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Influence of Chronic Aminophylline on Antielectroshock Activity of Diazepam and Aminophylline-Induced Convulsions in Mice

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WLAŹ, P., Z. ROLIŃSKI, Z. KLEINROK AND S. J. CZUCZWAR. *Influence of chronic aminophylline on antielectroshock activity of diazepam and aminophylline-induced convulsions in mice.* PHARMACOL BIOCHEM BEHAV **49**(3) 609–613, 1994.—The effects of chronic administration of aminophylline (AMPH; 50 mg/kg, twice daily for 14 consecutive days) were studied on both antielectroshock efficacy of diazepam (DZP) and convulsive activity of AMPH in mice. AMPH injected acutely at a dose of 50 mg/kg significantly reduced anticonvulsant action of DZP elevating ED_{50} from 10.9 (control) to 15.9 mg/kg ($p < 0.01$). After the administration of AMPH for 3 days, ED_{50} value was still higher compared with control. Chronic treatment with AMPH resulted in further increase of ED_{50} of DZP, which was 20.2 mg/kg, and this elevation was significant not only when compared with saline-treated animals, but also with acute and 3-day administration of the xanthine ($p < 0.01$, 0.05, and 0.001, respectively). Therefore, no tolerance to this AMPH-mediated effect was found, and even an enhancing influence was observed. On the other hand, chronic treatment with AMPH decreased convulsive activity of AMPH elevating ED_{50} for induction of clonic seizures from 218 to 252 mg/kg ($p < 0.01$). The remaining seizure parameters were unaffected. Furthermore, in both cases pharmacokinetic interactions were excluded, at least in terms of total plasma levels of the drugs. The results suggest that the mechanisms governing AMPH-induced reversal of the anticonvulsant efficacy of DZP qualitatively differ from those underlying AMPH-induced convulsions. Moreover, these data support the claim that AMPH should be avoided in patients suffering from different types of epilepsy.

Diazepam Chronic aminophylline Maximal electroshock Aminophylline-induced convulsions Tolerance

METHYLXANTHINES such as theophylline and caffeine are widely prescribed antiasthmatic drug and, undoubtedly, are the most frequently used over-the-counter stimulants, respectively (23). Both these drugs possess many undesirable actions, among them the ability to evoke dangerous convulsions in humans (32) and severe ones in laboratory animals (9), and to decrease the protective effectiveness of common antiepileptic drugs [for review, see (11)].

The way in which theophylline diminishes the anticonvulsant potency of antiepileptics is not known, and several mechanisms of this reversal have been discussed (11). Difficulties in the elucidation of this phenomenon derived from different involvement of antiepileptic drugs in purinergic transmission (21,24,29).

After chronic administration of methylxanthines, tolerance rapidly develops to their various behavioral actions (13,23). However, recently we studied the effects of chronic aminophylline (AMPH; theophylline$_2$ · ethylenediamine) treatment on anticonvulsant efficacy of a number of antiepileptic drugs in mice and observed no tolerance to the AMPH-induced reversal of this efficacy and observed that this AMPH effect was even enhanced over time (30,31).

Among the antiepileptic drugs, diazepam (DZP) is regarded to be a drug of choice for emergency treatment of

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status epilepticus. It has been postulated that some of the pharmacological actions of benzodiazepines may be due to interactions with adenosine (15). Anticonvulsant potency of DZP was diminished by acute aminophylline in a dose-dependent manner (5,6). Further, this antiepileptic, apart from valproate, was the only one conventional anticonvulsant efficiently blocking AMPH-induced seizures in mice (9).

Some patients suffering from epilepsy are simultaneously medicated with AMPH or theophylline for pulmonary reasons for prolonged periods. In such cases, from a clinical point of view it is essential to know whether AMPH chronic treatment still impairs the protective efficacy of DZP.

Therefore, the aim of the present study was to examine the influence of chronic AMPH treatment upon protection offered by DZP against maximal electroshock seizures as well as on AMPH convulsive properties in mice, taking into account that chronic theophylline administration elevated seizure threshold to a variety of chemical convulsants (26). In addition, a possibility of a pharmacokinetic nature of such an interaction was also considered.

METHOD

Animals

Male Swiss mice, initially weighing 25–30 g, were used in this study. The animals were housed in colony cages on standard laboratory conditions (with chow pellets and tap water continuously available) and maintained on a 12 L : 12 D cycle (lights on at 0800 h).

Maximal Electroshock Seizures

Maximal electroshock seizures (MES) were produced as described elsewhere (25). Mice received electrical stimulus via corneal electrodes. Parameters of the stimuli were as follows: sine-wave alternating current at the frequency of 50 Hz, the intensity—50 mA, and the duration—200 ms. Electrical system of the generator (GE-01, COTM, Białystok, Poland) was self-adjustable and the changes in external resistance did not result in the alterations of the current intensity. The tonic hindlimb extension was taken as the end point. Consequently, abolition of the end point was considered as a protective action of an antiepileptic drug.

To evaluate the antielectroshock efficacy of DZP under each treatment at least four groups consisting of 8–10 animals were injected intraperitoneally (IP) with increasing doses of the drug (dosage range—8.8–25 mg/kg) and then challenged with MES. The percentage of mice lacking in the end point was noted and a dose-effect relationship was converted into ED_{50} values with 95% confidence limits by a computer program written on a basis of the method of Litchfield and Wilcoxon (16). The MES test was always performed between 0830 and 1000 h.

AMPH-Induced Convulsions

Mice were injected IP with increasing doses of AMPH (dosage range—190–280 mg/kg) and then observed for 60 min. Clonic and tonic convulsions as well as mortality were noted throughout this period. The details on convulsive procedure were described elsewhere (9). From percentage of convulsive responses the ED_{50} values were calculated by fitting the data by computer linear regression analysis according to the method of Litchfield and Wilcoxon (16). At least four groups consisting of 10 animals were used to evaluate each ED_{50} value.

Drugs

Diazepam (DZP, Relanium, Polfa, Warsaw, Poland) was suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria) and given IP 60 min prior to MES.

Aminophylline (AMPH, Aminophyllinum, Polfa, Poznań, Poland) was dissolved in sterile saline and administered IP according to the treatment protocol (see below). The last injections of AMPH were done 30 min before MES.

Treatment Protocol

Animals were injected twice daily at 0800 and 2000 h with a) saline for 14 days, or b) saline for 11 days and then AMPH (50 mg/kg) for next 3 days, or c) AMPH (50 mg/kg) for 14 days.

On the fifteenth day mice from all groups received DZP (60 min before the test) and AMPH (30 min), and were challenged with MES.

Similarly, in the case of AMPH-induced seizures, on the fifteenth day group 3 was injected with increasing doses of AMPH.

Estimation of plasma levels of DZP and AMPH was carried out in mice derived from group 3.

In all cases, a group served as a control that was administered only saline, including the last injection.

Estimation of DZP and Theophylline Plasma Levels

The measurements of DZP and theophylline plasma levels were performed by fluorescence polarization immunoassay using an Abbott TDx Analyzer (Abbott, Irving, TX). The detailed procedure was described previously (8,30,31). Plasma levels of both drugs were estimated in animals receiving saline for 14 days (controls) and AMPH for the same period. DZP was given 60 min and AMPH 30 min before blood sample collection.

Statistics

Statistical comparisons of the respective ED_{50} values (with 95% confidence limits) were done with the help of computerized method of Litchfield and Wilcoxon (16). Concentrations of DZP and theophylline were expressed in $\mu g/ml$ as arithmetic means ± SD of at least eight determinations per experimental group, and compared by means of Student's *t*-test for unpaired observations.

RESULTS

Influence of Chronic AMPH on the Anticonvulsant Activity of Diazepam

Acute administration of AMPH in a dose of 50 mg/kg resulted in a significant elevation of the ED_{50} of DZP. Specifically, ED_{50} rose from 10.9 (8.8–13.5) (control) to 15.9 (13.5–18.6) (Fig. 1). Three-day treatment with AMPH (50 mg/kg, twice daily) did not increase significantly ED_{50} above the level obtained after single injection of AMPH (Fig. 1). Chronic treatment with AMPH (14 days, 50 mg/kg, twice daily) further decreased anticonvulsant potency of DZP which resulted in the elevation of ED_{50} value up to 20.2 (18.1–22.6) (Fig. 1). It was statistically significant not only when compared with control, but also with acute and 3-day administration of AMPH ($p < 0.001$, 0.05, and 0.001, respectively).

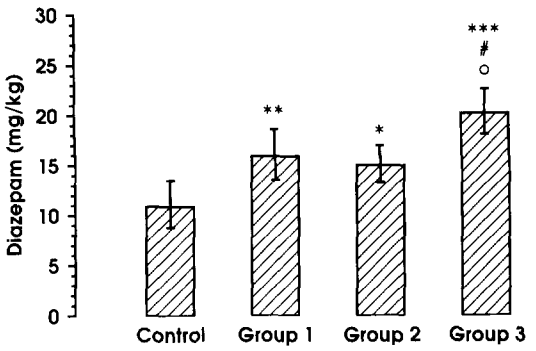

FIG. 1. Influence of chronic aminophylline (AMPH) treatment upon the antielectroshock activity of diazepam (DZP) in mice. Bars represent ED_{50} values and respective 95% confidence limits (vertical lines). Group 1 was treated with saline for 14 days twice daily; group 2 with saline for 11 days, and with AMPH (50 mg/kg) for next 3 days; group 3 with AMPH for 14 days. On the 15 day groups 1-3 received AMPH and DZP, 30 and 60 min before testing, respectively. Control group was given saline for a whole period, and saline + DZP on the fifteenth day. Statistical evaluation was carried out according to the method of Litchfield and Wilcoxon (16). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. control; $\#p < 0.05$ vs. group 1; $^{\circ}p < 0.001$ vs. group 2.

Effect of Chronic Administration of AMPH Upon AMPH-Induced Convulsions

Fourteen-day treatment with AMPH (50 mg/kg, twice daily) influenced susceptibility of mice to AMPH only with reference to clonic phase of convulsions. Specifically, ED_{50} for clonic seizures was elevated from 218 (201-237) to 252 (235-270) mg/kg; $p < 0.01$ (Fig. 2). The remaining seizure parameters were not significantly affected (Fig. 2).

Plasma levels of DZP and Theophylline After Chronic Treatment With AMPH

AMPH administered chronically for 14 days (50 mg/kg, twice daily) did not alter either the total plasma level of diazepam (18 mg/kg, 60 min prior to blood sampling) or theophylline (AMPH 200 mg/kg, 30 min). Both drugs were injected on the fifteenth day.

The mean (±SD from eight determinations) control values for DZP and theophylline were 3.92 ± 0.67 and 202.3 ± 42.2 μg/ml, respectively. In chronically AMPH-treated mice, plasma levels of DZP and theophylline reached 3.44 ± 0.72 and 211.4 ± 22.0 μg/ml, respectively.

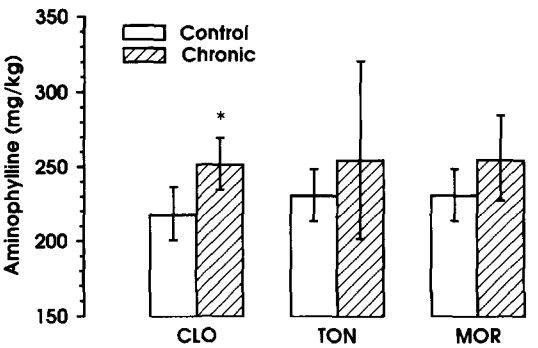

FIG. 2. Effect of chronic aminophylline (AMPH) on its own convulsive potential in mice. Mice from chronic group received AMPH twice daily for 14 days while those from control saline for the same period. On the fifteenth day animals from both groups were injected with increasing doses of AMPH. CLO—clonic seizures; TON—tonic seizures; MOR—mortality; Statistical evaluation was performed according to the method of Litchfield and Wilcoxon (16). $*p < 0.01$ vs. control. For further details see also legend of Fig. 1.

DISCUSSION

The present experiments cogently show that long-term treatment with AMPH led to a further reduction of the anticonvulsant action of DZP in comparison with the acute AMPH injection. A possibility of the pharmacokinetic origin of this interaction was excluded at least in terms of the total DZP plasma level, which was not affected by chronic exposure to AMPH. Moreover, in our previous paper we documented that theophylline plasma level after prolonged AMPH administration was even significantly lower than that obtained after acute AMPH injection (30). It should be underlined, that the AMPH dose of 50 mg/kg utilized in the present study, calculated on the basis of surface area, well approximates that in clinical conditions in humans. Furthermore, 50 mg/kg of AMPH is far below its CD_{50} for induction of clonic seizure activity (9), and this dose is well within the range normalizing bronchial constrictions in animal models of asthma (18).

Chronic treatment with methylxanthine derivatives leads to the rapid development of tolerance to their various behavioral actions (13). With reference to the reversal of DZP anticonvulsant activity caused by chronic AMPH, no such phenomenon was observed.

The fact that the protective activity of DZP was reversed by AMPH is in line with available data. A number of studies have demonstrated an ability of AMPH and caffeine to diminish the anticonvulsant potency of DZP in pentylenetetrazol-induced seizures (2), maximal electroshock convulsions (5), and amygdala-kindled seizures in rats (1). Conversely, benzodiazepine agonists and/or antagonist (flumazenil) suppressed the convulsive effects of AMPH (9) and caffeine (19,28), although most of classic antiepileptics were ineffective in this respect (9). AMPH also partially antagonized the myorelaxant action of DZP in genetically spastic rats (27).

Another finding from the present study is that chronic AMPH partially decreased the susceptibility of mice to the convulsive properties of AMPH, only clonic phase of convulsions being significantly affected. This is consistent with data published by Szot et al. (26), showing that chronic theophylline reduced the susceptibility of rats to a series of chemical convulsants. Thus, the mechanisms governing AMPH-induced reversal of the anticonvulsant efficacy of DZP qualitatively differ from those underlying AMPH-induced convulsions.

Mechanisms of AMPH-induced phenomena have not been yet sufficiently clarified. It is suspected that blockade of adenosine receptors, inhibition of phosphodiesterase, mobilization of intracellular calcium ions, or finally, enhanced excitatory amino acids release may be of importance (4,14). However, in clinical conditions, theophylline concentrations capable of blocking the phosphodiesterase activity may only occasionally be reached (14). Caffeine and theophylline also competitively inhibit [^{3}H]diazepam binding to the benzodiazepine receptors in vitro (19). Nevertheless, this finding was not confirmed in vivo (22).

Our recent studies mentioned in the preamble (30,31) have provided evidence that after chronic AMPH treatment no tolerance developed to the AMPH-induced reversal of the an-

ticonvulsant efficacy of phenobarbital, valproate, carbamazepine, and diphenylhydantoin. Furthermore, two-week treatment increased ED_{50}s of antiepileptic drugs studied vs. acute AMPH injection.

Tolerance that develops to various behavioral actions of caffeine and related methylxanthines after long-term administration is commonly considered as a result of adenosine receptor upregulation (3). On the other hand, an increase in the adenosine receptor number in mouse cerebral cortex was not associated with the enhanced adenosinergic neurotransmission and even downregulation of adenosine receptor function was found (17). Having in mind that methylxanthines are potent adenosine receptor blockers (14,23), a hypothesis may arise that the impairment of the purinergic transmission might be responsible for the decreased efficacy of DZP which was actually documented to inhibit adenosine uptake (21). However, this may not seem to be the case because CGS 15943A (a nonxanthine adenosine antagonist) was completely ineffective upon MES inhibition by DZP (7).

As a conclusion, it can be postulated that DZP may not provide sufficient protection against convulsive events when coadministered with AMPH, and the hazardous influence of this methylxanthine may be further enhanced when AMPH is administered chronically. This strongly supports the notion that AMPH should be avoided in patients with different types of epilepsy (6,11,12). An alternative could be enprofylline (3-propylxanthine), possessing even stronger bronchodilatory properties than AMPH and remaining without influence on the protection offered by common antiepileptic drugs (10,12). However, chronic enprofylline has not been studied as yet in this regard.

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