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Differential Effects of Various Antiinflammatory Drugs on Theophylline Neurotoxicity

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HOFFMAN, A., M. AFARGAN, E. PINTO, D. GILHAR AND J. BACKON. *Differential effects of various antiinflammatory drugs on theophylline neurotoxicity.* PHARMACOL BIOCHEM BEHAV 49(2) 335-339, 1994.—The purpose of the present investigation was to evaluate whether antiinflammatory drugs affect the pharmacodynamics of theophylline-induced seizures. Adult male Lewis rats were treated with either dexamethasone (DEX), hydrocortisone (HYD), ibuprofen (IBU), or mefenamic acid (MFA), for 4 consecutive days. On the fourth day they received a constant infusion of theophylline (2 mg/min IV) until the onset of maximal seizures. Then, blood and cerebrospinal fluid (CSF) were obtained for theophylline concentration determinations by HPLC. It was found that pretreatment with the corticosteroids DEX and HYD elevated the CSF theophylline concentration required to induce maximal seizures in comparison to the untreated rats (242 ± 6, 232 ± 6, and 203 ± 10 mg/l, respectively, $n = 10$, $p < 0.05$). MFA also increased the CSF theophylline concentration at that endpoint in comparison to the controls ($p < 0.01$), whereas pretreatment with IBU had no effect (280 ± 10 MFA, 225 ± 9 IBU vs. 220 ± 8 controls, $n = 12$). The data suggests that concomitant treatment with antiinflammatory drugs, together with theophylline, do not increase the risk for theophylline-induced seizures. Moreover, in certain cases they may elevate the seizure threshold and protect against these hazardous episodes.

Theophylline Antiinflammatory Steroids NSAID Dexamethasone Hydrocortisone Ibuprofen Mefenamic acid Induced seizures Neurotoxicity Pharmacodynamics Drug interaction

THEOPHYLLINE is widely used in the treatment of asthma and other reversible obstructive airway diseases that are often associated with inflammatory processes (2,21,15). This bronchodilator drug has a relatively narrow therapeutic range of plasma concentration, the upper limit being imposed by the risk of adverse cardiovascular and neurotoxic effects (16). One of the most serious manifestations of theophylline toxicity is life-threatening generalized seizures which occur in a wide range of plasma concentrations of this drug. This variability indicates that the pharmacodynamics (i.e., concentration–pharmacologic effect relationship) of theophylline neurotoxicity is not fixed and depends on variables, most of which are as yet unrecognized (14). These variables include certain (patho)-physiologic conditions [e.g., acute renal failure (5) and hyperthermia (28)] as well as concomitant drug therapy that modulates central nervous system (CNS) function. The effect of other xenobiotics taken concomitantly with theophylline may either increase the CNS sensitivity to theophylline-induced seizures [e.g., ephedrine (19) and papaverine (12)] or elevate the seizure thresholds [e.g., antiepileptic agents (6)].

The concurrent administration of theophylline with corticosteroids is very common in the treatment of inflammatory-derived respiratory disease (19,21). Similarly, patients being prescribed theophylline as bronchodilator therapy may also use self-prescribed NSAIDs as a cough/cold or headache remedy. Antiinflammatory drugs, either steroids or nonsteroidal antiinflammatory drugs (NSAIDs), affect eicosanoids (i.e., prostaglandin and leukotriene) synthesis in the central nervous system (CNS), as well as in other tissues (22-24,26,27). A marked increase of eicosanoid levels in the brain was found

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during experimentally induced convulsions (3,7,20). Furthermore, other studies demonstrated a positive correlation between the duration of seizures and prostaglandin formation in brain regions primarily involved in the epileptic process (7). Less is known about the association between leukotrienes and the pathophysiology of convulsions (3).

Inhibition of brain eicosanoid formation by antiinflammatory drugs was associated, in certain preclinical investigations, with a decreased susceptibility to seizure episodes (3). On the other hand, certain antiinflammatory drugs produced CNS excitation, and can on their own be epileptogenic in high doses (1). Accordingly, it is probable that CNS seizure thresholds could be attenuated by antiinflammatory drugs.

The available information concerning the impact of antiinflammatory agents on the pharmacodynamics of theophylline convulsive activity is insufficient. One clinical epidemiologic study suggested a possible protective effect of steroids against theophylline-related CNS disturbances (19). Several preclinical investigations of acute treatment with either a steroid (hydrocortisone) (29) or NSAIDs (13), or indomethacin (15) failed to demonstrate any alteration in the pharmacodynamics of theophylline-induced seizures. However, whether the repeated use of these drugs affects theophylline toxicity is not clear. The aim of this investigation was, therefore, to expand on the knowledge and understanding of possible pharmacodynamic interactions between steroids and nonsteroid antiinflammatory agents and theophylline convulsant activity.

Epidemiological surveys can, at best, identify potential risk factors of theophylline-induced seizures. More direct prospective investigations are substantially facilitated by the use of an appropriate animal model. Ramzan and Levy (14) previously developed such an experimental model. This unique model determines concentration-effect relationships and is superior to the customary method of assessing the dose-response relationship (e.g., LD_{50}) to evaluate susceptibility to drug toxicity. This unique animal model permits a clear distinction between pharmacokinetic (i.e., drug concentration-time relationship) and pharmacodynamic variables that the customary method does not provide. Ramzan and Levy (14) have shown that when rats were infused intravenously to onset of maximal seizure, the theophylline concentrations in the cerebrospinal fluid (CSF) (but not in serum and brain) at the pharmacological endpoint were independent of the drug infusion rate and showed little interindividual variation (c.v. < 10%). Thus, the rat appears to be a good model to explore pharmacodynamic interactions of theophylline neurotoxicity.

METHOD

Male Lewis rats (LEW/HSD Indianapolis, IN), weighing 175–220 g, were used in this investigation. During the experimental period, all animals were housed in individual metal cages in an environment of constant humidity (50–55%) and temperature (20–22°C) and in a light-controlled room (lights on from 0700 to 1900 h). They were maintained on laboratory chow and water ad lib. The rats were allowed 2 weeks to adjust to the new environment and to overcome possible stress incurred during transport. One day before the pharmacodynamic experiment an indwelling cannula was implanted in the right external jugular vein (25), under light ether anesthesia. The cannulas were filled with heparin-free saline solution.

The pharmacodynamic investigations were divided into two separate experiments to evaluate the effects of: a) corticosteroids and b) NSAIDs on theophylline neurotoxicity. In the first experiment, the rats received a daily dose of either dexamethasone free base (DEX) 10 mg/kg/day or hydrocortisone (HYD) 20 mg/kg/day, for 4 consecutive days. In the second experiment, the animals were pretreated with either ibuprofen (IBU) 30 mg/kg/day or mefenamic acid (MFA) 20 mg/kg/day. All the drugs used for pretreatment were suspended in a normal saline solution freshly prepared before administration and were administered IP between 1100 and 1300 h. The control group in each experiment received an equivalent volume (0.2 ml) of saline solution. DEX, HYD, IBU, MFA were purchased from Sigma, Chemical Co., St. Louis, MO. Each of the groups in the two experiments consisted of 12 rats, and the pretreated and control rats in each pharmacodynamic experiment were studied in a random order.

To determine the effect of pretreatment with steroids or NSAIDs on theophylline convulsive action, 45 min after the last pretreatment dose a theophylline solution (100 mg/ml base as aminophylline) was infused intravenously at a constant rate of 2 mg theophylline/min delivered into unanesthetized (and unrestrained) rats by a microprocessor-based syringe pump (Pump 22, Harvard Apparatus, South Natick, MA) until the onset of maximal seizures. This pharmacological endpoint was evidenced by forelimb flexion and usually tonic hindlimb extension. At that time, the rats were lightly anesthe-

TABLE 1

EFFECT OF REPEATED DEXAMETHASONE AND HYDROCORTISONE ADMINISTRATION FOR 4 DAYS ON THEOPHYLLINE DOSES REQUIRED TO INDUCE MAXIMAL SEIZURES, AND SERUM THEOPHYLLINE CONCENTRATIONS AT ONSET OF THIS EFFECT

Variable	Control Rats	Dexamethasone Pretreated Rats	Hydrocortisone Pretreated Rats
Number of animals	12	12	12
Rectal Temp. (°C)	37.2 ± 0.1	37.6 ± 0.1	37.3 ± 0.1
Body Weight (g)	309 ± 5	262 ± 36*	289 ± 6†
Infusion Time (min)	48.3 ± 1.7	45.0 ± 1.2	48.5 ± 1.5
Total Dose (mg/kg)	309 ± 7	340 ± 7†	331 ± 6†
Serum Theophylline Concentration (mg/l)	398 ± 8	438 ± 8*	424 ± 6†

Rats were infused IV with theophylline at 2 mg/min until onset of maximal seizure 45 min following the last IP administration with either dexamethasone 10 mg/kg/day or hydrocortisone 20 mg/kg/day. Results reported as mean ± SE.

*Significantly different from control value by Mann–Whitney test $p < 0.005$; †Significantly different from control value by Mann–Whitney test $p < 0.03$.

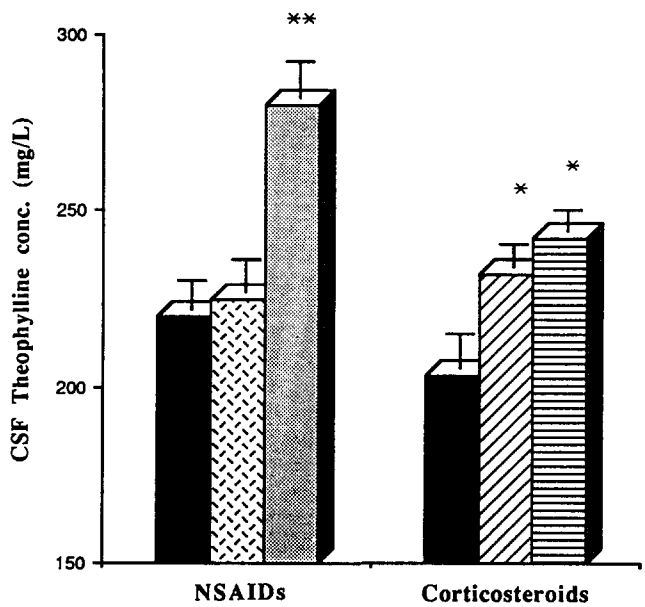

FIG. 1. Effect of pretreatment with NSAIDs or corticosteroids for 4 days on CSF theophylline concentrations at onset of maximal seizure. NSAIDs (n = 12): control ■, ibuprofen 30 mg/kg/day ⊠, mefenamic acid 20 mg/kg/day ⊡. Corticosteroids (n = 10): control ■, dexamethasone 10 mg/kg/day ▨, hydrocortisone 20 mg/kg/day ⊟. *Significantly different from control group $p < 0.05$, **$p < 0.01$. Data represent mean ± SE.

tized with ether (unless they died following maximal seizures) and the cerebrospinal fluid (CSF) from the cisterna magna (by a cisternal puncture) and blood (for serum) from the abdominal vena cavae were collected promptly, in that order, and stored at −20°C pending analysis. Theophylline concentrations in these biological samples were determined by HPLC method after selective extraction with ethyl acetate, according to a procedure previously described (14). The standard curve was linear in the range of 50–500 mg/l ($r > 0.995$). The rectal temperatures were monitored just before the pharmacodynamic study, and the body temperature during theophylline infusion was maintained by placing the rats on isothermal pads.

The doses of the antiinflammatory drugs employed in this investigation were all proven to be effective in inhibition of eicosanoid formation in the brain. Specifically, DEX (22,26), HYD (29), IBU (24), and MFA (7).

The nonparametric Mann-Whitney test evaluated the statistical significance of differences between each group and the corresponding control values ($p < 0.05$ was considered statistically significant). Results were reported as mean ± SE.

RESULTS

The effect of repeated pretreatment with DEX and HYD on theophylline dose required to induce maximal seizures and theophylline concentrations in the serum at the onset of seizures are summarized in Table 1. Both steroids effectively increased the theophylline dose (normalized by body weight) required to produce maximal seizures, whereas no significant differences in theophylline infusion time between the three groups were noted. The serum theophylline concentrations at onset of maximal seizures in the DEX as well as HYD pretreated rats were elevated in comparison to control values. The CSF theophylline concentrations at onset of the pharmacological endpoint, which is the pharmacodynamic indicator in this experimental strategy, were also increased (242 ± 6 DEX, 232 ± 6 HYD vs. 203 ± 10 controls) (Fig. 1). The body weight of the rats pretreated with both DEX and HYD was significantly lower while rectal temperature was not affected.

Repeated pretreatment with either IBU 30 mg/kg/day or MFA 20 mg/kg/day for 4 days had no apparent effect on body weight or rectal temperature (Table 2). Pretreatment with MFA significantly increased the dose of theophylline needed to produce the convulsive episodes and increased the serum theophylline concentrations determined at onset of maximal seizures. On the other hand, pretreatment with IBU did not attenuate these parameters (Table 2). Similarly, the CSF theophylline concentrations at the onset of the pharmacological endpoint were also elevated following pretreatment with MFA but not with IBU (Fig. 1).

DISCUSSION

Antiinflammatory drugs are effective inhibitors of eicosanoid synthesis in the CNS (22–24,26,27). In addition, these medications were reported to affect the CNS sensitivity to experimentally induced seizures in very different patterns (13,23,24). However, it is still not clear if any metabolites of

TABLE 2

EFFECT OF REPEATED IBUPROFEN AND MEFENAMIC ACID ADMINISTRATION FOR 4 DAYS ON THEOPHYLLINE DOSES REQUIRED TO INDUCE MAXIMAL SEIZURES, AND SERUM THEOPHYLLINE CONCENTRATIONS AT ONSET OF THIS EFFECT

Variable	Control Rats	Ibuprofen Pretreated Rats	Mefenamic Acid Pretreated Rats
Number of animals	12	12	12
Rectal Temp. (°C)	37.9 ± 0.5	37.7 ± 0.2	38.1 ± 0.3
Body Weight (g)	226 ± 9	211 ± 7	240 ± 7
Infusion Time (min)	35.3 ± 2.0	36.5 ± 1.4	45.4 ± 2.5*
Total Dose (mg/kg)	312 ± 9	346 ± 14	381 ± 17*
Serum Theophylline Concentration (mg/l)	404 ± 10	425 ± 9	507 ± 24*

Rats were infused IV with theophylline at 2 mg/min until onset of maximal seizure 45 min following the last IP administration with either ibuprofen 30 mg/kg/day or mefenamic acid 20 mg/kg/day. Results reported as mean ± SE.

*Significantly different from control value by Mann-Whitney test $p < 0.01$.

arachidonic acid play a role in the induction or if they are merely byproducts of seizures.

Since the effect of antiinflammatory agents on the CNS sensitivity to convulsive activity is not consistent and depends on the specific experimental model (23,24), it is impractical to extrapolate the results obtained from studies with other chemically induced seizures and use them to understand the role of antiinflammatory agents in the pathophysiology of theophylline neurotoxicity. The few available studies concerning theophylline neurotoxicity do not lead to a conclusive understanding of the potential pharmacodynamic interaction between antiinflammatory agents and theophylline neurotoxicity. It is important to investigate this specific interaction in view of the clinical relevance in assessing parameters affecting the CNS sensitivity to theophylline neurotoxicity (14).

A unique experimental strategy was employed in this investigation to assess such changes in the concentration–convulsive effect relationship of theophylline. It is based on the fact that at onset of maximal seizures, theophylline concentrations in the CSF represent the theophylline concentrations at the site of action in the brain. Therefore, theophylline concentrations in the CSF, at the pharmacological endpoint, can serve as pharmacodynamic indicators for comparison between pretreated rats and corresponding controls. Thus, the anticonvulsant activity of specific pretreatments is evidenced by higher CSF theophylline concentrations at onset of maximal convulsions in comparison to controlled group. On the other hand, proconvulsant activity of the investigated pretreatment is presented by lower CSF theophylline concentration at the pharmacological endpoint.

Daily administration of HYD for 4 days elevated the seizure threshold as indicated by the CSF theophylline concentrations (Fig. 1). A more profound seizure protective effect was evidenced following pretreatment with DEX. This outcome correlates with the differences in intensity of pharmacological activity of these two steroids (18) as is also evidenced by their effect on body weight. In addition, it has been demonstrated that DEX has a strikingly different pattern of uptake and retention in the brain as compared to other glucocorticoids (17). The anticonvulsant activity of these two corticosteroids in theophylline-induced seizures parallels clinical observations of minor incidences of paroxysmal EEG activity seen in children treated with combined therapy of theophylline with steroids rather than with theophylline therapy alone (19). There is a deviation between the results of pretreatment with a single dose of HYD which did not affect the pharmacodynamics of theophylline convulsive effect (29) and the results of the present investigation. This discrepancy helps to demonstrate that the protective effect of the steroid is time dependent and presumably mediated by the formation of a protein [e.g., lipocortin that inhibits the activity of phospholipase A_2 (26,27)]. Steroid hormone metabolites were reported before to act as a barbiturate-like modulator of the GABA receptor (9) [a mechanism that was previously proposed for the elevation of theophylline-induced seizures threshold by anticonvulsants (6)]. Therefore, if the metabolites of the steroid drug accumulate in the body, they may also contribute to the time dependency phenomenon of the anticonvulsant activity.

An intriguing finding is the demonstration of an anticonvulsant effect of MFA in this model. Controversy surrounds the exact mechanism of the anticonvulsant action of MFA exhibited in several chemically induced seizure models (7). In contrast to other NSAIDs, fenamates possess certain unique pharmacological properties such as being antagonistic to the excitatory prostaglandin $PGF_2\alpha$ and inhibiting phospholipase A_2 in various tissues (4). These two distinct activities offer some help in understanding how this drug modulates the seizure thresholds of theophylline-induced seizures. In other interactions between NSAIDs and convulsive agents the seizure modulating action of NSAIDs has been interpreted in terms of inhibition of brain prostaglandin synthesis (7,23,24). However, the failure of IBU to modulate the threshold of theophylline-induced seizures, as well as the lack of a modifying effect of acute treatments with both aspirin (13) and indomethacin (15) on theophylline neurotoxicity (as assessed by a similar experimental design), do not corroborate this explanation. This is true, provided that the brain prostaglandins levels have been attenuated by these NSAIDs at the doses used in our study and un the two other investigations (13,15,24).

As yet, the mechanism of theophylline convulsant activity is not fully clear (6). A mechanism that was suggested to play a role in theophylline-induced seizures is cerebral vasoconstriction (8,11). The anticonvulsant activity of both steroids and MFA demonstrated in the present study can also be attributed to this mechanism. This is connected to the fact that both steroids and MFA cause inhibition of phospholipase A_2 activity and thereby reduce both leukotrienes and prostaglandin levels. Leukotrienes are potent vasoconstrictors that can affect cerebral blood flow (3,20), and may also elicit a prolonged excitation of cerebral neurons (10). Therefore, the inhibition of leukotriene formation rather than of prostaglandins may play a role in the pathophysiology of theophylline and other chemically induced seizures. Further investigation has to be performed to elucidate the exact mechanism of these pharmacodynamic interactions.

The results of the present investigation may have a direct clinical relevance, indicating that concomitant treatment with antiinflammatory drugs together with theophylline do not increase the risk for theophylline-induced seizures. Moreover, in certain cases they may elevate the seizure threshold and protect against these hazardous episodes.

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