induced myoelonus whereas AOAA was effective in inhibiting only picrotoxin-induced myoclonus. The protective effect of clonazepam against picrotoxin-induced myoclonus was potentiated by AOAA pretreatment. Moreover, clonazepam afforded protection against picrotoxin-induced myoclonus in rats pretreated with a submyoclonic GABA reducing dose of allylglycine. These findings indicate that a GABA independent mechanism may also be involved in the antimyoclonic action of clonazepam.

Key words : clonazepam picrotoxin allylglycine aminooxyacetic acid

INTRODUCTION

Clinical (1, 2) and experimental (3) findings reveal that myoclonic syndrome is causally related to an impairment of gamma aminobutyric acid (GABA) activity in the brain. The GABA potentiating action of clonazepam is considered to be mainly responsible for its clinical (4, 5) and experimental (3) antimyoclonic effect. In the present study we have investigated whether clonazepam exerts its antimyoclonic action through a GABA-independent mechanism too. We have therefore studied the effect of clonazepam pretreatment against myoclonus induced by picrotoxin, a GABA receptor antagonist (6, 7) and that produced by allylglycine, a drug which inhibits synthesis (8) and release (9) of GABA. In another set of experiments we have investigated the antimyoclonic potency of clonazepam against picrotoxin-induced myoclonus in rats pretreated with allylglycine (a GABA reducing dose with no provocation of myoclonus) or aminooxyacetic acid (AOAA) which elevates brain GABA levels by inhibiting GABA-transaminase (10, 11).

METHODS

Adult Wistar strain male albino rats weighing

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150–200 g were used. Clonazepam (Roche, Switzerland) was dissolved in propylene glycol. Allylglycine, picrotoxin and AOAA (Sigma, U.S.A.) were dissolved in distilled water. All solutions were freshly prepared prior to injection (0 2 ml/100 g, ip). All experiments were carried out on fresh groups of rats at a room temperature of 30–33°C.

In the first set of experiments, groups of rats (6 in each group) were treated with clonazepam and 30 min later challenged with the myoclonic dose of picrotoxin (3 mg/kg) or allylglycine (150 mg/kg).

In the second set the animals received either AOAA, 6 hr prior to, or allylglycine (100 mg/kg, submyoclonic dose) 100 min prior to clonazepam. Thirty min after clonazepam treatment they were challenged with the myoclonic dose of picrotoxin (3 mg/kg).

In the third set, the animals were treated with AOAA and challenged 6.5 hr or 6 hr later with the myoclonic dose of picrotoxin (3 mg/kg) or allylglycine (150 mg/kg) respectively. Control groups received requisite volume of the vehicle (ip).

Picrotoxin and allylglycine-challenged animals were caged singly and observed for 1 hr and 2 hr, respectively. The onset of the first jerky movement (myoclonic latency) was recorded in each rat. Picrotoxin produced intermittent myoclonus every 5–10 min. Each animal was observed over a 10 min period at 10 min intervals (10–20, 30–40 and 50–60 min) and the intensity of the jerking movement was assessed according to the scoring method of 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. Since allylglycine produced myoclonus of lesser frequency (2 or 3 bouts in 2 hr), each bout was scored. The mean score of each animal was taken to compute the mean value of the group. The number of rats not responding to picrotoxin or allylglycine (total protection during the test period) was also noted. The latency data were statistically analysed by the Student's t-test and the scoring data by the Mann-Whitney U-test.

RESULTS

The myoclonic latency of picrotoxin was significantly prolonged in clonazepam pretreated animals. In these rats a dose-dependent inhibition of the intensity of myoclonic movements was found. Clonazepam, at 0.1 and 0.2 mg/kg dose levels produced total protection in 1 and 3 animals, respectively (Table I A). Though clonazepam pretreatment did not alter the myoclonic latency of allylglycine (150 mg/kg) it however, did reduce the myoclonic score in a dose-dependent manner (Table II A). AOAA pretreatment, in a dose-dependent manner, inhibited picrotoxin (Table I B) but not allylglycine (Table II B)-induced myoclonus. A potentiation of the effect of clonazepam against picrotoxin-induced myoclonus was found in AOAA pretreated animals (Table I B). Pretreatment with submyoclonic dose (100 mg/kg) of allylglycine resulted in a shortening of the myoclonic latency of picrotoxin. In these animals clonaxepam was effective in signifieantly prolonging the myoclonic latency of picrotoxin and in reducing the myoclonic score (Table I C).

TABLE I : Effect of clonazepam alone (A) and in rats pretreated with AOAA (B) or submyoclonic dose of allylglycine (C) against picrotoxin-induced myoclonus. Clonazepam was injected 6 hr after AOAA or 100 min after allylglycine. Picrotoxin (3 mg/kg) was injected 30 min after clonazepam. Values given are means±SEM (n=6). Figures in parenthesis indicate the number of rats in the group not responding to picrotoxin.

Pretreatment (mg/kg)	*Dose of clonazepam (mg/kg)*	*Myoclonic Latency (min) (Mean±SEM)*	*Myoclonic Score (Mean±SEM)*		
			Min after picrotoxin		
			10-20	*30-40*	*50-60*
A. Control		9.6 ±0.5	3.0 ±0.3	3.2 ±0.1	2.4 ±0.2
	0.05	15.3+ ±1.1	2.4 ±0.5	2.2* ±0 2	1.5* ±0 4
	0.1	22.2++ (1) ±1.6	1.4** ±0.3	1.2* ±0.2	0.0**
	0.2	27.6++ (3) ±1.3	0.0**	0.3** ±0.2	0.0**
B Control		9.8 ±0.8	3.2 ±0.4	3.0 ±0.3	2.4 ±0.2
AOAA					
2.0		11.3 ±1.0	2.3* ±0.1	2.3* ±0.1	1.1* ±0.1
4.0		13.1+ (1) ±1.1	1.5* ±0 5	1.6* ±0.5	0.7* ±0.3
8.0		21.3++ (3) ±1.0	0.5** ±0.2	0.9** ±0.3	0.0**
2.0	0.05	28.0‡ (3) ±1.0	0.0≠≠	0.8≠ ±0.3	0.0≠≠
C. Control		9.6 ±0.8	3.0 ±0.2	2.8 ±0.3	2.0 ±0.4
Allylglycine					
100		5.6+ ±0.2	3.4 ±0.4	3.2 ±0.3	2 8 ±0.2
100	0.1	15.3+'° ±1.1	2.2●'* ±0.2	2.0●'* ±0.2	0.8●'* ±0.2

+ P<0.05, ++ P<0.01 compared to respective control value (t-test). ‡ P<0.05 compared to that produced by the same dose of clonazepam alone as in A (t test). * P<0.05. ** P<0.01 compared to the respective control value (Mann-Whitney test). ≠ P<0 05, ≠≠ P<0.01 compared to that produced by clonazepam alone as in A (Mann-Whitney test). o P<0.05 compared to that produced by the same dose of clonazepam alone as in A (t test). ● P<0.05 compared to that produced by the same dose of clonazepam alone as in A (Mann-Whitney test).

TABLE II : Effect of clonazepam (A) and AOAA (B) against allylglycine-induced myoclonus. Allylglycine (150 mg/kg) was injected 30 min after clonazepam and 6 hr after AOAA. Values given are means ±SEM (n=6). Figures in parenthesis indicate the number of rats in each group not responding to allylglycine.

Treatment	*Dose (mg/kg)*	*Myoclonic latency (min, mean±SFM)*	*Myoclonic score (Mean±SEM)*
A. Control		106± 4.8	3.5±0.2
Clonazepam	0.05	109±13.1	2.8±0.2*
	0.1	117±13 2 (1)	1.5±0.3**
	0.2	121±16.2 (2)	0.8±0.3**
B. Control		105± 5 5	3.4±0.3
AOAA	2.0	109± 5.0	3.2±0.2
	4.0	107± 5 0	3.0±0.2
	8.0	108± 7.0	2.5±0.3

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

DISCUSSION

Picrotoxin, which is known to produce myoclonus by antagonizing GABA receptor activity (3), is unlikely to produce this effect if GABA-ergic activity is enhanced in the brain. The GABA-elevating action of AOAA (10, 11) and the GABA potentiating action of clonazepam (3) have been proposed for their protective effect against picrotoxin-induced myoclonus. A synergism between these two agents is likely and the same has been demonstrated in this study. The present study also shows the failure of AOAA to suppress myoclonus that resulted from an impairment by allylglycine of GABA synthesis and

release indicating that AOAA produces antimyoclonic action solely through a GABA-ergic mechanism. Interestingly, clonazepam, unlike AOAA, has inhibited allylglycine-induced myoclonus. Moreover, clonazepam afforded protection against picrotoxin-induced myoclonus in rats pretreated with a sub-myoclonic GABA reducing dose of allylglycine. Taken together, these findings suggest that clonazepam, in addition to its GABA potentiating action, might also be acting through a GABA-independent mechanism for its antimyoclonic effect. In support of this proposal, the benzodiazepine compounds have been shown to produce myorelaxant action without the involvement of GABA (13). Electrophysiological evidence (14, 15) has further indicated that these compounds are able to produce neuronal inhibition without the participation of GABA.

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