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β-Kainic acid is not an amino acid antagonist

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β-Kainic acid has been reported to have anticonvulsant properties. In view of its structural relationship to α-kainic acid it has therefore been tested for antagonist activity at excitatory receptors for *N*-methylaspartate, quisqualate and α-kainate using anaesthetized rats and brain slice preparations. No antagonism was found against these three agonists though β-kainate itself caused excitation. If β-kainate interferes with excitatory amino acid neurotransmission it probably acts presynaptically.

In trying to clarify the functional role of excitatory amino acids and their receptors in the central nervous system, a number of groups have studied the ability of agonists to produce convulsions, or of antagonists to prevent seizure activity. It has been shown that antagonists acting at the receptor responding primarily to *N*-methylaspartate (NMA) (Croucher et al 1982; Meldrum et al 1983), as well as antagonists acting at the receptors for α-kainate and quisqualate (Croucher et al 1984), have anticonvulsant activity including an ability to inhibit sound-induced seizures in genetically susceptible mice.

A recent report by Collins et al (1984) has demonstrated that a series of kainic acid derivatives, particularly β-kainic acid, are also able to prevent sound-induced seizures in mice. The present study was therefore designed to investigate the actions of β-kainate at amino acid receptors in the CNS using both in-vivo microiontophoresis and brain slice methodology.

Methods

For microiontophoresis (Stone 1985) male rats were anaesthetized with urethane, 1·5 g kg^{-1} i.p. and the somatosensory regions of cerebral cortex exposed and covered with saline. Drugs were applied from seven-barrelled micropipettes filled with solutions of the following (mM): NMA 20, pH 7; quisqualic acid 10, pH 8; α-kainic acid 10, pH 7; β-kainic acid 10, pH 7. Neuronal activity was recorded by a separate microelectrode glued alongside the multibarrel. Action potentials were amplified, displayed on oscilloscopes, gated and counted before being recorded on a Grass polygraph as firing rate in spikes s^{-1}.

In the studies of hippocampal slices, rats were killed by a blow to the head and the hippocampi removed into ice-cold saline of the following composition: (mM) NaCl 115, KCl 2·0, KH_2PO_4 2·2, $MgSO_4.7H_2O$ 1·2, $CaCl_2.2H_2O$ 2·5, $NaHCO_3$ 25, glucose 10. Slices were cut at 500 μm on a McIlwain chopper and placed in an atmosphere of water-saturated oxygen at room temperature for 1–2 h before use. Individual slices were then transferred to a 1 ml chamber and superfused with the physiological solution at 30 °C.

CA_1 pyramidal cells were activated antidromically by a bipolar electrode, using pulses of 500 μs duration, 100–500 μA amplitude and 0·5 Hz. The evoked population spike was recorded by a single micropipette, tip resistance 5–10 mΩ, located in the stratum pyramidale. Groups of 16 responses were averaged and the average plotted onto a chart recorder. Agonist compounds were added to the superfusing medium for 2 min. When tested as an antagonist, β-kainate was superfused for 5 min before, as well as during agonist application.

Results and discussion

The slice experiments revealed an agonist action of β-kainate with a potency approximately one sixth that of α-kainate (Fig. 1). However β-kainate had no antagonist activity against α-kainate, quisqualate or NMA. Indeed a combination of β-kainate with any of these agonists resulted in an additive response.

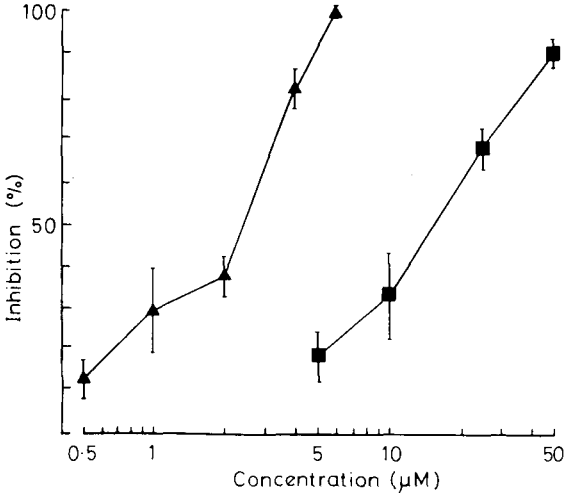

FIG. 1. Dose response relation for α-kainic acid (▲) and β-kainic acid (■) in producing depression of the CA_1 evoked population spike in rat hippocampal slices. Each point is the mean ± s.e.m. of observations on 8 slices. The ED50 values for the two compounds are 2·44 ± 0·26 μM (n = 8) and 16·1 ± 2·2 μM (n = 8), respectively.

* Correspondence.

The agonist activity of β-kainate was again evident from the microiontophoretic experiments in-vivo, where β-kainate produced responses very similar in profile to those of α-kainate though it was less potent. However, β-kainate showed no ability to antagonize excitation due to α-kainate, quisqualate or NMA. Indeed the application of β-kainate together with these agonists often produced merely a rising baseline. Even when β-kainate was applied at very low currents of about 5 nA, however, which was insufficient to produce an increase of baseline activity, the presence of background depolarization was seen as a rapid over-depolarization when the agonists were applied.

It may be concluded that β-kainate is *not* an antagonist of excitatory amino acids acting at the three main types of receptor currently recognized for NMA, quisqualic acid or α-kainate at least in the neocortex in-vivo or hippocampus in-vitro. The anticonvulsant properties of this isomer are presumably therefore unrelated to these receptors, or may involve a presynaptic action to reduce the release of endogenous substances mediating part of the convulsive behaviour.

The marked behavioural effects seen after β-kainate administration in-vivo (Collins et al 1984) could however, reflect the agonist properties of the compound. It is not yet clear whether these agonist effects are mediated by the α-kainate receptor, other excitatory sites, or a non-specific form of depolarization, as no selective antagonists at the α-kainate receptor are currently available. It would be of interest to examine the activity of β-kainate in kainate binding studies to help answer this question.

REFERENCES

Collins, J. F., Dixon, A. J., Badman, G., De Sarro, G., Chapman, A. G., Hart, G. P., Meldrum, B. S. (1984) Neurosci. Lett. 51: 371–376

Croucher, M. J., Collins, J. F., Meldrum, B. S. (1982) Science 21: 899–901

Croucher, M. J. Meldrum, B. S., Jones, A. W., Watkins, J. C. (1984) Brain Research 322: 111–114

Meldrum, B. S., Croucher, M. J., Badman, G., Collins, J. F. (1983) Neurosci. Lett. 39: 101–104

Stone, T. W. (1985) Microiontophoresis and Pressure Ejection. Wiley, Chichester

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Amiodarone does not affect digoxin kinetics in the rabbit

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It has been reported that amiodarone may interact with digoxin in man. We investigated the effects of amiodarone pretreatment (35 mg kg^{-1} day^{-1}) on the pharmacokinetics of a single dose of digoxin (50 μg kg^{-1}) in 6 rabbits. Total body clearance of digoxin was 138·84 ± 44·67 and 147·99 ± 29·17 ml min^{-1}, serum half life 187·9 ± 60·9 and 181·34 ± 25·57 min and volume of distribution 35·4 ± 8·54 and 37·8 ± 3·9 litres before and after amiodarone treatment, respectively. None of these changes were statistically significant. We conclude that the presence of an amiodarone-induced change in digoxin pharmacokinetics in the rabbit was not evident and that other animal models will be necessary for studying this interaction.

Amiodarone is a benzofuran iodine-containing compound which has been used effectively for a wide variety of cardiac arrhythmias (Marcus et al 1981). Recent reports indicate that it may interact with digoxin (Furlanello et al 1981; Moysey et al 1981; McGovern et al 1983) although not all studies have confirmed this. Since it is technically and ethically difficult to examine the mechanism of this interaction in man, we decided to investigate the effect of amiodarone pretreatment on digoxin clearance in the rabbit.

* Correspondence.

Methods

Six New Zealand white rabbits (2·5–3·75 kg) were caged separately in metabolism cages. On day 1 the rabbits were injected slowly with digoxin (Antigen Ltd) 50 μg kg^{-1} i.v. via the left ear marginal vein over 2 min. Venous blood samples (2 ml) were obtained at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min by incision of the right ear marginal vein and blood was collected into potassium EDTA collection tubes which were stored on ice until separation by centrifugation at 500g for 10 min. Plasma was removed and the samples were frozen at −20 °C until assayed (within 3 months).

At the end of day 1 and successively on days 2, 3 and 4, amiodarone (Cordarone-Labaz) 35 mg kg^{-1} was injected subcutaneously. On day 5, samples were collected as previously described and the animals killed by cervical dislocation.

Serum digoxin concentrations were measured using a commercially available radioimmunoassay kit (Amerlex-Amersham International). Amiodarone at a concentration of 4 mg $litre^{-1}$ showed no interference with the assay.

The decline in the post-distribution phase digoxin concentrations (pre- and post-amiodarone) was biex-