J. Pharm. Pharmacol. 1988, 40: 365-367
Communicated October 8, 1987

Differences in the development of tolerance to two anticonvulsant benzodiazepines in the amygdaloid kindled rat

N. A. YOUNG, S. J. LEWIS, Q. L. GG HARRIS, B. JARROTT, F. J. E. VAJDA, *University of Melbourne, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria 3084, Australia*

Abstract—The effects of chronic treatment with two benzodiazepines were studied on kindled amygdaloid seizures in rats. Clobazam (4 mg kg^{-1}), clonazepam (0·3 mg kg^{-1}) or vehicle (1 mL kg^{-1}) was administered, by intraperitoneal injection, to fully kindled rats twice daily for nineteen days. Each rat was electrically stimulated 30 min after the morning dose on alternate days of treatment. Tolerance developed rapidly to the anticonvulsant effects of clobazam after only three days of treatment, following which only a small residual protection was maintained. Tolerance to clonazepam developed gradually over the course of the experiment, although this effect was relatively minor.

A major problem that occurs in the treatment of clinical epilepsy with the benzodiazepines is the development of tolerance (Browne & Penry 1973). This is most marked with the 1,5-benzodiazepine, clobazam, but is also noted with the 1,4-benzodiazepines, clonazepam and diazepam.

In animal studies, tolerance to several benzodiazepines has been demonstrated using the pentetrazol infusion (Rosenberg et al 1985; Gent et al 1985) and kindling (Loscher & Schwark 1985; Young et al 1987) models of epilepsy. It has been recently reported that marked differences are seen in the characteristics of tolerance to the anticonvulsant actions of clonazepam and clobazam in the pentetrazol model (Gent et al 1985).

In contrast to the pentetrazol model of epilepsy, the generation of seizure activity in the kindled animal model does not require the administration of an exogenous chemical agent. Kindling (Goddard 1967) consists of administering daily, brief, low intensity electrical stimulation to discrete brain regions by means of an implanted depth electrode. Ongoing daily stimulations elicit progressively greater seizure activity until each stimulation produces a generalized convulsion. The fully kindled animal provides a useful and reliable model of epilepsy in which electroencephalographic and visual recordings provide measurements of seizure duration and severity, respectively. In addition, kindled amygdaloid seizures bear striking resemblance to human complex partial seizures progressing to generalized seizures (McNamara 1984; Engel & Cahan 1986).

The aim of the present study was to investigate the anticonvulsant effects of prolonged treatment with clobazam and clonazepam on amygdaloid kindled seizures in rats to investigate further what has been reported using pentetrazol infusions in mice (Gent et al 1985).

Methods

Thirty male Sprague-Dawley rats, 250–300 g, were stereotaxically implanted with a bipolar stainless steel electrode into the right basolateral amygdaloid nucleus (coordinates AP −2·8, L 4·5, DV −7·8 mm; flat skull position, reference from bregma; Paxinos & Watson 1982).

Following a two week post operative rest, a 1 s train of 60 Hz monophasic square-wave pulses (1 ms duration, 200 μA amplitude) was delivered to the rats once daily (5 days per week) until they were kindled, that is, showed a stage 5 (rearing and falling; Racine 1972) seizure on two consecutive daily stimulations (Young et al 1987).

Correspondence to: N. A. Young, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria 3084, Australia.

The fully kindled rats were stimulated at 200 μA for two days before the commencement of drug treatment. This substantiated that the animal had a stable, generalized (stage 5) seizure and provided baseline measurements of the after-discharge duration. On the following day the animals were randomly assigned to one of three groups and given an intraperitoneal injection (i.p.) of either vehicle (1 mL kg^{-1}), clobazam (4 mg kg^{-1}) or clonazepam (0·3 mg kg^{-1}). Clobazam (pure substance, Hoechst), 4·0 mg, was dissolved in a vehicle containing 0·4 mL propylene glycol, 0·1 mL ethanol and distilled water to 1 mL. Clonazepam (1 mg mL^{-1} ampoules, Roche) was diluted to a concentration of 0·3 mg mL^{-1} with the same vehicle. Vehicle/drugs were administered twice daily (0800 and 2000 h) for a total of 19 days. On alternating days of treatment each rat was stimulated 30 min after the morning dose and the after-discharge duration (measured on the EEG) and the seizure stage (Racine 1972) were determined. The choices of drug doses and time interval between drug administration and electrical stimulation were based upon data reported in the literature (Albright & Burnham 1980; Caccia et al 1980; Morton 1984) and also upon preliminary experiments in this laboratory (unpublished). At the end of the experiment all animals were killed and the brains sectioned to verify the electrode position.

The significance of within group effects of vehicle or drug treatment were determined by Friedman's non-parametric two way analysis of variance followed by the critical range method for testing for differences between treatment days (Colquhoun 1971). The significance of correlations between seizure stage and after-discharge duration for each group were determined by the Spearman rank correlation method (Snedecor & Cochran 1967). Between group differences in seizure stage and after-discharge duration were determined by the Kruskal-Wallis non-parametric one way analysis of variance followed by the critical range test (Colquhoun 1971).

Results

The effects of chronic treatment with vehicle, clobazam and clonazepam on the seizure stage (SS) and the after-discharge duration (AD) in amygdaloid kindled rats are shown in Fig. 1. Repeated injections of vehicle had no effect on the SS ($Chi^2 = 2·7$, 11 d.f., $P > 0·9$) or the AD ($Chi^2 = 8·0$, 11 df., $P > 0·7$) and there were no differences between pretreatment and any one treatment day ($P < 0·05$ for all comparisons of the critical range test).

Overall, clobazam significantly reduced the SS ($Chi^2 = 40·7$, 11 d.f., $P < 0·001$, compared with pretreatment) and AD ($Chi^2 = 37·0$, 11 d.f., $P < 0·001$). The SS and AD values on days 1 and 3 of clobazam treatment were significantly lower than pretreatment ($P < 0·01$ for both comparisons). After the third day the AD and SS showed a significant return to pretreatment values ($Chi^2 = 28·5$, $P < 0·008$ and $Chi^2 = 26·6$, $P < 0·002$, respectively). A strong correlation was seen between the SS and AD over the course of clobazam treatment ($r_s = 0·87$, 8 d.f., $P < 0·001$).

Clonazepam produced a significant overall reduction in SS ($Chi^2 = 60·5$, 11 d.f., $P < 0·001$) and AD ($Chi^2 = 66·3$, 11 d.f., $P < 0·001$). The SS and AD values on all individual treatment

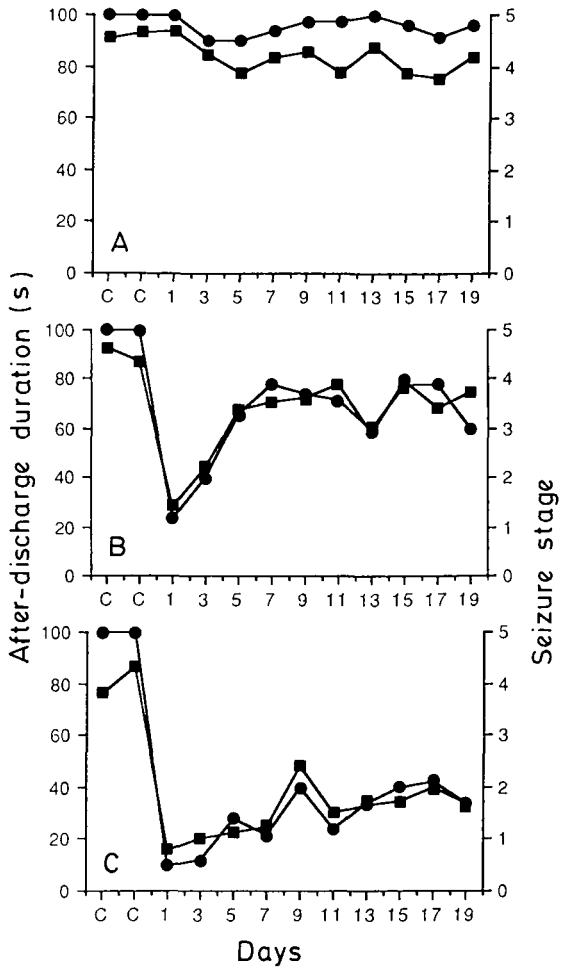

FIG. 1. Effects of chronic treatment with (A) vehicle (1 mL kg^{-1}), (B)clobazam (4 mg kg^{-1}) and (C) clonazepam (0·3 mg kg^{-1}) on the mean (n = 10) after-discharge duration (s) and mean (n = 10) seizure stage (arbitrary units) in amygdaloid kindled rats. C = control stimulations (no drug treatment). Key: ● seizure stage (arbitrary units), ■ after-discharge duration (s).

days remained significantly reduced from pretreatment values ($P < 0·01$ for all comparisons of the critical range test). Tolerance occurred with both the SS and AD values showing a return towards pretreatment ($Chi^2 = 25·3$, $P < 0·003$, and $Chi^2 = 34·7$, $P < 0·002$, respectively). Over the course of the experiment, a strong correlation was seen between SS and AD ($r_s = 0·91$, 8 d.f., $P < 0·001$).

There was a significant ($P < 0·05$ for all comparisons) difference between the clobazam- and clonazepam-treated groups, which would be expected since the development of tolerance was very different in each case. Due to the relatively rapid development of tolerance of clobazam, the AD values did not differ from those of the vehicle-treated animals but the SS values did differ from the corresponding vehicle values ($P < 0·05$). In contrast, tolerance to clonazepam was more gradual than that of clobazam and on the final day of treatment the clonazepam SS and AD values were still lower than pretreatment and, therefore, the clonazepam-treated group differed significantly ($P < 0·01$ for all comparisons) from the vehicle-treated values.

Discussion

The present results in amygdaloid kindled rats show that the initial treatment with either clobazam or clonazepam is very effective in attenuating the severity (SS) and duration (AD) of kindled seizures and that, over prolonged treatment, tolerance develops more markedly to the effects of clobazam than those of clonazepam. The ways in which the tolerance developed to the anticonvulsant effects of clobazam and clonazepam differed significantly. More specifically, the effects of clobazam diminished in a matter of three days treatment, whereas tolerance to the anticonvulsant effects of clonazepam developed more gradually and was still relatively minor after thirteen days of treatment. This difference in the acquisition of tolerance to these two benzodiazepines has also been noted using pentetrazol infusions in mice (Gent et al 1985).

On initial examination of our results and those of previous studies (Gent et al 1985) it would appear that the differences in the development of tolerance to clobazam and clonazepam may be due to the presence or absence of active metabolites, respectively. Indeed the benzodiazepine diazepam, which also shows rapid tolerance and is demethylated to an active metabolite in the same way as clobazam, would support this theory (Gent et al 1985). However, studies using both the pentetrazol and kindling animal models have disputed this theory (Frey et al 1984; Loscher & Schwark 1985).

In more recent studies, cross tolerance has been shown to exist between the benzodiazepines and sodium valproate and it has been suggested that tolerance is due to a 'functional' mechanism, although a metabolic component may exist (Gent et al 1986; Young et al unpublished data).

In a recent review (File 1985) it was proposed that 'behavioural tolerance', that is, learned adaptation, could underlie or influence the rate of development of tolerance. However, it is difficult to see how compensatory responses to the anticonvulsant actions of benzodiazepines could become operantly conditioned. In particular, it would seem impossible to determine to what extent the cues associated with injections could serve as conditioned stimuli.

The reason(s) for tolerance occurring to the anticonvulsant effects of the benzodiazepines remains unclear and poses a confounding problem in the treatment of clinical epilepsy with these drugs. The use of pentetrazol as a model of tolerance to the anticonvulsant effects of the benzodiazepines in animals is well established. However, the development of the kindled animal as a model of tolerance is likely to be advantageous, since this model bears striking resemblance to human complex partial seizures progressing to generalized seizures (McNamara 1984).

We would like to thank Dr F. Rudolph, Hoechst, Australia, for the gift of clobazam and Reckitt and Colman (Australia) for generous support with these studies. Q. L. G. H. and S. J. L. are supported by the National Health and Medical Research Council of Australia.

References

Albright, P. S., Burnham, W. M. (1980) Development of a new seizure model: effects of anticonvulsants on cortical- and amygdala-kindled seizures in the rat. Epilepsia 21: 681–689

Browne, T. R., Penry, J. K. (1973) Benzodiazepines in the treatment of epilepsy. A review. Ibid. 14: 277–310

Caccia, S., Carli, M., Grattini, S., Poggesi, E., Rech, R., Samanin, R. (1980) Pharmacological activities of clobazam and diazepam in the rat: relation to drug brain levels. Arch. Int. Pharmacodyn. 243: 275–283

Colquhoun, D. (1971) Lectures on biostatistics, Clarendon Press, Oxford.

Engel, J., Cahan, L. (1986) Potential relevance of kindling to human partial epilepsy. in: Wada, J. (ed.) Kindling 3, Raven Press, New York, pp 37–54

File, S. E. (1985) Tolerance to the behavioural actions of benzodiazepines. Neurosci. Biobehav. Rev. 9: 113–121
Frey, H., Philippin, H., Scheuler, W. (1984) Development of tolerance to the anticonvulsant effect of diazepam in dogs. Eur. J. Pharmacol. 104: 27–38
Gent, J. P., Feely, M. P., Haigh, J. R. M. (1985) Differences between the tolerance characteristics of two anticonvulsnt benzodiazepines. Life Sci. 37: 849–856
Gent, J. P., Bentley, M., Feely, M., Haigh, J. R. M. (1986) Benzodiazepine cross-tolerance in mice extends to sodium valproate. Eur. J. Pharmacol. 128: 9–15
Goddard, G. V. (1967) Development of epileptic seizures through brain stimulation at low intensity. Nature 214: 1020–1021
Loscher, W., Schwark, W. S. (1985) Development of tolerance to the anticonvulsant effect of diazepam in amygdala-kindled rats. Exp. Neurol. 90: 373–384
McNamara, J. O. (1984) Kindling: an animal model of complex partial epilepsy. Ann. Neurol. 16 (Suppl): S75–S76
Morton, D. J. (1984) Pharmacokinetics of twelve anticonvulsant drugs in the rat. Neuropharmacology 23: 1125–1127
Paxinos, G., Watson, C. (1982) The rat brain in stereotaxic coordinates, Academic Press, N.S.W., Australia
Racine, R. J. (1972) Modification of seizure activity by electrical stimulation: II motor seizure. Electroencephalogr. Clin. Neurophysiol. 32: 281–294
Rosenberg, H. C., Tietz, E. I., Chiu, T. H. (1985) Tolerance to the anticonvulsant action of benzodiazepines. Relationship to decreased receptor density. Neuropharmacology 24: 639–644
Snedecor, G. W., Cochran, W. G. (1967) Statistical methods, 6th Edition, Iowa State University Press, Ames, Iowa
Young, N. A., Lewis, S. J., Harris, Q. L. G., Jarrott, B., Vajda, F. J. E. (1987) The development of tolerance to the anticonvulsant effects of clonazepam, but not sodium valproate, in the amygdaloid kindled rat. Neuropharmacology 26: 1611–1614

J. Pharm. Pharmacol. 1988, 40: 367–369
Communicated October 12, 1987

Intracerebroventricularly administered bradykinin augments carrageenan-induced paw oedema in rats

S. K. BHATTACHARYA, P. J. R. MOHAN RAO, NEETA DAS, G. DAS GUPTA, *Neuropharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India*

Abstract—Intracerebroventricular (i.c.v.) administered bradykinin (2·5 and 5·0 μg/rat) was found to augment carrageenan-induced acute paw oedema throughout the 4 h post-carrageenan observation period. The effect was statistically significant with the higher dose. The pro-inflammatory effect of i.c.v. bradykinin was antagonized following pretreatment with hemicholinium and atropine ethoiodide administered i.c.v., drugs that reduce central cholinergic activity. Similarly, central administration of drugs that inhibit the synthesis of eicosanoids, hydrocortisone, diclofenac and paracetamol, also attenuated the pro-inflammatory effect of bradykinin. The findings indicate that the inflammation-promoting effect of centrally administered bradykinin involves the central prostaglandin and cholinergic neurotransmitter systems.

Bradykinin is known to be released in the periphery and has been implicated in a variety of physio-pathological conditions, including inflammation (Colman & Wong 1979). It is now evident that the kinin exerts discernible effects on the mammalian central nervous system (CNS), leading to the postulate that it functions as a central neuromodulator (Clark 1979). The kinin has been identified in the CNS of several animal species, including rats, and enzyme systems capable of synthesizing and inactivating bradykinin and other kinins have been demonstrated in the mammalian brain and cerebrospinal fluid (Clark 1979; Shisheva et al 1983).

There are indications that the CNS may modulate peripheral inflammation. Schizophrenics have an unusually low incidence of rheumatoid arthritis and show reduced inflammatory response to injury and infection (Horrobin 1977). Experimentally induced acute inflammation is attenuated by general anaesthetics (Griswold et al 1982; Bhattacharya et al 1987), narcotic analgesics, spinal transection, and acute or chronic denervation (Brown et al 1968). Patients with thalamic or spinothalamic lesions show a substantially decreased flare response to histamine, indicating that the vasodilator component of inflammation is modulated by the CNS (Appenzeller & McAndrews 1966).

In recent reports from this laboratory, the central cholinergic (Das & Bhattacharya 1985), prostaglandin (PG) E_2 (Bhattacharya & Das 1984) and some excitatory amino acid (Bhattacharya & Sarkar 1986) neurotransmitter systems have been shown to augment carrageenan-induced paw oedema in rats. On the contrary, the central noradrenergic (Bhattacharya & Das 1986), 5-hydroxytryptaminergic (Bhattacharya & Das 1985a), histaminergic (Bhattacharya & Das 1985b), $PGF_2\alpha$ (Bhattacharya & Das 1984) and inhibitory amino acid (Bhattacharya & Sarkar 1986) neurotransmitter systems attenuate carrageenan-induced acute inflammation. Since it has been postulated that bradykinin functions as a neuromodulator (Clark 1979), the effect of i.c.v. administered bradykinin has been investigated on carrageenan-induced paw oedema, taken as the experimental model of acute inflammation, in rats.

Materials and methods

The studies were conducted on inbred Wistar strain albino rats (150–200 g) of both sexes. The rats were housed in colony cages at an ambient temperature of 25±2°C and 45–55% relative humidity, with a 12 h light-dark cycle. The rats were fed on standard pellet chow and given tap water through drinking bottles. Experiments were conducted at this ambient temperature between 0900 and 1400 h. Paw inflammation was induced by carrageenan (0·1 mL of 1% suspension in 0·9% saline) injected below the plantar aponeurosis of the hind paw (Winter et al 1962). The paw volume, up to the ankle joint, was measured before and at hourly intervals for 4 h after carrageenan administration, by means of a mercury plethysmograph. The increase in paw volume has been expressed in units, each unit representing 1 cm (volume = 0·075 mL) length of the displaced mercury column.

Intracerebroventricular (i.c.v.) cannulation of the right lateral

Correspondence to: S. K. Bhattacharya, Neuropharmacology Laboratory, Dept of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.