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THE EFFECTS OF CYCLIC DICARBOXYLIC ACIDS ON SPONTANEOUS AND AMINO ACID-EVOKED ACTIVITY OF RAT CORTICAL NEURONES

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1 A series of cyclic dicarboxylic acids were applied by microiontophoresis to neurones in the cerebral cortex of rats anaesthetized with urethane. The object was to examine effects on spontaneous firing rates and any ability to antagonize responses to excitatory amino acids.

2 At relatively low ejecting currents (10–25 nA) *cis*-2,3-piperidine dicarboxylic acid (*cis*-2,3-PDA) had no effect on spontaneous firing but selectively antagonized the excitation evoked by N-methyl-D-aspartate (NMDA) without affecting responses to quisqualate or kainate. At higher ejecting currents (60–100 nA) responses to all three agonists were reduced.

3 Other *cis*-piperidine dicarboxylic acids and piperazine-2, 3-dicarboxylic acid had only weak and variable effects on cell firing and responses to NMDA, quisqualate, kainate, glutamate and aspartate.

4 2, 3-Pyridine dicarboxylic acid (quinolinic acid) produced excitation of all cortical neurones tested.

5 2-Amino-5-phosphono-valeric acid, an NMDA antagonist, reduced responses to quinolinate, implying that this compound can act at NMDA receptors.

6 It is suggested that quinolinic acid may be of physiological interest as a potential endogenous excitant in the nervous system and that *cis*-2,3-PDA and its N-methyl derivative may be of use in studies of receptor pharmacology and the identification of synaptic transmitters.

Introduction

Neurochemical and electrophysiological data strongly suggest that one or more dicarboxylic amino acids may act as synaptic transmitters in the central nervous system (Curtis & Johnston, 1974; Krnjević, 1974; Stone, 1979a, b; DiChiara & Gessa, 1981; DeFeudis & Mandel, 1981). The most popular candidates for such a role are L-glutamate and L-aspartate.

It has been suggested that at least two kinds of receptor for such dicarboxylic amino acids may exist for which the most effective agonists are N-methyl-D-aspartate (NMDA) and quisqualic acid respectively (Davies & Watkins, 1979; Evans, Francis, Hunt, Oakes & Watkins, 1979). A third receptor type may exist for which the preferred agonist is kainic acid. In the search for specific agonists and antagonists at these receptors, numerous structure activity studies have been reported (Watkins & Evans, 1981). In a recent investigation Davies, Evans, Francis, Jones & Watkins (1981a) noted that *cis*-2,3-piperidine dicarboxylic acid (*cis*-2,3-PDA) is an antagonist at excitatory amino acid receptors in the spinal cord, though it seemed not to be selective as it reduced responses to agonists at all three types of receptors. The present study is an attempt to examine the actions of a series of cyclic dicarboxylic acids, including *cis*-2,3-PDA, as agonists or excitatory amino acid antagonists in the cerebral cortex, in order to assess the structural specificity of any effects.

Methods

Male Wistar rats were anaesthetized with urethane, (1.5 g/kg. i.p.) and held in a stereotaxic frame. The somatosensory areas of cerebral cortex around the bregma suture were exposed and the dura mater removed. The exposed surface of the cortex was covered with warmed saline throughout the experiment. The rectal temperature was maintained at 37°–38°C by an automatically controlled heating blanket.

All drugs were applied by microiontophoresis from seven-barrelled micropipettes containing glass fibres (Clark Electromedical) to permit rapid filling of the barrels immediately before use. The tip of the mic-

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ropipette was broken back to an overall size of 4–8 μm under microscopic observation. A separate single glass microelectrode was glued alongside the multibarrel assembly under microscopic control, for recording unit activity (Stone, 1973). The assembly was arranged so that the recording electrode protruded 0–5 μm beyond the multibarrel. Spikes were amplified, displayed on Tektronix oscilloscopes, gated by a window discriminator and counted and recorded on a Grass polygraph either by a resetting integrator (reset time 1 s) or by a continuous ratemeter with a time constant of 2 s.

The following compounds were used: L-glutamate sodium*, 100 mM, pH 8; L-aspartate potassium*, 100 mM, pH 8; kainic acid* 20 mM, pH 4; N-methyl D-aspartic acid, (NMDA) 20 mM, pH 5; (−)-D-2-amino-5-phosphono-valeric acid (2APV) 10 mM, pH 5; quisqualic acid, 10 mM, pH 5; *cis*-2,3-piperidine dicarboxylic acid (2,3-PDA); 20 mM, pH 5; *cis*-2,4-piperidine dicarboxylic acid, 10 mM, pH 5; *cis*-2, 5-piperidine dicarboxylic acid, 10 mM, pH 5; *cis*-2, 6-piperidine dicarboxylic acid, 10 mM, pH 5; 2,3-pyridine dicarboxylic acid*, (quinolinic acid), 10 mM, pH 5; 2,6-pyridine dicarboxylic acid* (dipicolinic acid), 10 mM, pH 5; phthallic acid*, 10 mM, pH 4; *cis*-2,3-piperazine dicarboxylic acid, 20 mM, pH 4; (Figure 1). Drugs marked with an asterisk were purchased from Sigma Chemicals. Quisqualic acid was obtained both as a gift from Dr H. Shinozaki, and after being extracted from *Quisqualis indica*. The remaining compounds were synthesized by S.B. and J.F.C. pH was adjusted where necessary by the addition of NaOH or HCl.

cis-2,3-PDA was prepared by hydrogenation for 24 h at 50 p.s.i. of 2,3-pyridine dicarboxylic acid in water using PtO_2 (BDH) as catalyst. The crude product was a 8:1 *cis-trans* mixture as judged from ^{13}C nuclear magnetic resonance (n.m.r.). The product was purified by crystallization from alcohol-water to yield pure *cis*-2,3-PDA. cis-2,4-PDA was prepared and purified in a similar way. cis-2,5-PDA was also prepared by hydrogenation but acetic acid was used as the solvent. *cis*-2,6-PDA was also prepared in this way, but here very little *trans*-product was obtained and the product was crystallized from alcohol : acetone : water.

cis-2,3-piperazine dicarboxylic acid was prepared by hydrogenation of pyrazine dicarboxylic acid hydrochloride in water, and the product crystallized from ethanol-water.

The purity of all products was confirmed by microanalysis, ^{13}C n.m.r. and mass spectroscopy.

Where compounds were tested as antagonists of amino acid evoked excitation, the criterion for describing responses as antagonized was that they were reduced by at least 50% of their control size, measured as the peak height of the responses (Figure 2). Where a compound is said to have produced only incomplete antagonism, this refers to a situation when full antagonism could not be obtained by increasing the size or duration of ejecting current used to apply the antagonist.

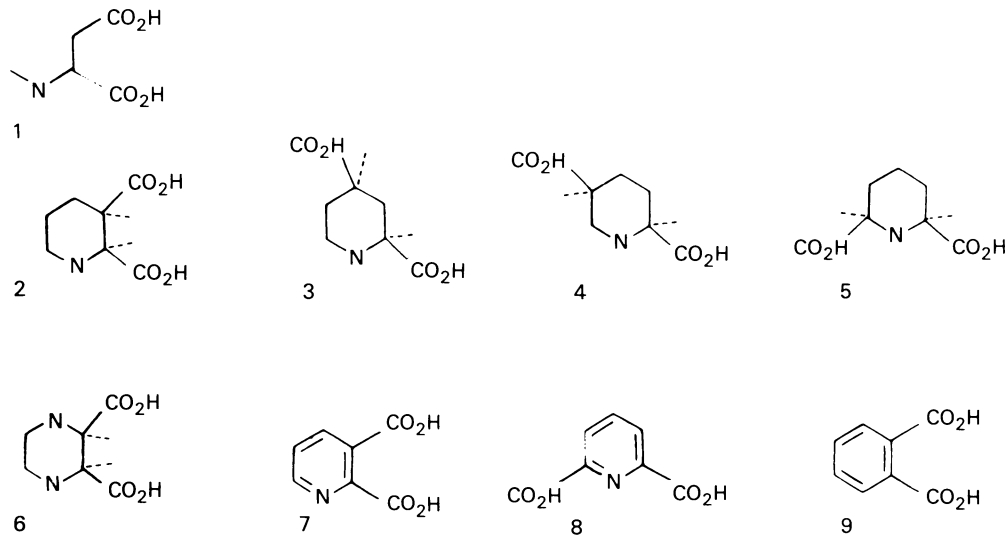

Figure 1 Structures of the dicarboxylic acid derivatives used in this study: (1) N-methyl-D-aspartic acid (NMDA); (2) *cis*-2,3-piperidine dicarboxylic acid (*cis*-2,3-PDA); (3) *cis*-2,4-piperidine dicarboxylic acid (*cis*-2,4-PDA); (4) *cis*-2,5-piperidine dicarboxylic acid (*cis*-2,5-PDA); (5) *cis*-2,6-piperidine dicarboxylic acid (*cis*-2,5-PDA); (6) *cis*-2,3-piperazine dicarboxylic acid; (7) 2,3-pyridine dicarboxylic acid (quinolinic acid); (8) 2,6-pyridine dicarboxylic acid (dipicolinic acid); (9) phthallic acid.

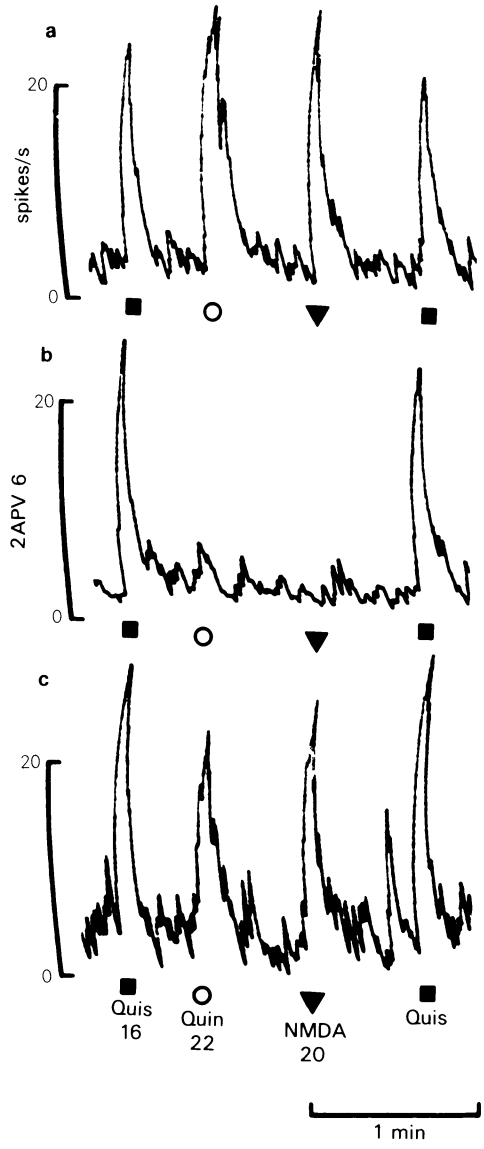

Figure 2 Record of the firing rate of a cortical neurone excited by the iontophoresis of quisqualic acid (Quis), 16 nA, quinolinic acid (Quin), 22 nA, and N-methyl-D-aspartate (NMDA), 20 nA. Control responses are shown in (a). The iontophoresis of 2-amino-5-phosphono-valeric acid (2APV), 6 nA, caused antagonism of responses to quinolinic acid and NMDA with almost no effect on the response to quisqualic acid, as seen in (b) 2 min after beginning ejection of the antagonist. Recovery of responses is seen in (c), begun 2 min after (b). Ordinate scale: spikes/s. Time bar: 1 min.

Results

Agonistic effects

On spontaneous activity *cis*-2,3-PDA had no detectable effect when applied by lower ejecting currents (less than 25 nA) but at higher ejecting currents (over 60 nA) a very slow and weak increase of firing was seen on 4 of 14 cells.

The other piperidine dicarboxylic acids had weak and variable effects on spontaneous neuronal activity. The *cis*-2,4-PDA weakly depressed 1 and excited 2 of 12 cells tested at ejecting currents of 60 nA or more. *cis*-2,5-PDA had no effect on the spontaneous activity of 14 cells. *cis*-2,6-PDA evoked only a weak excitation of 12 of 32 cells, when applied by ejecting currents of 50 nA or more.

cis-2,3-Piperazine dicarboxylic acid proved to have agonist activity and caused excitation of 9 of 10 cells.

2,3-Pyridine dicarboxylic acid (quinolinic acid) proved to be an effective excitant of cortical neurones, exciting all of 55 neurones studied when applied with currents of 20 to 65 nA for 5 to 15 s. Figure 2 illustrates the responses to quinolinic acid, quisqualic acid and NMDA. Figure 2 also illustrates the ability of the NMDA antagonist, (−)-D-2-amino-5-phosphono-valeric acid (2APV) (Davies, Francis, Jones & Watkins, 1981b; Perkins, Stone, Collins & Curry, 1981) to antagonize the excitation evoked by quinolinic acid together with the response to NMDA. 2APV abolished responses to NMDA and quinolinate without affecting responses to quisqualate on 16 of 19 cells.

2,6-Pyridine dicarboxylic acid (dipicolinic acid) caused a weak excitation of 4 of 12 cells tested when applied by higher currents (more than 60 nA).

Phthallic acid was applied to 15 cells using ejecting currents of up to 100 nA for 6 min but no effect on spontaneous firing was seen.

Antagonistic effects

At relatively high ejecting currents (60 to 100 nA) *cis*-2,3-PDA caused a nonselective reduction in the size of responses to NMDA, glutamate, kainate and quisqualate. The responses to these compounds were reduced to a similar extent on all 12 cells tested (Figure 3). At lower ejecting currents (10 to 25 nA) *cis*-2,3-PDA completely antagonized the responses to NMDA, with no change of responses to kainate or quisqualate, on 16 of 18 neurones. An example is shown in Figure 3.

cis-2,4-PDA and *cis*-2,5-PDA exhibited no antagonistic action towards any of the excitatory agonists NMDA, glutamate, kainate or quisqualate.

On 5 of 25 cells, *cis*-2,6-PDA appeared to reduce

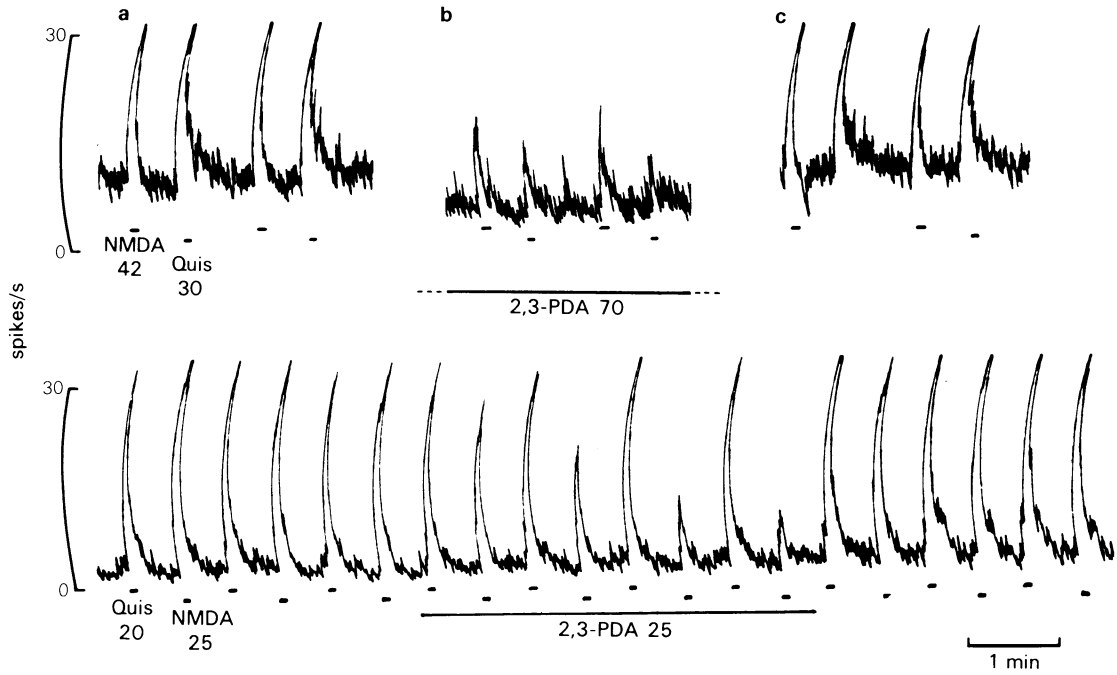

Figure 3 Upper traces: records of the firing rate of a cortical neurone excited by pulses of N-methyl-D-aspartate (NMDA), 42 nA, and quisqualic acid (Quis) 20 nA. The iontophoresis of *cis*-2,3-PDA with a current of 70 nA produces a fall of the background firing rate together with a non-selective reduction in the size of responses to the agonists. Record (b) was begun approximately 2 min after beginning the ejection of *cis*-2,3-PDA, and record (c), showing the recovery of control response size was begun 4.5 min after (b). Lower trace: record of the firing rate of a neurone in the cerebral cortex, excited by pulses of iontophoretically applied quisqualic acid (Quis), 20 nA, and N-methyl-D-aspartate (NMDA) 25 nA. The iontophoresis of *cis*-2,3-PDA with a current of 25 nA reduces selectively the responses to NMDA. Ordinate scale: spikes/s. Time bar: 1 min.

the excitation evoked by glutamate or quisqualate at currents of 40 to 60 nA, with no change in responses to NMDA. The reduction in the size of responses to glutamate or quisqualate was only partial, reaching about 50% on 4 cells and about 75% on one cell even with prolonged application (10 min). Increasing the ejecting current to 80 or 100 nA gave a greater reduction in the size of the response but it also reduced the response to NMDA.

cis-2,3-Piperazine dicarboxylic acid produced a partial, non-selective reduction in the size of responses to NMDA and quisqualic acid on 1 of 10 cells.

Quinolinic acid could not be tested as an antagonist of evoked excitation because of its own excitant activity, but dipicolinic acid caused a reduction to 50% in responses to NMDA, but not in responses to glutamate or to quisqualate, on 5 cells. Conversely, it antagonized responses to glutamate but not to NMDA on 3 cells and produced an approximately equal antagonism of responses to NMDA, quisqualate and kainate on 8 cells. Ejecting currents in excess of 60 nA were required to evoke all these effects.

Discussion

The present results show that *cis*-2,3-PDA exhibits antagonistic properties towards excitatory amino acids which are not shared by the *cis*-2,4-, 2,5- or 2,6-dicarboxylic analogues. Of some surprise, was the observation that this antagonism appeared to be relatively selective for NMDA, since Davies *et al.* (1981a) have reported that *cis*-2,3-PDA reduces excitation evoked by kainate and quisqualate in addition to excitation evoked by NMDA. Indeed, this group reported that *cis*-2,3-PDA was the most effective compound they had encountered in antagonizing responses to quisqualate. At present we can only suggest as an explanation of this difference that some subtle differences may exist between the spinal cord receptors and those in the cerebral cortex, in the sense that in cortex either quisqualate excitations are more resistant or NMDA is more susceptible to blockade by *cis*-2,3-PDA. Alternatively there may be some difference of cellular localization, the two receptor types being grouped on different parts of the

cell surface, and exhibiting apparent differences of sensitivity to antagonists when studied by the iontophoretic technique. It must be emphasised that this selectivity was apparent only at the lower currents used by *cis*-2,3-PDA, and at currents above this the non-selective antagonism seen by Davies *et al.* (1981a) was also noted in the present study.

It is not surprising that *cis*-2,3-PDA showed some selectivity against responses to NMDA as it is a highly structured, semi-rigid analogue of NMDA. The failure of 2,3-piperazine dicarboxylic acid to show any activity does, however, imply that the electronic distribution in the ring is of importance for activity, and the three-dimensional structure is not the only criterion.

The observation that *cis*-2,3-PDA could cause a weak increase of firing of a small proportion of neurones is consistent with the conclusion of Davies *et al.* (1981a) that this compound is a partial agonist.

It is of interest that the fully rigid analogue of NMDA, quinolinic acid, is an effective excitant of cortical neurones, though Watkins (1978) has found it to be a relatively weak excitant in the spinal cord. However, our present observations are consistent with the work of Lapin and his colleagues (Lapin, 1981) who have reported on the potent convulsant activity of kynurenines, including quinolinic acid. This compound is a normal product of cell metabolism (Mahler & Cordes, 1971), and thus it is possible that it may have some physiological role in modulating neuronal excitability. Besides occurring endogenously in mammalian tissues, quinolinic acid has also been detected at the lobster neuromuscular junction (Netherton & Gurin, 1980) where an excitatory amino acid or related compound (Shinozaki & Ishida, 1979; Ishida & Shinozaki, 1980) have long been suspected of being the neurotransmitter.

The present results also suggest that quinolinic acid activates at least one of the two main types of amino acid receptor thought to occur in the brain, namely those for NMDA. This conclusion follows from the ability of 2APV (Davies *et al.*, 1981b; Perkins *et al.*, 1981) to abolish responses to quinolinic acid in parallel with NMDA without affecting responses to quisqualate.

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