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N^6-2-(4-Aminophenyl)ethyl-adenosine enhances the anticonvulsive activity of antiepileptic drugs

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Abstract

N^6-2-(4-Aminophenyl)ethyl-adenosine (APNEA, a non-selective agonist of the adenosine A_3 receptors), at the subprotective dose of 1 mg/kg against electroconvulsions, significantly potentiated the anticonvulsive action of phenobarbital, diphenylhydantoin and valproate against maximal electroshock, being ineffective at lower doses. APNEA (0.0039–1 mg/kg) also enhanced the protective activity of carbamazepine. Aminophylline (5 mg/kg) and 8-cyclopentyl-1,3-dimethylxanthine (8-CPX, 5 mg/kg), reversed the APNEA (1 mg/kg)-induced enhancement of the anticonvulsive action of phenobarbital, diphenylhydantoin and valproate, but not that of carbamazepine produced by APNEA at 0.0039 mg/kg. The adenosine agonist did not alter the plasma levels of antiepileptic drugs studied, so a pharmacokinetic interaction is not probable. Finally, APNEA (0.0156 and 1 mg/kg) administered alone or in combination with carbamazepine significantly decreased the body temperature and impaired long-term memory. Our results suggest that APNEA at low doses potentiates the protective activity of carbamazepine most likely through the A_3 subtype of adenosine receptors. At higher doses, APNEA seems to enhance the anticonvulsive effect of other antiepileptics via adenosine A_1 receptors.

Keywords: APNEA (N^6-2-(4-aminophenyl)ethyl-adenosine); Adenosine receptor; Adenosine A_3 receptor; Maximal electroshock; Antiepileptic drug; Seizure

1. Introduction

The pharmacological effects of adenosine analogues include inhibition of locomotor activity and motor coordination (Barraco et al., 1983), sedation (Yarbrough and McGuffin, 1981; Dunwiddie and Worth, 1982), hypothermia (Zarrindast and Heidari, 1993), suppression of food intake (Levine and Morely, 1982) and depression of cardiovascular and respiratory functions (Collis and Hourani, 1993). In the brain, adenosine inhibits Ca^{2+} influx and opens presynaptic K^+ channels causing a significant reduction of the release of glutamate and other neurotransmitters (Ribeiro, 1991). Hence, adenosine is considered to provide an inhibitory tone in the mammalian central nervous system (Harms et al., 1978). The study of Young and Dragunow (1994) supports the hypothesis that status epilepticus and brain injury could result from a loss or impairment of the adenosine seizure termination mechanism. These effects are mediated via cell surface receptors, although high concentrations of adenosine can also inhibit adenyl cyclase via the intracellular P-site (Collis and Hourani, 1993). The purine could either inhibit (via the adenosine A_1 receptor subtype) or stimulate (via the adenosine A_2 receptor subtype) adenyl cyclase activity in cultured nerve cells, adipocytes and hepatocytes (Van Calker et al., 1979). Adenosine A_1 receptors are G protein-linked and can act through effectors other than adenyl cyclase, including K^+ channels, Ca^{2+} channels, phospholipases A_2 or C, and guanylyl cyclase (Collis and Hourani, 1993). Binding affinity at adenosine A_1 sites has commonly been assessed using [^{3}H]*R*-N^6-phenylisopropyladenosine (R-PIA). Ligand affinity at adenosine A_2 sites has been assessed by the displacement of [^{3}H]5′-*N*-ethylcarboxamido-adenosine (NECA) from rat striatal membranes. The majority of the antagonists developed have been xanthines and a number have been claimed to exhibit selectivity for the adenosine A_1 receptor subtype. 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX) and 8-cyclopentyl-1,3-dimethylxanthine (8-CPX), however are the only currently available antagonists that show a consis-

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tent and marked selectivity for the adenosine A_1 receptor subtype. Selectivity for the adenosine A_2 receptor has been claimed for the triazoloquinoxaline, CGS 15943, and the triazoloquinazoline CP 66713 (Collis and Hourani, 1993; Williams et al., 1987).

Recent studies have demonstrated that N^6-2-(4-aminophenyl)ethyl-adenosine (APNEA; a non-selective agonist of adenosine A_3 receptors) lowers blood pressure and that response is resistant to blockade of adenosine A_1 and A_2 receptors by the xanthine adenosine (A_1 and A_2) receptor antagonist 8-(*p*-sulfophenyl)-theophylline (Fozard and Carruthers, 1993). The first report indicating that seizure protection can be obtained through chronic stimulation of the newly discovered adenosine A_3 receptors was published by Von Lubitz et al. (1995).

In the present study, we investigated the influence of APNEA upon the anticonvulsive activity of conventional antiepileptic drugs. Moreover, the effects of APNEA alone or in combination with antiepileptics on the performance of mice in the chimney test, passive avoidance task and body temperature were studied.

2. Materials and methods

2.1. Animals and experimental conditions

The experiments were carried out on female Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experimental temperature was 21 ± 1°C and mice were on a natural light-dark cycle. The experimental groups consisting of 8–12 animals, were chosen by means of a randomized schedule.

2.2. Drugs

Diphenylhydantoin, carbamazepine (both drugs purchased from Sigma, St. Louis, MO, USA), valproate magnesium (Polfa, Rzeszów, Poland), phenobarbital sodium (Polfa, Kraków, Poland), aminophylline (Polfa, Poznań, Poland), 8-CPX and APNEA (N^6-2-(-aminophenyl)ethyl-adenosine), both compounds from Research Biochemicals International (Natick, MA, USA), were used in this study. Diphenylhydantoin, carbamazepine and 8-CPX were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). Valproate, phenobarbital aminophylline and APNEA were brought into solution with sterile saline. All drugs were administered intraperitoneally, in a volume of 10 ml/kg, diphenylhydantoin 120 min, phenobarbital, and 8-CPX 60 min, valproate, carbamazepine, aminophylline and APNEA 30 min before electroconvulsions and behavioral tests.

2.3. Electroconvulsions

Electroconvulsions were produced according to Swinyard et al. (1952), using ear-clip electrodes and alternating current delivered by a Hugo Sachs (Type 221, Freiburg, Germany) generator, the stimulus duration being 0.2 s. Tonic hindlimb extension was taken as the endpoint. The electroconvulsive 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. To estimate the convulsive threshold, at least four groups of mice (8–10 animals per group) were challenged with electroshocks of various intensities. Subsequently, an intensity-response curve was calculated on the basis of the percentage of mice convulsing. In order to evaluate the respective ED_{50} values (in mg/kg) mice pretreated with various doses of antiepileptic drugs were challenged with maximal electroshock (25 mA). Again, at least four groups of mice, consisting of 8–10 animals were used to estimate each ED_{50} value. A dose–effect curve was constructed, basing on the percentage of mice protected.

2.4. Chimney test

The effects of antiepileptic drugs on motor impairment were quantified with the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up the plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of the mice to climb backwards the tube within 60 s and the results were shown as a percentage of animals which failed to perform the test.

2.5. Passive avoidance task

The mice were placed in an illuminated box ($10 \times 13 \times 15$ cm), connected to a large dark box ($25 \times 20 \times 15$ cm) which was equipped with an electric grid floor. Entrance into the dark box was punished by an electric footshock (0.6 mA for 2 s; facilitation of acquisition). The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the next day (24 h later), the same animals were put into the illuminated box and observed up to 180 s. The control (saline-treated) animals did not enter the dark box for over 180 s. According to Venault et al. (1986), the step through passive avoidance task is recognized as a measure of long-term memory.

2.6. 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, APNEA or vehicle were administered and, at the time of the convulsive test, the final temperature was recorded. Body temperature alterations are presented as the means of differences (Δt; $\pm$S.D. of at least 8

measurements) between the reference temperature and the temperature after treatment.

2.7. Estimation of the plasma levels of antiepileptic drugs

The animals were administered a vehicle + an antiepileptic or APNEA with the respective antiepileptic drug. Mice were killed by decapitation at times scheduled for the convulsive test and samples of blood of approximately 1 ml were collected into Eppendorf tubes. Samples of blood were centrifuged at 10 000 rpm (Abbott centrifuge, Irving, TX, USA) for 3 min and plasma samples of 70 μl were transferred into Abbott system cartridges and the rest of the plasma was put to system MPS-1 (Amicon, Danvers, MA, USA) for separation of free from protein-bound microsolutes. Then the MPS-1 tubes were centrifuged at 3000 rpm (MPW-360 centrifuge; Mechanika Precyzyjna, Warsaw, Poland) for 10 min and the filtrate samples of 50 μl were put into Abbott system cartridges. The total and free plasma levels of antiepileptic drugs were estimated by immunofluorescence, using an Abbott Tdx analyzer (Abbott). Plasma levels of antiepileptic drugs were expressed as means ± S.D. of at least 8 determinations.

2.8. Statistics

Both CS_{50} and ED_{50} values and statistical analysis of the data, obtained in the electroconvulsive tests, were estimated by computer probit analysis, according to Litchfield and Wilcoxon (1949). The results from the chimney test were compared statistically by using Fisher's exact probability test, and from passive-avoidance task by Mann-Whitney's test. Plasma levels of antiepileptic drugs and body temperature were evaluated with unpaired Student's *t*-test.

3. Results

3.1. Effects of APNEA upon the electroconvulsive threshold

APNEA (2 mg/kg), applied 30 min before the test, significantly raised the electroconvulsive threshold and this effect was reversed by two adenosine receptor antagonists, aminophylline (5 mg/kg) and 8-CPX (5 mg/kg; Table 1). Both, aminophylline and 8-CPX (at the dose of 5 mg/kg) did not affect the threshold, but 8-CPX at 10 mg/kg, significantly lowered it from 6.1 to 4.8 mA (result not shown in Table 1). APNEA at 0.25 and 1 mg/kg did not significantly influence the threshold (Table 1). In subsequent experiments, this non-selective adenosine A_3 receptor agonist was used up to the subprotective dose of 1 mg/kg.

3.2. Influence of APNEA upon the protective activity of antiepileptic drugs against maximal electroshock-induced seizures in mice

APNEA (0.0039, 0.0078, 0.0156, 0.0625, 0.25 and 1 mg/kg) significantly diminished the ED_{50} of carba-

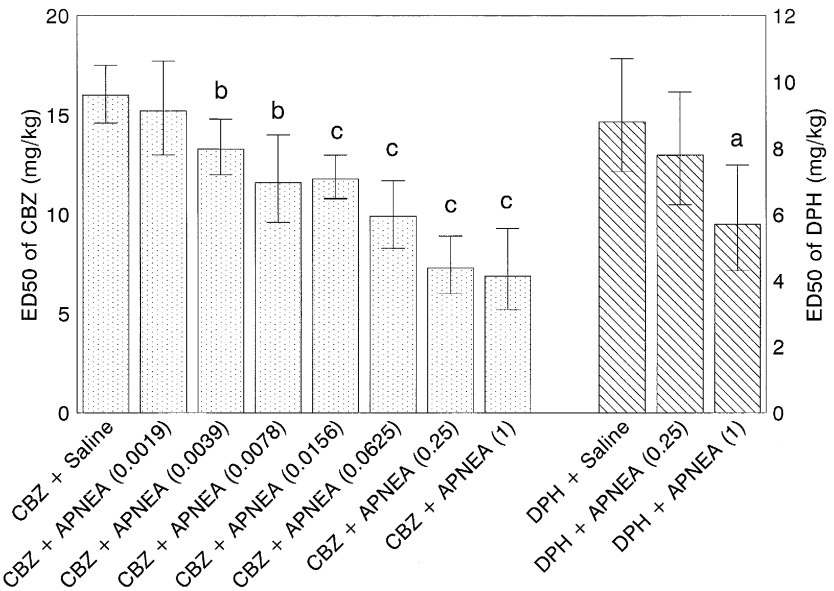

Fig. 1. Effect of N^6-2-(4-aminophenyl)ethyl-adenosine (APNEA) on the anticonvulsive action of carbamazepine or diphenylhydantoin against maximal electroshock-induced seizures in mice. Bars represent ED_{50} values in mg/kg. Error bars show 95% confidence limits for the ED_{50} values. All compounds were injected intraperitoneally in a single dose, diphenylhydantoin 120 min, carbamazepine and APNEA 30 min before the test. The doses of APNEA are shown below each bar in mg/kg. The calculation of the ED_{50} values and statistical analysis were performed according to Litchfield and Wilcoxon (1949). [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. respective control group. Abbreviations: CBZ, carbamazepine; DPH, diphenylhydantoin.

Table 1
Influence of N^6-2-(4-aminophenyl)ethyl-adenosine (APNEA) upon the electroconvulsive threshold in mice

Treatment (mg/kg)	CS_{50} (mA)
Vehicle-1	5.5 (5.1–6.8)
APNEA (0.0039)	5.9 (5.0–7.0)
APNEA (0.25)	5.5 (5.1–6.8)
APNEA (1)	6.2 (5.7–6.8)
APNEA (2)	7.1 (5.9–8.6) [a]
APNEA (2) + AMI (5)	6.2 (6.4–7.2)
Vehicle-2	6.1 (5.7–6.4)
APNEA (2)	6.9 (6.5–7.3) [b]
APNEA (2) + 8-CPX (5)	6.0 (5.5–6.6)

CS_{50} (in mA; with 95% confidence limits in parentheses) is a current strength necessary to produce convulsions in 50% of mice tested. APNEA, AMI, and 8-CPX were administered i.p., 30, 30, and 60 min prior to the electroconvulsive test. AMI, aminophylline; 8-CPX (8-cyclopentyl-1,3-dimethylxanthine). Calculation of CS_{50} values and statistical comparisons were performed according to Litchfield and Wilcoxon (1949). [a] $P < 0.05$, [b] $P < 0.01$ vs. respective vehicle.

mazepine from 16.0 to 13.3, 11.6, 11.8, 9.9, 7.3 and 6.9 mg/kg, respectively. APNEA (at 0.0019 mg/kg) did not influence the protective activity of this antiepileptic drug (Fig. 1). APNEA (1 mg/kg) also potentiated the antielectroshock efficacy of valproate, phenobarbital and diphenylhydantoin, reducing their ED_{50} values from 256 to 228, from 17.2 to 9.9 and from 8.8 to 5.7 mg/kg, respectively. At a lower dose of 0.25 mg/kg, APNEA did not affect the anticonvulsive action of these three antiepileptics (Figs. 1 and 2).

3.3. Influence of aminophylline or 8-CPX on the APNEA-induced enhancement of the protective activity of antiepileptic drugs

Aminophylline (5 mg/kg) reversed the APNEA (1 mg/kg)-induced potentiation of the anticonvulsant activity of phenobarbital, diphenylhydantoin and valproate. On the contrary, neither aminophylline (5 mg/kg) nor 8-CPX (5 mg/kg) affected the influence of APNEA (0.0156 mg/kg) upon the protective activity of carbamazepine against maximal electroshock-induced seizures. The potentiating effect of APNEA (1 mg/kg) upon carbamazepine was partially inhibited by aminophylline (5 mg/kg) or 8-CPX (5 mg/kg; Table 2).

3.4. Chimney test

When applied at doses equal to their ED_{50}s against maximal electroshock, phenobarbital (17.2 mg/kg), diphenylhydantoin (8.8 mg/kg) and carbamazepine (16 mg/kg) did not influence the performance of mice in this test. Only valproate (256 mg/kg) caused motor impairment in 33.3% of the animals (Table 3). APNEA (1 and 0.0156 mg/kg) did not significantly affect the motor coordination of mice tested (results not shown in Table 3). The combined treatment of APNEA (1 mg/kg) with diphenylhydantoin (5.7 mg/kg), carbamazepine (6.9 mg/kg) or phenobarbital (9.9 mg/kg), did not cause any significant motor impairment. The concomitant treatment of carbamazepine (11.8 mg/kg) and APNEA (0.0156

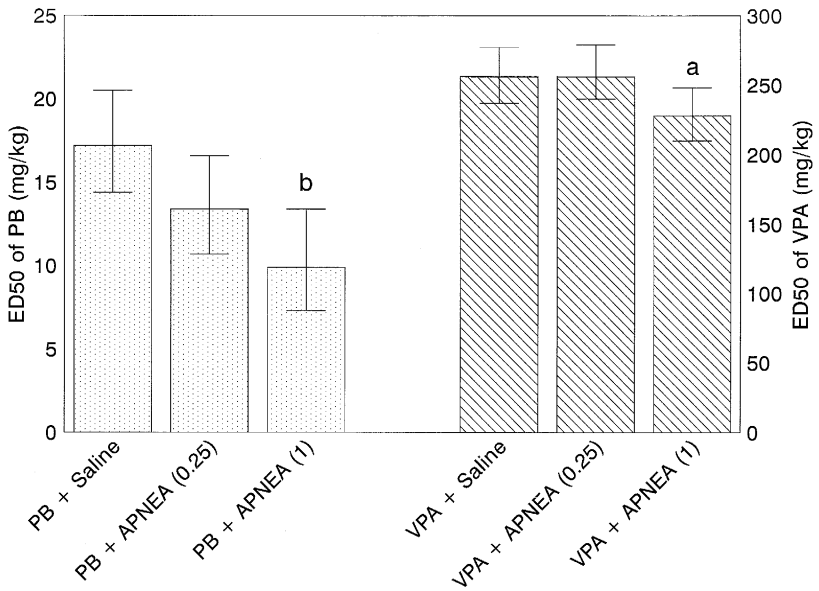

Fig. 2. Effect of N^6-2-(4-aminophenyl)ethyl-adenosine (APNEA) on the anticonvulsive activity of phenobarbital or valproate against maximal electroshock-induced seizures in mice. Bars represent ED_{50} values in mg/kg. Error bars show 95% confidence limits for the ED_{50} values. All compounds were injected intraperitoneally in a single dose, phenobarbital 60 min, valproate and APNEA 30 min before the test. The doses of APNEA are shown below each bar in mg/kg. The calculation of the ED_{50} values and statistical analysis were performed according to Litchfield and Wilcoxon (1949). [a] $P < 0.05$, [b] $P < 0.01$ vs. respective control group. Abbreviations: PB, phenobarbital; VPA, valproate.

Table 2
Influence of aminophylline or 8-CPX on the APNEA-induced potentiation of protective activity offered by antiepileptics

Treatment (mg/kg)	ED_{50} (mg/kg)
Valproate + saline	255 (239–271)
Valproate + APNEA (1)	226 (214.5–246) [a]
Valproate + APNEA (1) + AMI (5)	258.8 (239–281)
Phenobarbital + saline	17.2 (14.4–20.5)
Phenobarbital + APNEA (1)	10.1 (7.5–13.3) [b]
Phenobarbital + APNEA (1) + AMI (5)	15.2 (12.1–19.0)
Diphenylhydantoin + saline	8.7 (7.3–10.3)
Diphenylhydantoin + APNEA (1)	5.6 (4.4–7.1) [a]
Diphenylhydantoin + APNEA (1) + AMI (5)	8.5 (7.3–10.0)
Carbamazepine + saline	16.1 (14.5–17.8)
Carbamazepine + APNEA (1)	7.1 (5.4–9.0) [c]
Carbamazepine + APNEA (1) + AMI (5)	11.3 (10.0–12.8) [c,d]
Carbamazepine + APNEA (0.0156)	11.8 (10.9–12.9) [c]
Carbamazepine + APNEA (0.0156) + AMI (5)	11.4 (9.4–13.8) [c]
Carbamazepine + saline	14.8 (12.6–17.4)
Carbamazepine + APNEA (1)	6.8 (5.4–8.7) [c]
Carbamazepine + 8-CPX (5)	14.1 (12.2–17.1)
Carbamazepine + APNEA (1) + 8-CPX (5)	10.4 (8.9–12.2) [b,d]
Carbamazepine + APNEA (0.0156)	8.8 (7.3–10.7) [c]
Carbamazepine + APNEA (0.0156) + 8-CPX (5)	9.5 (8.2–11.1) [c]

Table data are ED_{50} values (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). All drugs were administered i.p., diphenylhydantoin 120 min, phenobarbital and 8-CPX 60 min, valproate and APNEA 30 min before the test. AMI, aminophylline; 8-CPX, 8-cyclopentyl-1,3-dimethylxanthine. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. respective control group; [d] $P < 0.01$ vs. carbamazepine + APNEA (1 mg/kg)-treated group. See also Table 1.

Table 3
Motor impairment after administration of antiepileptic drugs, APNEA or a combination of APNEA with an antiepileptic

Treatment (mg/kg)	Mice impaired (%)
Vehicle	0
Carbamazepine (16)	16.6
Carbamazepine (11.8)	0
Carbamazepine (11.8) + APNEA (0.0156)	16.6
Carbamazepine (6.9)	0
Carbamazepine (6.9) + APNEA (1)	0
Diphenylhydantoin (8.8)	8.3
Diphenylhydantoin (5.7)	0
Diphenylhydantoin (5.7) + APNEA (1)	16.6
Phenobarbital (17.2)	8.3
Phenobarbital (9.9)	0
Phenobarbital (9.9) + APNEA (1)	16.6
Valproate (256)	33.3 [a]
Valproate (228)	8.3
Valproate (228) + APNEA (1)	50.0 [b]

The results are expressed in percentage of animals that failed to perform the chimney test (see Section 2). The experimental groups consisted of 12 mice. [a] $P < 0.05$, [b] $P < 0.01$ vs. vehicle (Fisher's exact probability test). Antiepileptics at higher doses and combined treatment provide a 50% protection against maximal electroshock. See also Tables 1 and 2.

mg/kg), still being an equivalent of an ED_{50} value for carbamazepine alone against maximal electroshock, resulted in no motor disturbances. Valproate (228 mg/kg) injected simultaneously with APNEA (1 mg/kg), which provided a 50% protection against maximal electroshock, produced significant motor deficit in 50% of the animals (Table 3).

3.5. Dark avoidance task

Phenobarbital, diphenylhydantoin and carbamazepine, when given at the doses equal to their ED_{50}s against maximal electroshock, caused a moderate impairment of long-term memory. Valproate at its ED_{50} of 256 mg/kg caused a strong worsening of the performance of mice in this memory task. APNEA (0.0156–1 mg/kg) also produced memory deficits. The combined treatment of APNEA (1 mg/kg) with phenobarbital (9.9 mg/kg), diphenylhydantoin (5.7 mg/kg), valproate (228 mg/kg) and carbamazepine (6.9 mg/kg), providing a 50% protection against maximal electroshock, resulted in the pronounced impairment of long-term memory when compared to the effects of these antiepileptics alone given at their ED_{50}s against maximal electroshock. Nevertheless, co-administration of APNEA (0.0156 mg/kg) with carba-

Table 4
Memory impairment after administration of antiepileptic drugs, APNEA or a combination of APNEA with an antiepileptic

Treatment (mg/kg)	Time (s)
Vehicle	> 180
APNEA (1)	80.0 ± 18.84 [c]
APNEA (0.25)	95.6 ± 16.54 [b]
APNEA (0.0625)	117.1 ± 14.71 [b]
APNEA (0.0156)	138.9 ± 13.66 [b]
Carbamazepine (16)	133.3 ± 13.39 [b]
Carbamazepine (11.8)	159.2 ± 8.95 [b]
Carbamazepine (6.9)	> 180
Carbamazepine (6.9) + APNEA (1)	50.6 ± 13.14 [c,f]
Carbamazepine (11.8) + APNEA (0.015625)	138.9 ± 11.9 [b]
Diphenylhydantoin (8.8)	124.5 ± 14.96 [c]
Diphenylhydantoin (5.7)	131.2 ± 15.29 [b]
Diphenylhydantoin (5.7) + APNEA (1)	73.9 ± 12.62 [c,d]
Phenobarbital (17.2)	141.7 ± 12.73 [a]
Phenobarbital (9.9)	147.8 ± 12.73 [a]
Phenobarbital (9.9) + APNEA (1)	69.1 ± 15.04 [c,e]
Valproate (256)	57.9 ± 13.14 [c]
Valproate (228)	75.8 ± 16.00 [c]
Valproate (228) + APNEA (1)	27.4 ± 5.08 [c,e]

Presented values are the means of 12 determinations ± S.E. The retention was quantified as a time period, the animals were avoiding the dark compartment. Mann-Whitney's test was used for statistical analysis of the data. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. vehicle; [d] $P < 0.05$ vs. diphenylhydantoin (5.7 mg/kg), [e] $P < 0.01$ vs. phenobarbital (9.9 mg/kg) or valproate (228 mg/kg), [f] $P < 0.001$ vs. carbamazepine (6.9 mg/kg). See also the legends to Tables 2, 3 and 4.

mazepine (11.8 mg/kg) did not significantly worsen the performance of mice in passive-avoidance task when compared to carbamazepine (11.8 mg/kg) alone-treated group (Table 4).

3.6. Influence of APNEA and carbamazepine alone or in combination upon the body temperature

APNEA significantly decreased the body temperature. The respective Δt values for APNEA (0.0156 and 1 mg/kg) were: -1.67 ± 0.55 and -2.2 ± 0.04°C, control Δt being -0.61 ± 0.26°C. Both values were significantly different from control Δt at $P < 0.01$ and $P < 0.001$, respectively.

Carbamazepine (6.9 mg/kg) did not significantly affect the body temperature (Δt: 0.17 ± 0.15°C). However, a combination with APNEA (0.0156 mg/kg) resulted in a hypothermic effect (Δt: -1.67 ± 0.26°C; $P < 0.01$ versus carbamazepine alone and versus control group). Similarly, co-administration of carbamazepine with APNEA (1 mg/kg) caused more pronounced hypothermia (Δt: -3.0 ± 0.52°C; $P < 0.001$ versus carbamazepine alone and versus control).

3.7. Influence of APNEA on the plasma levels of antiepileptic drugs

APNEA (1 mg/kg) did not alter the total and free plasma levels of carbamazepine (11.8 mg/kg), valproate (228 mg/kg) and diphenylhydantoin (5.7 mg/kg). APNEA (1 mg/kg) did not also affect the total plasma levels of phenobarbital (9.9 mg/kg) (Table 5).

3.8. Influence of aminophylline in combination with APNEA on the plasma levels of antiepileptic drugs

Aminophylline (5 mg/kg), administered together with APNEA (1 mg/kg), did not alter the plasma levels of antiepileptic drugs studied (Table 6).

Table 5
Influence of APNEA upon the total and free plasma levels of antiepileptic drugs

Treatment (mg/kg)	Plasma level	
	Total	Free
Carbamazepine (7.1)	5.79 ± 1.30	1.12 ± 0.14
Carbamazepine (7.1) + APNEA (1)	5.62 ± 1.22	1.05 ± 0.09
Diphenylhydantoin (5.7)	3.40 ± 0.65	0.44 ± 0.09
Diphenylhydantoin (5.7) + APNEA (1)	3.52 ± 0.59	0.56 ± 0.05
Phenobarbital (10.1)	11.54 ± 0.94	N.D.
Phenobarbital (10.1) + APNEA (1)	11.16 ± 0.75	N.D.
Valproate (226)	289.2 ± 22.84	249.3 ± 19.69
Valproate (226) + APNEA (1)	284.2 ± 23.44	245.6 ± 20.46

Presented values are the means (in μg/ml of plasma) of 8 determinations ± S.D. Unpaired Student's *t*-test was used for statistical analysis of the data. N.D., not determined. See also the legends to Tables 1 and 2.

Table 6
Influence of the combined treatment of APNEA with methylxanthines upon the plasma levels of antiepileptic drugs

Treatment (mg/kg)	Plasma level
Carbamazepine (7.1)	1.47 ± 0.30
Carbamazepine (7.1) + APNEA (1) + AMI (5)	1.25 ± 0.12
Carbamazepine (6.8)	1.24 ± 0.40
Carbamazepine (6.8) + APNEA (1) + 8-CPX (5)	1.28 ± 0.41
Diphenylhydantoin (5.6)	0.44 ± 0.03
Dipenylhydantoin (5.6) + APNEA (1) + AMI (5)	0.46 ± 0.03
Phenobarbital (10.1)	11.96 ± 1.68
Phenobarbital (10.1) + APNEA (1) + AMI (5)	12.19 ± 0.96
Valproate (226)	240.5 ± 28.81
Valproate (226) + APNEA (1) + AMI (5)	235.3 ± 23.89

Only the plasma level of phenobarbital is total; the remaining plasma levels are free. AMI, aminophylline. See also the legends to Tables 1, 2 and 5.

3.9. Effect of 8-CPX combined with APNEA on the free plasma level of carbamazepine

The free plasma level of carbamazepine was no affected by co-administration of 8-CPX (5 mg/kg) + APNEA (1 mg/kg; result not shown in Table 6).

4. Discussion

The present study clearly demonstrates that APNEA, at the subprotective dose of 1 mg/kg against electroconvulsions, enhanced the anticonvulsive activity of all antiepileptics studied. But only in the case of carbamazepine, its protective action was potentiated by APNEA in lower doses than 1 mg/kg. The potentiating effect of APNEA upon the antiepileptics tested was not associated with an increased plasma level of any antiepileptic drug so a pharmacokinetic interaction may be excluded.

APNEA is a non-selective adenosine A_3 receptor agonist which possesses a substantial affinity towards adenosine A_1 receptors as well (Jacobson et al., 1995). However, it has been demonstrated that this compound lowers blood pressure and this response is resistant to blockade by the xanthine adenosine receptor antagonist, 8-(*p*-sulfophenyl)theophylline (Fozard and Carruthers, 1993). According to Linden (1994), human and sheep, but not rat adenosine A_3 receptors are potently blocked by certain xanthines, notably acidic 8-phenylxanthines. Jacobson et al. (1993) provided evidence that intraperitoneal injection of a selective adenosine A_3 receptor agonist N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide (IB-MECA) into mice produced a 50% reduction in locomotor activity. This effect was not blocked by adenosine A_1 and A_{2A} receptor antagonists and seemed to be mediated by adenosine A_3 receptors.

Activation of adenosine A_1 receptors is most likely responsible for the reduced seizure activity in mice

(Dragunow, 1991; Marangos and Miller, 1991; Von Lubitz and Marangos, 1992). Acute systemic administration of IB-MECA resulted in the protective effect against chemically, but not electrically induced seizures (Von Lubitz et al., 1995). According to these authors, this fact may suggest that amelioration of convulsions induced by *N*-methyl-D-aspartate (NMDA) may be based upon pharmacokinetic phenomena rather than a direct action of the drug. IB-MECA, as an adenosine A_3 receptor agonist, exerts vasoconstricting properties, which may be followed by a decrease in the absorption of NMDA, so the plasma concentration of this chemoconvulsant could not reach the epileptogenic level. On the other hand, the protective effect of chronically administered IB-MECA was evident in both chemically and electrically evoked seizures. It remains unclear, whether the results of chronic stimulation of adenosine A_3 receptor was related to the cerebral blood perfusion, neuronal mechanisms, or both. Nevertheless, it was demonstrated that chronic treatment with IB-MECA caused a slight but significant hypertension (Von Lubitz et al., 1994). Our data suggest that the protective effect of this non-selective adenosine A_3 receptor agonist seems dependent upon its central effects. Any decrease in the penetration of antiepileptic drugs into the brain would result in their reduced anticonvulsive effects.

It was reported that carbamazepine exerts both antagonistic- and agonistic-like influence on adenosine receptors (Phillis, 1984; Fujiwara et al., 1986, respectively). Some authors propose that this antiepileptic blocks both adenosine A_1 and A_2 receptor populations (Skerrit et al., 1983; Weir et al., 1984) whilst Fujiwara et al. (1986) classifies carbamazepine as an adenosine A_1 receptor agonist and adenosine A_2 receptor antagonist. Diphenylhydantoin was shown to inhibit adenosine uptake into presynaptic terminals which resulted in an increase in the extracellular level of this neurotransmitter (Phillis, 1984). Phenobarbital, like carbamazepine, is a potent displacer of R-PIA. Diphenylhydantoin was less potent and valproate remained ineffective in this respect (Skerrit et al., 1982). According to Weir et al. (1984), the most effective displacer of cyclohexyladenosine was carbamazepine, phenobarbital exerted a weaker potency, diphenylhydantoin and valproate were quite ineffective. Several authors reported that phenobarbital may be rather an adenosine A_1 receptor antagonist (Skerrit et al., 1983; Weir et al., 1984). There is so far no evidence on the relationship between antiepileptic drugs and adenosine A_3 receptors. The complete reversal by aminophylline (5 mg/kg) of the APNEA (1 mg/kg)-induced potentiation of the protective activity of diphenylhydantoin, phenobarbital, and valproate may suggest an involvement of adenosine A_1 receptor-mediated events in this effect. This assumption may be based upon the finding that the enhancement of the protective action of conventional antiepileptic drugs against maximal electroshock-induced seizures was dependent upon adenosine A_1 receptor stimulation. In fact, activation of adenosine A_2 receptors led to an opposite effect (for discussion, see Czuczwar et al., 1990). On the other hand, the APNEA (1 mg/kg)-evoked enhancement of the anticonvulsive efficacy of carbamazepine was only partially inhibited by aminophylline or 8-CPX. The protective activity of carbamazepine after this partial inhibition was equal to that observed after combination with APNEA at a low dose of 0.0156 mg/kg, probably activating preferentially adenosine A_3 receptors.

Alkylxanthines block both adenosine A_1 (Daly et al., 1981) and A_2 (Van Calker et al., 1979) receptor subtypes. Aminophylline (50 mg/kg) impaired the anticonvulsive action of phenobarbital, valproate, diphenylhydantoin, and carbamazepine against maximal electroshock in mice (Czuczwar et al., 1986; Czuczwar et al., 1987; Czuczwar et al., 1989). In contrast, 8-(*p*-sulfophenyl)theophylline (a theophylline derivative unable to cross the blood-brain barrier) did not influence the protective activity of conventional antiepileptics (Borowicz et al., 1993). This indicates that the aminophylline-induced impairment of the anticonvulsive action of antiepileptics results from the central effects of this methylxanthine. However, the aminophylline (50 mg/kg)-induced impairment of the protective activity of conventional antiepileptic drugs against electroconvulsions is probably not associated with the blockade of adenosine A_1 receptors (for discussion see Czuczwar and Kleinrok, 1990). Actually, aminophylline at 5 mg/kg was ineffective upon the protection provided by antiepileptic drugs against maximal electroshock (Czuczwar et al., 1989).

Our findings may supplement previous reports. Aminophylline does not seem to be an adenosine A_3 receptor antagonist in mice. APNEA at high doses may stimulate A_1 and A_3 adenosine receptor subtypes, and its subsequent anticonvulsive action seems to be mediated by the former. Such an assumption may be supported by the reversal of its anticonvulsive activity not only by aminophylline, but by the selective adenosine A_1 receptor antagonist, 8-CPX, as well. APNEA administered at low doses may be assumed to activate mainly adenosine A_3 receptors, since its potentiating effect upon carbamazepine was neither blocked by aminophylline nor 8-CPX.

Adenosine A_1 receptor activation resulted in an impairment of acquisition while adenosine A_2 receptor activation was ineffective upon this parameter (Zarrindast and Bijan, 1994). Our study, in addition to the data obtained by Zarrindast and Bijan (1994), provided evidence that a probable stimulation of adenosine A_3 receptors by APNEA in a low dose, also resulted in the impairment of acquisition in the passive avoidance task. Finally, we found that APNEA, also in the low dose range, caused hypothermia. Previously, Zarrindast and Heidari (1993) noticed a hypothermic effect after stimulation of adenosine A_1 receptors. Interestingly, APNEA (up to 1 mg/kg) did not affect motor coordination evaluated in the chimney test, in spite to the well known central depressant effects of

adenosine A_1 receptor agonists upon a number of behavioral parameters in rodents (see Section 1).

Dragunow (1991) has suggested that adenosine might be the brain's natural anticonvulsant neurotransmitter since many convulsive procedures led to a considerable rise in extracellular levels of adenosine. During and Spencer (1992) provided a clear-cut evidence that this is really the case in epileptic patients, too. Their studies, with the use of microdialysis probes implanted in the hippocampi of epileptic patients with intractable complex partial epilepsy, revealed that during seizures, extracellular levels of adenosine in the dialysate were elevated by 6- to 31-fold.

In conclusion, APNEA (in the low dose range) appears to enhance the protective activity of carbamazepine against maximal electroshock via adenosine A_3 receptor-mediated effect. In our opinion, APNEA or more selective adenosine A_3 receptor agonists may be a valuable pharmacological tool to investigate the dependence of the protective effects of various anticonvulsive drugs and agents on adenosine A_3 receptor-mediated events. The preferential sensitivity of carbamazepine, among other conventional antiepileptic drugs, to adenosine A_3 receptor stimulation should be especially underlined and may bear a potential clinical significance. The only limitations might be a memory deficit and hypothermia observed upon a combined treatment of carbamazepine and APNEA. The advantage of the combined treatment of APNEA with the remaining antiepileptic drugs seems questionable. One could hypothesize that the efficacy of carbamazepine might be low in patients with disturbed adenosine functions.

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