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Differential effects of agents enhancing purinergic transmission upon the antielectroshock efficacy of carbamazepine, diphenylhydantoin, diazepam, phenobarbital, and valproate in mice

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Summary. L-phenylisopropyladenosine (L-PIA; a preferential A 1 adenosine agonist—0.05 mg/kg) offered no protection against electroconvulsions in mice but potentiated the anticonvulsant action of diazepam and valproate against maximal electroshock-induced seizures, decreasing the respective ED_{50} values from 9.5 to 4.0 mg/kg and from 250 to 185 mg/kg. However, it remained without effect on the protective activity of phenobarbital, carbamazepine and diphenylhydantoin. 5′-N-ethylcarboxamidoadenosine (NECA; a preferential A 2 adenosine agonist—0.5 mg/kg) potentiated the efficacy of valproate. On the other hand, NECA (1 mg/kg) diminished the anticonvulsant action of phenobarbital (ED_{50} was elevated from 16.5 to 20.5 mg/kg), possessing no effect upon the protective action of carbamazepine. In addition, papaverine (20 mg/kg) significantly enhanced the protective efficacy of valproate and up to 40 mg/kg remained without influence upon the protective action of carbamazepine. However, papaverine (20 and 40 mg/kg) inhibited the anticonvulsive potential of phenobarbital.

In the light of the results obtained A 1 and A 2 adenosine receptor-mediated events seem to possess different influences upon the protective effects of antiepileptic drugs.

Keywords: Antiepileptics, adenosine, seizures.

Introduction

Adenosine and its direct and indirect agonists have been shown to possess anticonvulsant activity in many tests of experimental epilepsy. Specifically, adenosine inhibited audiogenic seizures in mice (Maitre et al., 1974) and its direct agonists protected rodents against kindled, pentetrazol-, pilocarpine-, and electroshock-induced convulsions (Bortolotto et al., 1985; Dragunov et al., 1985; Dunwiddie and Worth, 1985; Murray et al., 1985; Turski et al., 1985).

Papaverine, an adenosine uptake inhibitor (Bender et al., 1980), also offered some protection against amygdala-kindled seizures in rats (Dragunov et al., 1985). Adenosine agonists were also found effective in an in vitro model of epilepsy. O'Shaughnessy et al. (1988) revealed that L-phenylisopropyladenosine (L-PIA), 2-chloroadenosine and adenosine itself attenuated burst activity induced in rat cortical slices by the removal of magnesium ions from the superfusing medium. Furthermore, adenosine agonists inhibited epileptiform activity in the hippocampus produced by low calcium media, penicillin and bicuculline (Ault and Wang, 1986; Lee et al., 1984). Conversely, adenosine antagonists were found to lower the convulsant threshold in a variety of experimental models of epilepsy and to impair the protective efficacy of common antiepileptic drugs, in higher doses being potent convulsant agents (Chu, 1981; Czuczwar et al., 1985, 1986, 1987a–d; Skerrit et al., 1983b).

Skerrit et al. (1982) documented an interaction of some antiepileptics with A 1 adenosine receptors, carbamazepine and phenobarbital possessing the highest affinity. Diphenylhydantoin was considerably weaker and valproate did not influence L-PIA binding to A 1 adenosine receptors at all. Moreover, carbamazepine was also reported to bind to the A 2 adenosine receptor population since it displaced an A 2 receptor agonist, 5′-N-ethylcarboxamidoadenosine (NECA; Skerrit et al., 1983a). Similar results, as regards the interaction of antiepileptic drugs with A 1 receptors, were obtained by Weir et al. (1984) with the use of cyclohexyladenosine as an A 1 receptor ligand. The most potent displacer was carbamazepine, phenobarbital appeared less potent and diphenylhydantoin, valproate, primidone, and ethosuximide remained without any significant action within the therapeutic levels. Fujiwara et al. (1986) found that carbamazepine in the therapeutic range bound preferentially to A 1 adenosine receptors whilst phenobarbital was less active in this respect. A number of antiepileptic drugs affected adenosine uptake very potently. Benzodiazepine derivatives, diazepam and clonazepam, as well as diphenylhydantoin, whose IC_{50} concentrations were within the micromolar range, seemed the most active agents in this respect. Phenobarbital and carbamazepine exhibited considerably weaker potency as adenosine uptake inhibitors (Phillis, 1984; Phillis and Wu, 1982).

Taking into consideration the interaction of some antiepileptics with endogenous adenosine and its receptors as well as the ability of adenosine agonists to suppress seizure activity, the present authors have studied the influence of agents enhancing purinergic transmission upon the anticonvulsant activity of a number of common antiepileptic drugs in mice.

Materials and methods

General

Experiments were carried out on Albino Swiss female mice weighing 22–27 g. The animals were housed in standard laboratory conditions (colony cages, unlimited access to chow pellets and tap water, temperature of 21 ± 1 °C) with a natural light-dark cycle. Each mouse

was used only once and the experimental groups were completed by means of a randomized schedule. The convulsive test was performed between 10.00 a.m. and 1.00 p.m.

Convulsive procedure

Electroconvulsions were induced according to Swinyard et al. (1952) with the use of corneal electrodes and alternating current (50 Hz), the stimulus duration being 0.2 s. Tonic hindlimb extension was taken as the endpoint. The convulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. Mice pretreated with antiepileptic drugs were challenged with maximal electroshock (50 mA) in order to evaluate the respective ED_{50} values (in mg/kg). At least 32 animals were used to estimate each CS_{50} or ED_{50} value.

Temperature measurements

Temperature measurements were performed at a constant environmental temperature of 21 ± 1 °C. The body temperature was measured in the rectum with a thermistor thermometer (Ellab, Copenhagen, Denmark), the probe being inserted to a depth of 15 mm. The reference temperature was the mean of three preliminary measurements taken at 20 min intervals. After the third measurement, the respective drugs, substances or vehicles were administered and at the time of the electroshock-induced seizures the final temperature was recorded. Body temperature alterations were presented as differences (Δ t) between the reference temperature and the mean temperature after treatment.

Drugs

The following antiepileptic drugs were used throughout the study: carbamazepine, diphenylhydantoin (both drugs purchased from Sigma, St. Louis, MO, U.S.A.), diazepam (Relanium, Polfa, Poznan, Poland), phenobarbital sodium (Luminalum, Polfa, Warsaw, Poland), and valproate sodium (Depakine, Labaz, Ambarez, France). Carbamazepine, diphenylhydantoin and diazepam were suspended in a 3% solution of Tween 81 (Loba Chemie, Vienna, Austria) and administered 60, 120, and 60 min respectively, prior to electroconvulsions. Phenobarbital and valproate were dissolved in saline and their doses refer to the free acid forms.

To influence central purinergic transmission L-PIA (Boehringer Mannheim, Mannheim, F.R.G.), NECA (Byk Gulden, Konstanz, F.R.G.), and papaverine hydrochloride (Polfa, Warsaw, Poland) were used. L-PIA was brought into solution with 3% Tween 81 and the remaining agents were sufficiently soluble in saline. L-PIA was injected 30 min, papaverine 20 min, and NECA 15 min before the test. Also two xanthine derivatives, aminophylline (theophylline$_2$ · ethylenediamine; Sigma, St. Louis, MO, U.S.A.) and enprofylline (3-propylxanthine; Draco, Lund, Sweden) were included in the present study. Aminophylline was dissolved in saline and enprofylline was dissolved in a minimum quantity of 1 N NaOH and made up to the desired volume with saline at pH 10.0. All drugs and agents were administered intraperitoneally in the volume of 0.05 ml/10 g body weight.

Calculation of data and statistics

The CS_{50} and ED_{50} values as well as statistical significances were calculated according to the method of Litchfield and Wilcoxon (1949). The original method was modified in that the computer construction of the dose-effect relationship was performed.

The differences between mean Δ t values were assessed statistically using Student's t-test.

Results

Effects of L-PIA, NECA, and papaverine upon the threshold for electroconvulsions in mice

L-PIA (up to 0.05 mg/kg) did not influence the convulsant threshold and in the range of 0.1–4.0 mg/kg it had a protective action (Table 1). L-PIA (0.1 mg/kg) raised the threshold from 9.1 to 10.6 mA and the maximal increase (to 13.3 mA) was observed after 4.0 mg/kg. However, the anticonvulsant activity of L-PIA at this relatively high dose was accompanied by pronounced sedation, ataxia and myorelaxation. L-PIA (0.1 mg/kg) produced only moderate sedative effect and at 0.05 mg/kg it evoked practically no obvious behavioral abnormalities. NECA (up to 2 mg/kg) and papaverine (up to 40 mg/kg) remained without effect upon the threshold for electroconvulsions. NECA (2 mg/kg) caused sedation and myorelaxation whilst papaverine (40 mg/kg) was only a slightly myorelaxant agent. It was impossible to estimate the threshold for papaverine in doses of 60 and 80 mg/kg because of the profound toxic action consisting of loss of righting reflex in about 50% of the animals, strong myorelaxation and mortality reaching 20%.

Table 1. Effects of adenosine agonists on the threshold for electroconvulsions in mice

Treatment	Dose (mg/kg; i.p.)	Tr. time (min)	CS_{50} (mA)	P<
Tween 81	–		9.1 (8.5–9.7)	–
L-PIA	0.01		9.1 (8.5–9.7)	NS
L-PIA	0.05		9.2 (8.5–9.9)	NS
L-PIA	0.1	30	10.6 (9.6–11.7)	0.05
L-PIA	1.0		11.3 (10.3–12.4)	0.05
L-PIA	2.0		12.5 (11.9–13.1)	0.02
L-PIA	4.0		13.3 (12.2–14.5)	0.01
Saline	–		9.2 (8.6–9.8)	–
NECA	0.1		9.1 (8.3–9.9)	NS
NECA	0.5	15	9.2 (8.7–9.8)	NS
NECA	1.0		9.4 (8.7–10.2)	NS
NECA	2.0		9.8 (9.2–10.5)	NS
Papaverine	10		9.2 (8.6–9.8)	NS
Papaverine	20	20	8.8 (8.1–9.5)	NS
Papaverine	40		9.9 (9.1–10.8)	NS
Papaverine	60 and 80		toxic effects	–

L-PIA L-phenylisopropyladenosine; *NECA* 5-N'-ethylcarboxamidoadeonsine; *NS* not significant

CS_{50} values and statistical analysis of the results were carried out according to Litchfield and Wilcoxon (1949)

Table 2. Effects of adenosine agonists upon the anticonvulsant efficacy of carbamazepine, diphenylhydantoin, and phenobarbital against MES-induced convulsions in mice

Treatment	L-PIA (mg/kg)			NECA (mg/kg)			Papaverine (mg/kg)			
	0	0.05	0.1	0	0.5	1.0	0	10	20	40
CBZ	17 (14–20)	18 (15–21)	17 (15–20)	19.5 (18–21)	20 (18–22)	19.5 (18–21)	16 (13–19)	17 (15–19)	17 (15–19)	15.5 (13–18)
DPH	8.0 (7.3–8.8)	6.9 (6.3–7.6)	5.6* (4.6–6.8)	8.0 (7.1–9.0)	7.0 (5.5–8.9)	7.3 (5.9–9.0)	8.0 (7.1–9.0)	7.8 (6.5–9.4)	7.3 (6.0–8.8)	7.4 (5.8–9.5)
PB	16.5 (14–19)	17.5 (15–20)	16 (12–20)	16.5 (14–19)	18 (15–21)	20.5* (18–24)	16.5 (14–19)	17.5 (15–21)	22* (18–27)	20.5* (18–24)

MES maximal electroshock; *CBZ* carbamazepine; *DPH* diphenylhydantoin; *PB* phenobarbital

All drugs were administered i.p.; DPH and PB 120 min; CBZ 60 min, L-PIA 30 min, papaverine 20 min, and NECA 15 min prior to electroconvulsions. Table data are ED_{50} (in mg/kg) with 95% confidence limits in parentheses. ED_{50} values and statistical analysis of the data were calculated according to Litchfield and Wilcoxon (1949)

* $P < 0.05$ vs. respective control group

Table 3. Effects of adenosine agonists upon the anticonvulsant efficacy of VPA against MES-induced seizures in mice

VPA + treatment (mg/kg)	ED_{50} of VPA (mg/kg)	P<
VPA + 3% Tween 81	250 (232–270)	–
VPA + L-PIA (0.01)	250 (217–288)	NS
VPA + L-PIA (0.025)	220 (183–264)	NS
VPA + L-PIA (0.05)	185 (171–200)	0.01
VPA + saline	250 (216–290)	–
VPA + NECA (0.1)	230 (211–251)	NS
VPA + NECA (0.25)	225 (205–248)	NS
VPA + NECA (0.5)	200 (171–234)	0.05
VPA + saline	260 (230–267)	–
VPA + papaverine (10)	255 (230–283)	NS
VPA + papaverine (20)	220 (204–238)	0.05
VPA + papaverine (40)	220 (204–238)	0.05

MES maximal electroshock; *VPA* valproate

All drugs were injected i.p.; VPA and L-PIA 30 min, papaverine 20 min, and NECA 15 min prior to electroconvulsions

ED_{50} values and statistical analysis of the results were calculated according to Litchfield and Wilocoxon (1949)

See also legend to Table 1

Effect of aminophylline and enprofylline upon the anticonvulsant action of L-PIA (2 mg/kg)

Aminophylline (5 mg/kg; 0.024 mmol of anhydrous theophylline/kg) partially reversed the protective activity of L-PIA, lowering the CS_{50} value from 12.5 to 10.7 mA. Enprofylline in the equimolar dose of 4.62 mg/kg (0.024 mmol/kg) was ineffective but, when injected at the higher dose of 46.2 mg/kg (0.24 mmol/kg), it was also able to reduce the protection offered by L-PIA.

Influence of L-PIA, NECA and papaverine on the efficacy of carbamazepine, diphenylhydantoin, diazepam, phenobarbital and valproate against electroconvulsions in mice

L-PIA (up to 0.1 mg/kg), NECA (up to 1.0 mg/kg) and papaverine (up to 40 mg/kg) remained without effect upon the antielectroshock efficacy of carbamazepine (Table 2). There were also no behavioral changes in animals receiving the com-

Table 4. Influence of purinergic agonists upon the protective activity of DZP against MES-induced convulsions in mice

DZP + treatment (mg/kg)	ED_{50} of DZP (mg/kg)	P<
DZP + 3% Tween 81	9.5 (8.2–11)	–
DZP + L-PIA (0.01)	8.5 (6.8–11)	NS
DZP + L-PIA (0.025)	6.0 (5.0–7.2)	0.02
DZP + L-PIA (0.05)	4.0 (3.5–4.6)	0.001
DZP + saline	9.7 (8.5–11)	–
DZP + NECA (0.1)	9.0 (7.3–11)	NS
DZP + NECA (0.25)	6.8 (5.7–8.2)	0.05
DZP + NECA (0.5)	5.1 (4.2–6.1)	0.01
DZP + saline	9.5 (8.4–11)	–
DZP + papaverine (10)	8.4 (6.8–10)	NS
DZP + papaverine (20)	toxic effects	–

DZP diazepam (given i.p. 60 min before electroconvulsions)

See also legends to Tables 1 and 2

bination of carbamazepine with a purinergic agonist as compared with mice given carbamazepine alone. Generally, the above agents were without effect upon the action of diphenylhydantoin and only L-PIA (0.1 mg/kg) was able to potentiate its anticonvulsant activity. L-PIA (up to 0.1 mg/kg), NECA (0.5 mg/kg) and papaverine (10 mg/kg) did not modify the protective efficacy of phenobarbital either. However, both NECA (1.0 mg/kg) and papaverine (20 and 40 mg/kg) led to a significant increase of phenobarbital ED_{50} value from 16.5 to 20.5, 22, and 20.5 mg/kg, respectively (Table 2). Practically, behavioral alterations could hardly be seen after combined treatment of phenobarbital with agents enhancing purinergic transmission when compared with phenobarbital alone. The anticonvulsant effect of valproate was distinctly modified by adenosine agonists (Table 3). L-PIA (0.05 mg/kg) and NECA (0.5 mg/kg) decreased the respective ED_{50} values from 250 to 185 and 200 mg/kg. Papaverine (20 mg/kg) also potentiated the effectiveness of valproate, lowering its ED_{50} value from 260 to 220 mg/kg. Potentiation of the anticonvulsant activity of valproate was accompanied by moderate enhancement of sedation. Valproate-induced myorelaxation was not influenced and ataxia or loss of righting reflex were never observed. The protective efficacy of diazepam against electroconvulsions was modified by both L-PIA and NECA and unaffected by papaverine (10 mg/kg; Table 4). Specifically, L-PIA (0.025 and 0.05 mg/kg) and NECA (0.25 and 0.5 mg/kg) lowered diazepam ED_{50} value from 9.5 to 6.0, 4.0, 6.8, and 5.1 mg/

Table 5. Effects of AMPH and ENPR upon the potentiation of adenosine agonists of the protective action of VPA against MES-induced seizures in mice

VPA + treatment (mg/kg)	ED_{50} of VPA (mg/kg)	P1<	P2<
VPA + 3% Tween 81 + saline	250 (223–280)	–	–
VPA + L-PIA (0.05) + saline	180 (165–196)	0.01	–
VPA + L-PIA (0.05) + AMPH (5)	225 (208–243)	NS	0.05
VPA + L-PIA (0.05) + ENPR (4.62)	190 (173–209)	0.01	NS
VPA + L-PIA (0.05) + ENPR (46.2)	240 (216–266)	NS	0.01
VPA + saline + saline	260 (236–286)	–	–
VPA + NECA (0.5) + saline	200 (174–230)	0.05	–
VPA + NECA (0.5) + AMPH (5)	230 (204–260)	NS	NS
VPA + NECA (0.5) + ENPR (4.62)	200 (174–230)	0.05	NS
VPA + NECA (0.5) + ENPR (46.2)	240 (214–269)	NS	NS
VPA + papaverine (20) + saline	215 (197–234)	0.05	–
VPA + papaverine (20) + AMPH (5)	280 (257–305)	NS	0.01
VPA + papaverine (20) + ENPR (4.62)	220 (204–238)	0.05	NS
VPA + papaverine (20) + ENPR (46.2)	260 (236–286)	NS	0.05

AMPH aminophylline; *ENPR* enprofylline (given 30 min prior to test) P 1 – vs groups treated with VPA alone; P 2 – vs groups receiving VPA combined with one of adenosine agonists

See also legends to Tables 1, 2, and 3

kg, respectively (Table 4). L-PIA remained essentially without influence upon the behavioral effects of diazepam while NECA moderately increased sedation and myorelaxant activity. Combination of papaverine (20 mg/kg) with diazepam resulted in a pronounced toxic reaction, including loss of righting reflex, profound myorelaxation and mortality in the range of 20–30% within 10 min of the papaverine injection.

Effects of aminophylline and enprofylline upon the anticonvulsant action of valproate, diazepam, and phenobarbital modified by adenosine agonists

Aminophylline (5 mg/kg) and enprofylline (46.2 mg/kg) reversed the potentiating effect of both L-PIA (0.05 mg/kg) and NECA (0.5 mg/kg) upon the anti-electroshock activity of diazepam (Table 5). Both xanthine derivatives also attenuated NECA-induced enhancement of diazepam sedation and myorelaxation. In the above doses the xanthine derivatives reversed the increase in the protective efficacy of valproate (Table 6) induced by L-PIA (0.05 mg/kg) and papaverine (20 mg/kg). L-PIA-, NECA- and papaverine-induced potentiation of valproate sedation was also attenuated by xanthine pretreatment. Aminophylline (5 mg/kg) and enprofylline (46.2 mg/kg) also partly affected NECA

Table 6. Effects of AMPH and ENPR upon the anticonvulsant activity of DZP enhanced by adenosine agonists in MES-induced seizures in mice

DZP + treatment (mg/kg)	ED_{50} of DZP (mg/kg)	P1<	P2<
DZP + 3% Tween 81 + saline	9.7 (8.8–11)	–	–
DZP + L-PIA (0.05) + saline	4.2 (3.8–4.6)	0.001	–
DZP + L-PIA (0.05) + AMPH (5)	7.0 (6.3–7.8)	0.05	0.01
DZP + L-PIA (0.05) + ENPR (4.62)	4.5 (4.0–5.0)	0.001	NS
DZP + L-PIA (0.05) + ENPR (46.2)	6.8 (6.1–7.6)	0.01	0.01
DZP + saline + saline	9.5 (8.2–11)	–	–
DZP + NECA (0.5) + saline	5.4 (4.8–6.0)	0.01	–
DZP + NECA (0.5) + AMPH (5)	7.2 (6.5–8.0)	0.05	0.02
DZP + NECA (0.5) + ENPR (4.62)	5.5 (5.0–6.1)	0.01	NS
DZP + NECA (0.5) + ENPR (46.2)	7.2 (6.4–8.1)	0.05	0.02

P1 – vs groups treated with DZP alone; P2 – vs groups given DZP in combination with either L-PIA or NECA

See also legends to Tables 1, 2, 4, and 5

Table 7. Effect of AMPH upon the PB anticonvulsant activity modified by NECA and papaverine in MES-induced convulsions in mice

PB + treatment (mg/kg)	ED_{50} of PB (mg/kg)	P1<	P2<
PB + saline + saline	16.5 (14.2–19.1)	–	–
PB + NECA (1) + saline	20.5 (17.7–23.8)	0.05	–
PB + NECA (1) + AMPH (5)	17.0 (15.0–19.2)	NS	0.05
PB + papaverine (40) + saline	20.5 (17.7–23.8)	0.05	–
PB + papaverine (40) + AMPH (5)	17.5 (14.2–21.5)	NS	NS

P1 – vs group administered with PB alone; P2 – vs group receiving PB with either NECA or papaverine.

See also legends to Tables 1, 2, and 5

(0.5 mg/kg)-induced enhancement of anticonvulsant activity of valproate. Specifically, the obtained ED_{50} value was neither significant versus valproate alone nor valproate combined with NECA (Table 6). Morevoer, aminophylline (5 mg/kg) attenuated the ability of NECA (1.0 mg/kg) to impair the protective efficacy of phenobarbital (Table 7). Partial effect was also observed in the case of papaverine-induced decrease in phenobarbital activity.

Influence of adenosine agonists and antiepileptic drugs alone or in combination upon the body temperature

All adenosine agonists significantly decreased the body temperature. The respective Δt values for L-PIA (0.05 and 0.1 mg/kg), NECA (0.5 and 1 mg/kg) and papaverine (40 mg/kg) were -1.7 ± 0.87, -2.45 ± 0.9, -2.31 ± 0.6, -2.39 ± 0.53, and -0.42 ± 0.87 °C, control Δt being $+0.34 \pm 0.43$ °C. All values were significantly different from control Δ t at $p<0.001$ except that for papaverine where the level of significance was $p<0.05$.

Valproate (185 mg/kg) did not significantly affect the body temperature (Δt: 0.0 ± 0.52 °C). However, combination with L-PIA (0.05 mg/kg) resulted in a hypothermic effect (Δt: -2.33 ± 0.57 °C; $p<0.001$ versus valproate alone and not significant versus L-PIA alone). A similar trend was observed when valproate (200 and 220 mg/kg) was combined with NECA (0.5 mg/kg) and papaverine (40 mg/kg). However, the hypothermic effect observed after the last combined treatment was significant in comparison with both valproate and papaverine-treated groups.

Diazepam (4 mg/kg) itself lowered the body temperature (Δt: -0.63 ± 0.62 versus control Δt: $+0.34 \pm 0.43$ °C; $p<0.01$) and in combination with L-PIA (0.05 mg/kg) resulted in Δt: -2.48 ± 0.88 °C ($p<0.001$ versus diazepam alone and not significant versus L-PIA alone). Diazepam (5.1 mg/kg)-induced hypothermia (Δt: -0.64 ± 0.59 versus control Δt: $+0.2 \pm 0.39$ °C; $p<0.01$) was further increased by NECA at the dose of 0.5 mg/kg (Δt: -2.57 ± 0.64 °C; $p<0.001$ versus diazepam alone) but this effect was not statistically different from hypothermia produced by NECA alone. It is remarkable that adenosine agonists influenced the body temperature in mice pretreated with phenobarbital in the same way. Specifically, phenobarbital alone (20.5 mg/kg) remained without effect upon this parameter (Δt: -0.21 ± 0.93 versus control Δt: -0.043 ± 0.47 °C) and combined treatment with either NECA (1 mg/kg) or papaverine (40 mg/kg) produced distinct hypothermia (Δt: -2.44 ± 0.68 and -1.68 ± 0.71 °C; $p<0.001$ and $p<0.01$, respectively). L-PIA (0.1 mg/kg), NECA (1 mg/kg) and papaverine (40 mg/kg) led also to further decreases in the body temperature in mice pretreated with carbamazepine, which alone in doses of 17 and 19.5 mg/kg produced significant hypothermia. However, the hypothermic response after combined treatment was never significantly different from the effects of adenosine agonists alone.

In some cases the effects of aminophylline (5 mg/kg) on hypothermia induced by the combined treatment of antiepileptic drugs and adenosine agonists were investigated. It remained generally without effect on the response produced by the combination of L-PIA (0.05 mg/kg) with either valproate (185 mg/kg) or diazepam (4 mg/kg).

Discussion

Among the agents affecting purinergic transmission used in the present study, only L-PIA displayed a moderate protective action against electroconvulsions

in mice. This effect was significantly diminished by both aminophylline and enprofylline (adenosine receptor blockers) which could suggest the involvement of A 1 adenosine receptor-mediated events since NECA (a potent A 2 receptor agonist and a weak A 1 receptor agonist; Van Calker et al., 1979), was devoid of any protective activity against electroconvulsions in mice. It is remarkable that the inhibitory potential of L-PIA seems mainly dependent upon its central action and the involvement of peripheral effects (e.g. hypotension) appears less important (Bruns et al., 1983).

Among the antiepileptic drugs studied, the anticonvulsant potency of valproate was the most susceptible to modification by drugs enhancing purinergic transmission. Both aminophylline and enprofylline significantly reversed L-PIA- and papaverine-induced potentiation of valproate's protective action. NECA-produced potentiation was affected to a lesser degree. Furthermore, L-PIA and NECA considerably enhanced the antielectroshock activity of diazepam which was significantly blocked by both xanthine derivatives. L-PIA, in the dose of 0.1 mg/kg, also decreased the ED_{50} value of diphenylhydantoin. The enhancement of antiepileptic drug efficacy by purinergic agonists appears dependent upon A 1 adenosine receptor-mediated events. This suggestion may be supported by the analysis of L-PIA, NECA, and papaverine actions on the antielectroshock activity of phenobarbital and carbamazepine. In contrast to valproate, diazepam, and diphenylhydantoin, the anticonvulsant potentials of carbamazepine and phenobarbital were not modified by L-PIA up to 0.1 mg/kg. Further, NECA and papaverine remained without influence on the anticonvulsant efficacy of carbamazepine and even weakened that of phenobarbital. Keeping in mind that phenobarbital is a selective A 1 adenosine receptor blocker (Lohse et al., 1985), one can assume that its reduced protective activity in combiation with either NECA or papaverine might result from direct or indirect stimulation of the A 2 adenosine receptor population. Carbamazepine interacts with both types of adenosine receptors (Skerrit et al., 1983a; Weir et al., 1984). Analysis of this interaction may lead to the assumption that carbamazepine is in fact an A 1 and A 2 adenosine receptor blocker which renders this drug insensitive to purinergic agonists. However, it is not clear what mechanism is responsible for NECA-induced potentiation of valproate and diazepam antielectroshock effects. It may be that weak A 1 adenosine receptor stimulation induced by NECA is sufficient to produce the observed enhancement of anticonvulsant activity of these antiepileptics.

One should also consider that the potentiation of the antelectroshock activity of some antiepileptic drugs by adenosine agonists might be a consequence of interaction with adenosine receptors unrelated to adenyl cyclase activity (Ribeiro and Sebastiao, 1986). However, at present there is no evidence available on the affinity of antiepileptic drugs towards this type of adenosie receptors.

It is noteworthy that aminophylline, in a low dose of 5 mg/kg, antagonized both potentiating and inhibitory effects of purinergic agonists upon the protective efficacy of the antiepileptics used. This gives support to an assumption

that modification of anticonvulsant drugs action by L-PIA, NECA, and papaverine is a receptor specific process, dependent either on A 1 or A 2 adenosine receptor-mediated events.

Bowker and Chapman (1986) clearly demonstrated that there was a correlation between the anticonvulsant efficacy of adenosine agonists in mice against sound-induced seizures and the lowering of body temperature. The protective activity of adenosine agonists was lost after warming the mice to prevent the adenosine agonist-induced hypothermia. It should be accentuated that the hypothermic response observed after combined treatment with antiepileptic drugs and adenosine agonists in the present study does not seem essential in the enhancement of the protective potential of some antiepileptic drugs. Firstly, hypothermia was observed after combination of phenobarbital and carbamazepine with L-PIA, NECA and papaverine. However, the anticonvulsant activity of the latter was not affected by purinergic agonists and the protection offered by the former was even decreased by NECA and papaverine. Furthermore, low dose aminophylline prevented the L-PIA, NECA and papaverine-induced enhancement of the protective efficacy of valproate and diazepam but did not significantly affect the hypothermic effects resulting from the combined treatment with these antiepileptic drugs and adenosine agonists.

In conclusion, the results obtained indicate that purinergic agonists may differentially affect the protective activities of antiepileptic drugs. Whilst the anticonvulsant effects of valproate and diazepam were distinctly potentiated, the protective efficacy of phenobarbital was either unaffected or even decreased. The contribution of the purinergic system to the effects of carbamazepine is subject to discussion. Some authors propose that this antiepileptic blocks both A 1 and A 2 adenosine receptor populations (Skerrit et al., 1983a; Weir et al., 1984) whilst Fujiwara et al. (1986) classifies carbamazepine as an A 1 adenosine receptor agonist and A 2 adenosine receptor antagonist. Taking into consideration the inability of theophylline to affect the anticonvulsive potency of carbamazepine against amygdala-kindled seizures in rats, Weiss et al. (1985) are of the opinion that the effects of carbamazepine are not related to purinergic transmission. And although there are a great deal of data on the reversal of carbamazepine anticonvulsive activity by methylxanthine derivatives (Czuczwar et al., 1986, 1987c, d; Skerrit et al., 1983b) other mechanisms than adenosine receptor blockade are probably involved in this methylxanthine activity (Czuczwar et al., 1986, 1987c, d). The inability of purinergic agonists to affect the antielectroshock action of carbamazepine provides further evidence that the anticonvulsant activity of this antiepileptic is either unrelated to adenosine receptor-mediated events, or carbamazepine is in fact an adenosine receptor blocker. Studies of Marangos et al. (1985) showing that chronic carbamazepine treatment can induce increases in brain adenosine receptor density appear to support the latter possibility.

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