J. Pharm. Pharmacol. 1981, 33: 479–480
Communicated January 13, 1980

0022–3573/81/070479–02 $02.50/0

Aminophylline seizures in the rat

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Because of its rapid bronchodilator action, aminophylline is the drug most commonly given to patients who have acute respiratory insufficiency related to bronchoconstriction. Focal and generalized seizures occurring during status asthmaticus have traditionally been attributed to the hypoxia associated with such attacks. In recent years, many have come to suspect that the seizures may result from a direct effect of aminophylline on the brain but this has never been proved. Zwillich et al (1975) found that the occurrence of seizures in neurologically normal patients was observed only in patients with a serum theophylline level above 25 $\mu g\ ml^{-1}$. Repetitive generalized seizures, often with focal onset (Schwartz & Scott 1974; Yarnell & Chu 1975) may occur at lower dosages of aminophylline in neurologically impaired patients, and are commonly fatal despite high doses of intravenous antiepileptic drugs (Yarnell & Chu 1975). In fact, effective anti-epileptic therapy for this complication of aminophylline therapy has not been described. As a first step the efficacy of commonly used intravenous antiepileptic drugs in preventing this complication in animals given intravenous aminophylline has been assessed.

Aminophylline was given intravenously to male Sprague-Dawley rats 250–275 g, using the method of tail vein infusion of Man & Comsroe (1973). The tail was washed with warm soapy water, dried vigorously to produce vasodilation, and taped to the top of a wooden block. A 25 g needle from a scalp vein infusion set was then inserted into either lateral tail vein about one-third of the tail length from the tip of the tail. Once stroking the tail vein resulted in blood backing into the polyethylene tubing, a Harvard infusion pump was switched on at a rate of 1·85 ml min^{-1}, and a stop watch started to time the infusion at the instant the column of blood entered the needle. Aminophylline concentration in the infusion fluid was 25 mg ml^{-1}. The stop watch was stopped at the onset of tonic extension of the forelimbs. Since the seizures were uniformly fatal, different groups of animals were used for determination of control and experimental thresholds. The anticonvulsants were given intraperitoneally. Diazepam was given 45 min and phenytoin and phenobarbitone 1 h before aminophylline infusion. The data were analysed for significant differences using Dunnet's 't' test, (Dunnet 1964). At least six animals were used in each experimental group.

The first evident behavioural change in an animal given a convulsive dose of aminophylline was a cessation of normal exploratory behaviour (wandering, twitching nose, moving about), the animal standing still with its back legs spread out hypotonically. Subsequently, unlike electroshock seizures, there was tonic extension of all four limbs in a caudal direction associated with persistent apnoea and hyperaemia of the mucous membranes and distal limbs. The seizures were invariably fatal at an average dose of 351 mg kg^{-1} (n = 13) in the absence of prophylactic or resuscitative measures.

Table 1 indicates the effect of increasing doses of antiepileptic drugs on the aminophylline seizure threshold. Doses lower than those shown did not affect the threshold.

Diazepam significantly elevated the aminophylline seizure threshold only at doses that produced behavioural somnolence and ataxia. Phenytoin and phenobarbitone, however, significantly elevated the seizure threshold at doses that produced no obvious behavioural changes. At higher doses, both phenytoin and phenobarbitone raised the seizure threshold higher than did the maximal tolerable dose of diazepam. Phenobarbitone was effective at a lower dose (10 mg kg^{-1}) than phenytoin and produced the greater elevation of the seizure threshold at the higher doses used (30 and 50 mg kg^{-1}).

Aminophylline given intravenously to rats at a sufficient dose predictably produces severe seizures and

* (Supported by a grant from the Veterans Administration).

Table 1. Effect of commonly used antiepileptic drugs on aminophylline seizure threshold (mg kg^{-1} i.v.).

Antiepileptic dose (mg kg^{-1} i.p.)	0	1	2	10	30	50	100
Phenytoin	351 ± 62	—	—	—	430 ± 99	563 ± 79	644 ± 80
Phenobarbitone	351 ± 62	—	—	543 ± 27	558 ± 66	636 ± 23	Fatal
Diazepam	351 ± 62	484 ± 56	488 ± 77	526 ± 36	Fatal	—	—

Values represent mean ± standard deviation, all significant at $P<0·01$ n = 6 or more.

death. The commonly used parenteral antiepileptic drugs appear to be effective in raising the threshold, both phenobarbitone and phenytoin affording protection against seizures at doses producing no obvious behavioural change, while diazepam was effective only at doses producing behavioural toxicity (somnolence, ataxia). Since, of the drugs tested, phenobarbitone was effective at lower doses and had a greater maximal elevating effect on the aminophylline seizure threshold, it would appear to be the agent of choice in the rat model. Whether it would be effective in preventing seizures in patients treated with parenteral aminophylline remains to be resolved.

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J. Pharm. Pharmacol. 1981, 33: 480–482
Communicated February 3, 1981

0022-3573/81/070480-03 $02.50/0

Intrathecal injections in rats by percutaneous lumbar puncture

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Drugs can often best be delivered to the central nervous system intrathecally, the intracisternal or intraventricular routes usually being the most accessible in small animals (e.g. Jeffers & Griffith 1949; Noble et al 1967). However, for some quantitative experiments on tetanus, we preferred to make lumbar punctures, because they *are used more commonly in man*, incur no risk of mechanical damage to the brain or brain stem, and need neither preparative surgery nor stereotaxic apparatus. A procedure for making and monitoring such injections in rats, the smallest animals likely to be technically suitable, is now described.

Male rats of an inbred Norwegian strain, 200–230 g, were first given an injection of about 8 LD50 of tetanus toxin in 100 mm^3 of gelatin-phosphate buffer into the right gastrocnemius muscle. The animals were then anaesthetized and an injection of tetanus antitoxin made into the lumbar cerebrospinal fluid (c.s.f.) either before the onset of tetanus, for prophylaxis, or after the first signs had appeared. This was followed by X-ray contrast oil via the same needle to permit radiographic monitoring. Rats in which tetanus progressed beyond a mild and apparently painless stage were killed by anaesthesia.

For lumbar punctures and radiography, the animals were anaesthetized with halothane in oxygen from an open-circuit apparatus similar in principle to that of Reese & Nunn (1961). The mixture was partially humidified by passage over water. Induction was rapid with 5% (v/v) halothane in oxygen at 3·25 l min^{-1}; anaesthesia was then maintained by 1–3% halothane in oxygen. The animal was placed prone on an operating-board carrying a fixed V-shaped support for the mandible and a loose bridge for the operator's hand to prevent compression of trachea and lungs (Fig. 1).

The injection was then made from an apparatus based on that of Jeffers & Griffith (1949) for intracisternal injections from a narrow graduated tube under known low pressures (Fig. 1). The injecting needle (IN of Fig. 1) was a double-ended cartridge-type Solila dental needle, 26 s.w.g. (0·45 mm) diam. and 1″ or 1¼″ (25 or 32 mm) from tip of long end to ball, and with a Hüber point on its long end (Amalgamated Dental Trade Distributors Ltd., London). The tubing for liquids was mostly polyethylene (Portex Ltd., Hythe, Kent), which could be permanently graduated with waterproof ink and which remained flexible, transparent and hydrophobic after repeated use, but 'Flexible Blue' (Portex) nylon tubing was used for MD of Fig. 1, because of its greater flexibility and oil- and pressure-resistance, while F.E.P. (Teflon: Dupont Ltd., Reading. Berks.) was used where neither graduation nor great flexibility were needed, because it was more durable and hydrophobic. The polytrimethylpentene (TPX: ICI Ltd., Welwyn Garden City, Herts.) 'T'- and 'X'-junction pieces shown in Fig. 1 were fabricated by drilling polished blanks and fitting them with stainless steel tube connections made from hypodermic needles. They were also used, together with clips on the plastic tubing, as 2- or 3-way stopcocks in situations where conventional stopcocks leaked unpredictably and/or had too much dead space; commercially available stopcocks were suitable for ATT and MTT of Fig. 1. The reservoirs for saline (NaCl, 154 mM), and for the dilutions of tetanus antitoxin in saline, were mostly sterile disposable polystyrene syringes, but polypropylene and glass ones were used both for storing and dispensing X-ray contrast oil (Myodil: Glaxo Ltd., Greenford, Middlesex) which attacks polystyrene, and when resterilization was desirable. They were connected to tubing via shortened and blunted hypodermic needles with nylon hubs. The apparatus was mounted in laboratory clamps and adjusted to exclude strain on the needle IN once inserted into the canal. Calibration was checked by making dummy injections of rat serum (followed by X-ray contrast oil: see below) into known volumes of saline; the E_{280} was then measured spectrophoto-

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