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INVOLVEMENT OF ADENOSINE IN POSTICTAL EVENTS IN RATS GIVEN ELECTROSHOCK

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Abstract : The effect of a selective adenosine antagonist, 8-cyclopentyl 1,3-dimethylxanthine (8-CPT) was used to examine involvement of adenosine in ictal and postictal events in rats subjected to maximal electroshock (MES). MES induces the ictal event of hindlimb tonic extension (HLTE) followed by postictal depression (PID). 8-CPT 10 mg/kg, ip produced maximal significant reduction of PID without affecting HLTE, further confirming involvement of adenosine in PID. Carbamazepine and sodium valproate were studied independently and were coadminstered with 8-CPT to determine if their anticonvulsant activity was modulated by adenosine and if they altered PID. 8-CPT did not antagonize the seizure protection afforded by CBZ or SV. CBZ significantly reduced postictal events whereas SV had no significant effect. These observations further confirm a role for adenosine in postictal phenomena.

Key Word : 8-cyclopentyl 1,3 dimethylxanthine carbamazepine sodium valproate adenosine adenosine antagonist rats post-ictal depression

INTRODUCTION

Recent studies have shown that extracellular brain adenosine concentration increases dramatically following enhanced energy consumption, such as seizures (1) and/or hypoxia (2). The release of endogenous adenosine is reported to terminate ongoing seizure activity and is demonstrated to play a role in post-ictal depression (PID) (2,3). The use of pharmacological tools such as adenosine receptor antagonists to elucidate the role of adenosine in the brain has gained wide acceptance (4,5). Although the methylxanthines aminophylline and caffeine are classical adenosine A1 and A2 receptor antagonists and block PID (4,6), they are non-specific and do not distinguish between A1 and A2 adenosine receptor subtypes.

8-cyclopentyl 1,3-dimenthyl-xanthine/8 cyclopentyl theophylline (8-CPT) shows potent A1 selective adenosine receptor blockade *in vivo* following parenteral administration and crosses the blood brain barrier (4). It attains maximal brain levels in 7 to 10 min after ip. administration and declines by 30 min (7). This study is concerned with the role of adenosine in modulating PID in rats subjected to electroshock. We focussed our attention on the following -[a]: 8-CPT was used to determine if it blocked the appearance of PID; [b] : The effects of anticonvulsant drugs were also examined on postictal events. Carbamazepine (CBZ) and sodium valproate (SV) were selected as it is reported that, CBZ exhibits adenosine A1 receptor antagonistic properties (8) whereas SV is not known to interact with adenosinergic mechanisms (9); [c]: Interaction of 8-CPT and CBZ and 8-CPT and SV were studied in order to determine if 8-CPT altered their seizure protective ability.

METHODS

Maximal electroshock seizures (MES) were induced in Wistar rats (160-200 g) of either sex with 150 mA AC given for 0.2 Sec. (6) and animals were

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randomly assigned to groups of 6 for different drug/ vehicle treatments and for various time cuts.

Following MES, the duration of the hindlimb tonic extension (HLTE), representing the ictal event, was determined in seconds. Rats not displaying HLTE, during pre-selection sessions were rejected and abolition of HLTE by anti-epileptic drugs was taken as the criterion for anticonvulsant activity. The 100% seizure protective dose (ED100) of carbamazepine (CBZ) was 10 mg/kg, ip and for SV was 300 mg/kg, ip. Peak efficacy time of CBZ was 45 min. and of SV was 10 min in MES test as established in a previous study (6).

8-CPT, (Research Biochemicals, Natick, MA), 2.5 and 10 mg/kg, ip, was dissolved in a drop of 95% ethanol was made upto final volume by adding propylene glycol (5). CBZ 10 mg/kg, ip, was dissolved in 50% propylene glycol and 50% saline with slight warming, SV was dissolved in saline. The volume of the vehicle for 8-PTC, CBZ and SV was adjusted so that each rat received. 0.1 ml/100 g body weight. The respective vehicles had no effect on any of the parameters examined and control rats received appropriate amounts of the vehicle. Intraperitoneal administration of 8-CPT is reported to reach maximal levels in the brain by 7 min and declines by 30 min (7). Hence, the effects of 8-CPT *per se* and when combined with CBZ or SV were studied at time cuts of 8 and 30 min.

In interaction studies with CBZ and SV, the time of administration of 8-CPT was adjusted at 8 and 30 min prior to MES. Following the tonic extensor seizure, a period of post-ictal immobility and depression ensued, and the time taken for the recovery of the following reflexes was measured in seconds : head and forelimb righting reflex (FLRR), which invaribly occured prior to the recovery of the hindlimb righting reflex. The total period of post-ictal depression (PID) was measured as the time taken from the end of tonic and clonic movements, till the hindlimb and body righting reflexes recovered and the rat could walk away.

Data are presented as means ± SEM and tests of significance were made by Student's paired t test. P values less than 0.05 were considered significant. A one way ANOVA test was used to establish that the control values for different parameters were not significantly different from each other.

RESULTS

Effect of 8-cyclopentyl theophylline (8-CPT) on behaviour, ictal and postictal events: 8-CPT, 2.5 m/kg ip, produced no behavioural changes and had no significant effect on the parameters examined. At 10 mg/kg ip, increased self-grooming was seen but no effect was seen on spontaneous motor activity; nor did it exhibit proconvulsant activity. Increased irritability, as evidenced by exaggerated responses to light tactile stimuli (puff of air) or to sharp sound (startle response) was observed. Hyperpnea was seen in all rats. After MES, marked excitement and jumping movements were seen.

Table 1[A] shows the effects of 8-CPT 10 mg/ kg, ip on HLTE and postictal events after 8 min and 30 min in rats subjected to maximal electroshock. At both time cuts the HLTE was not influenced demonstrating absence of anticonvulsant activity. On the other hand at 8 min, FLRR and PID were highly significantly reduced in duration ($P < 0.0001$). Reduction of FLRR and PID were seen at 30 min though to a less significant extent.

Effect of carbamazepine (CBZ) and sodium valproate (SV): CBZ 10 mg/kg, ip, produced an altered behavioural pattern of light sedation and quiescence without ataxia. Following MES, there was abolition of HLTE and the rats exhibited behavioural excitement together with increased vocalization. SV 300 mg/kg, ip, apart from abolishing HLTE, produced mild to moderate ataxia and the rats remained ataxic after MES.

Table I [B] shows the anticonvulsant effect of CBZ and SV manifested by the absence of the ictal event (HLTE). Following MES in CBZ pretreated rats, there was immediate recovery of the FLRR in all rats, while PID was reduced though not to a significant exent. After SV, neither FLRR nor PID were significantly changed. However, PID duration was prolonged.

Interaction studies of 8-CPT 10 mg/kg, ip with CBZ, 10 mg/kg, ip and with SV, 300 mg/kg, ip: Table II shows the comparative effects of CBZ and SV *per se* and together with 8-CPT on ictal and postictal events. Data were established 8 min and 30 min after 8-CPT administration. At 8 min, 8-CPT had no effect on the

TABLE I : Effect of 8-cyclopentyl theophylliine (CPT), carbamazepine (CBZ) and sodium valproate (SV) on ictal and post ictal events in rats subjected electroshock.

[A] B-CPT, 10 mg/kg, ip,

Parameter	*MES : 8 mins after 8-CPT*			*MES : 30 mins after 8-CPT*		
	Control	*8-CPT*	*P*	*Control*	*8-CPT*	*P*
HLTE	8.0 ± 0.5	6.9 ± 0.8	NS	7.8 ± 0.6	7.7 ± 0.0	NS
FLRR	151.6 ± 9.5	94.0 ± 8.6	< 0.0001	164.8 ± 12.2	46.5 ± 4.7	<0.005
PID	224.2 ± 15.2	136.4 ± 8.57	< 0.0001	350.8 ± 52.1	220.5 ± 28.4	< 0.01

[B] CBZ, 10 mg/kg, ip and SV 300 mg/kg, ip

	MES : 45 mins after CBZ			*MES : 10 mins after SV*		
	Control	*CBZ*	*P*	*Control*	*SV*	*P*
HLTE	7.6 ± 0.7	0	<0.0017	8.3 ± 0.4	0	< 0.0001
FLRR	138.0 ± 36.3	0	< 0.03	170.0 ± 8.6	137.5 ± 67.1	NS
PID	332.3 ± 150.3	309 ± 35.3	NS	390.8 ± 55.5	570.0 ± 93.3	NS

In Table IA, IB and A II, values are X ± SEM and are in seconds. See text for abbreviations for parameters.

Significances by paired t test are with respect to controls in Table 1 and to CBZ and SV in Table II. NS = Not significant.

The control values for various parameters in Table I [A] and [B] were not significantly different when compared using one way ANOVA.

TABLE II : Interaction of 8-CPT, 10 mg/kg, ip with CBZ 10 mg/kg, ip and SV 300 mg/kg ip on ictal and post ictal events.

MES : 8 min after 8-CPT.

Parameter	*CBZ*	*CBZ + 8-CPT*	*P*	*SV*	*SV + 8-CPT*	*P*
HLTE	0	0	NS	0	0	NS
FLRR	0	0	NS	137.5 ± 67.1	22.8 ± 8.5	NS
PID	309 ± 35.3	136.7 ± 10.9	< 0.002	570.0 ± 93.3	29.5 ± 7.4	< 0.01

anticonvulsant efficacy (on HLTE) of both CBZ and SV. 8-CPT did not significantly alter the effect of either CBZ or SV on duration of FLRR, but did reduce the duration of PID with both drugs. Similar effects on all parameters were observed 30 min after 8-CPT, though the reduction of PID was to a lesser extent than after 8 min.

DISCUSSION

Recent studies suggest that the brain adenosine system may subserve endogenous seizure termination in convlusive states (3) and may also be involved in postictal depression (PID) (1). Although the function of postictal depression is unknown, it may serve in triggering inhibitory activity, which arrests ongoing seizure activity (1). Interaction of adenosine with A1 receptors has been suggested to mediate its neurodepressant effects (10) and if PID is mediated by adenosine release, then selective adenosine receptor A1 antagonists, such as 8-CPT, should block the appearance of PID. The major finding in this study is that centrally active A1 adenosine receptor antagonists

8-CPT, has been shown to antagonise postictal events in a significant manner, without influencing the actual ictal event. In previous studies we showed that non-specific adenosine receptor antagonists aminophylline and caffeine significantly reduced the period of postictal recovery (11).

There have been limited studies on the effects of antiepileptic drugs on PID. From a pharmacological and clinical standpoint, attention has been focused on their ability to abolish the ictal event, viz. seizures. Further, studies on the role of adenosine in modulating PID following generalised seizure activity has recently gained wide acceptance. During a seizure, a dramatic build up of brain adenosine levels occurs (2) which ultimately terminates the ongoing seizure, producing widespread inhibition of neuronal activity, resulting in PID. Subjects are refractory to further seizure activity during PID. In this study interestingly, CBZ showed an immediate recovery of the FLRR, a reflex mediated by cerebellar and rostral spinal pathways. This property of CBZ somewhat resembles that of 8-CPT which also highly significantly reduced FLRR. Recent results from *in vitro* studies suggest that CBZ is an antagonist at A1 adenosine receptors in cerebellum, cerebral cortical and hippocampal membranes and is a selective ligand for brain adenosine A1 receptors (8). CBZ and adenosine both exhibit anticonvulsant and sedative properties. However, there are several recent reports which show that CBZ has adenosine A1 receptor antagonistic properites (8). It is possible that there are different subtypes of the A1 receptors (2). It can be speculated that this adenosine antagonistic effect of CBZ is more relevant to it's antidepressant action in view of it's tricyclic structure (12). SV had no significant effect on FLRR, but prolonged PID whereas CBZ reduced PID though not to a significant extent.

Another significant finding was that when 8-CPT was administered with either CBZ or SV, there was no impairment of their seizure protective ability, suggesting that their mechanism of action as anticonvulsants may not involve adenosinergic mechanisms. It has been reported that adenosine antagonists fail to block the anticonvulsant effect of CBZ (9). The combined effect of the anticonvulsants and 8-CPT on post-ictal phenomena were less convincing and the significant reduction in PID may have been due to the presence of 8-CPT.

In conclusion, the data confirm the observation on the role of adenosine in PID (1) and lend support to the previous evidence that the anticonvulsant action of CBZ and SV is not mediated by an agonistic effect through the adenosine receptor (9).

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