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# Effect of nicotinamide on theophylline seizures in rats

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Nicotinamide administered exogenously to animals and humans has many pharmacologic actions including sedative [1–5], anticonvulsant [6, 7], muscle relaxant [7] and tranquilizing effects [8]. It is also a normal brain constituent and has neurochemical properties common with benzodiazepines [7, 9] and barbiturates [10] and has been suggested as an endogenous ligand for the benzodiazepine–γ-aminobutyric acid (GABA) receptor complex [7]. Nicotinamide may also play a role in the etiology of seizures since it provides partial protection alone and complete protection in combination with adenosine against audiogenic seizures [4].

In investigating the various risk factors associated with theophylline-induced seizures, it was deemed important to examine the effect of nicotinamide administration on theophylline seizures. Specifically, a recently developed model of theophylline-induced convulsions in rats [11] was used to assess if nicotinamide pretreatment increases theophylline concentrations in serum, cerebrospinal fluid, and brain at which maximal seizures occur.

*Methods*

Male Sprague–Dawley rats, housed individually in an environmentally controlled room for at least 1 week before experimentation, were anesthetized with ether 1 day before the study and a cannula was placed in the jugular vein. On the study day, theophylline solution (100 mg/ml base as aminophylline) was infused into unanesthetized (and unrestrained) rats at 0.0206 ml/min via this cannula to elicit a seizure in approximately 30 min. One group of animals was pretreated with i.v. bolus nicotinamide, 1000 mg/kg in saline (2 ml/kg), 15 min prior to the start of the theophylline infusion, while the other (control) group received the same volume of saline. Food and water were withheld only during testing, and normal body temperature was maintained with isothermal pads. Animals from the two groups were tested randomly.

Immediately following the maximal seizure as evidenced by forelimb flexion and usually tonic hindlimb extension, samples of CSF (by cisterna magna puncture), blood (for arterial serum from abdominal aorta) and brain (after decapitation) were obtained in that order and assayed for theophylline using liquid chromatography. Nicotinamide concentrations were also estimated using the assay for theophylline. All these procedures have been described in detail previously [11]. Variables from the two groups of animals were compared using Student's *t*-test for unpaired data.

*Results and discussion*

Onset times to seizure, cumulative doses and concentrations of theophylline at onset of seizures are summarized in Table 1. The animals pretreated with nicotinamide took the same time and needed the same dose to elicit a seizure as saline-treated controls. More importantly, serum and CNS concentrations of theophylline at which the maximal seizures occurred did not change with nicotinamide pretreatment, i.e. nicotinamide did not offer any protection against theophylline-induced convulsions. This lack of protection was despite very high nicotinamide concentrations being achieved in both serum and CNS in rats pretreated with this agent (Table 2). In addition, nicotinamide serum concentrations were identical to those in CSF and brain, consistent with its negligible serum protein binding (Table 2).

Theophylline use is often associated with serious toxicity including seizures and death. In many instances these seizures are not preceded by warning signs and are associated with wide-ranging serum theophylline concentrations [12]. As a result, there is a great need to examine risk factors associated with theophylline seizures and to identify agents that may offer protection against such seizures. Recently a model for theophylline-induced seizures has been developed in rats [11], and this study was our initial attempt at

Table 1. Theophylline doses and concentrations at onset of maximal seizure

| Variables | Pretreatment | |
|---|---|---|
| | Saline | Nicotinamide (1000 mg/kg) |
| No. of animals | 9 | 9 |
| Body weight (g) | 307 ± 10 | 300 ± 11 |
| Infusion rate (mg/min) | 2.06 | 2.06 |
| Infusion rate (mg/min/kg) | 6.7 ± 0.2 | 6.9 ± 0.3 |
| Onset time to seizure (min) | 32 ± 4 | 32 ± 4 |
| Dose to seizure (mg) | 65 ± 7 | 65 ± 7 |
| Dose to seizure (mg/kg) | 213 ± 24 | 217 ± 30 |
| Concentrations at onset of seizure (mg/l or mg/kg) | | |
| Serum total | 332 ± 43 | 341 ± 45 |
| CSF* | 210 ± 26 | 211 ± 47 |
| Brain | 178 ± 32 | 187 ± 30 |
| Serum free | 286 ± 30 | 272 ± 33 |
| Serum free (unbound) fraction | 0.865 ± 0.046 | 0.798 ± 0.017† |

Values are given as means ± SD.
* N = 7 and 6, respectively, due to failure to obtain CSF.
† Significantly different from saline-treated group, $P < 0.001$.

Table 2. Nicotinamide concentrations in rats pretreated with nicotinamide

| | Nicotinamide (mg/l or mg/kg) |
|---|---|
| Serum total | 1295 ± 57 (9) |
| CSF | 1276 ± 83 (6) |
| Brain | 1240 ± 132 (9) |
| Serum free | 1274 ± 59 (9) |
| Serum free (unbound) fraction | 0.984 ± 0.041 (9) |

Values are means ± SD, with the number of animals given in parentheses.

finding agents that may be beneficial against theophylline seizures.

Nicotinamide was chosen as a potential beneficial agent since it offers protection against audiogenic seizures with its effect being enhanced by adenosine [4] and since theophylline's neurotoxicity is presumably related to its effect at adenosine receptors [13], although it is also a GABA and benzodiazepine antagonist [14]. In view of this, the lack of protective effect noted in the current study was surprising. However, this is unlikely to be due to the dose of nicotinamide used since the chosen dose was high and similar to the claimed optimum dose of 400 mg/kg [4]. Similarly, the pretreatment time is probably not inappropriate since the rats received nicotinamide 15 min prior to the start of theophylline infusion giving a pretreatment period of 45 min in most rats which is also the time when nicotinamide showed its maximum protective effect in the audiogenic seizure study [4]. Our negative results may more appropriately reflect the fact that the primary neurochemical action of nicotinamide is on a receptor distinct from the adenosine system, since nicotinamide's anticonvulsant effect has been demonstrated previously in assay systems sensitive to benzodiazepines [7] and GABAergic drugs [6].

In summary, the present study demonstrates that nicotinamide (1000 mg/kg) administered intravenously 45 min before onset of seizures in rats failed to provide protection against theophylline seizures.

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## Excretion and metabolism of injected ecdysone in the white mouse

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Ecdysteroids represent a class of steroids which have retained the C-27 skeleton of cholesterol (or in some cases the C-28 or C-29 skeleton of some phytosterols). They are widespread in invertebrates and plants [1–3]. By contrast, they have not yet been found in vertebrates, where steroid biosynthesis and metabolism use different pathways which include an early side-chain cleavage between C-20 and C-22.

In invertebrates, at least in arthropods (there is some, as yet limited, evidence that this could perhaps also apply to annelids and nematodes), ecdysteroids are hormones which control development and reproduction [4–9]. In mammals, although the ecdysteroid structures strongly differ from those of Vertebrate-type steroids, there is evidence that these molecules have several pharmacological effects which include (1) a stimulation of protein synthesis in liver [10], (2) interference with glycaemia controlling factors [11, 12], (3) modifications of enzyme activities, e.g. protein kinase in rat liver [13], glutamic decarboxylase [14] or acetylcholinesterase [15] in rat brain, and (4) alterations in lipid metabolism [16]. These results raise an important question: do ecdysteroids interact with mammalian receptors normally used by other hormones with or without being previously converted into some metabolite(s)?

Moreover, it has been recently claimed that in mammals (including humans) infested with helminths it is possible to detect infestation by the unusual presence of ecdysteroids in blood or urine of infested individuals [17–20]. These compounds would be secreted by parasites in amounts high enough to account for noticeable immunoreactivity in host fluids. This problem is, however, rendered more complex due to the possible presence of ecdysteroids in the food of animals, as these molecules are present in so many plant species [2, 3]. In any case, it may be of interest to learn something about ecdysteroid metabolism in mammals: it is indeed highly probable that mammals are able to transform