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Modulators of metabotropic glutamate receptors microinfused into perirhinal cortex: Anticonvulsant effects in rats challenged with soman

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ABSTRACT

Examination of critical subreceptors in the seizure controlling perirhinal cortex has revealed that microinfusion of ionotropic glutamatergic antagonists can exert anticonvulsant efficacy against soman-induced seizures. The purpose of the present study was to investigate whether modulators of metabotropic glutamate (mGlu) receptors may ensure anticonvulsant effects when microinfused into the perirhinal cortex. The results showed that the mGlu5 receptor antagonist MPEP hydrochloride (2-Methyl-6-(phenylethynyl) pyridine hydrochloride) and the mGlu2/3 receptor agonist DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl) glycine) caused full protection against seizures or increased latency to onset of seizures, whereas the mGlu1 receptor antagonist LY367385 ((S)-(+)-α-Amino-4-carboxy-2-methylbenzeneacetic acid) did not produce anticonvulsant efficacy in response to systemically administered soman ($1.3\times LD_{50}$). Low doses of the above modulators had no anticonvulsant effects, whereas too high dose of MPEP resulted in proconvulsant effects. The results suggest that the perirhinal cortex is a likely site of cholinergic recruitment of glutamatergic hyperactivity after exposure to a convulsant dose of soman. Modulators of mGlu receptors may represent an alternative or supplement to ionotropic glutamate antagonists as anticonvulsants against nerve agent-evoked seizures.

1. Introduction

The nerve agent soman is a highly toxic organophosphate that irreversibly inhibits acetylcholinesterase, the enzyme hydrolysing acetylcholine. Accumulation of acetylcholine in the synaptic cleft results in respiratory dysfunction, prolonged limbic seizures, convulsive status epilepticus and death. The course of neurochemical events after nerve agent poisoning evoking seizures/convulsions can be divided into 3 phases. An early phase lasting from the time of exposure to about 5 min after seizure onset is dominated by excessive cholinergic activity. Then follows a transitional phase of cholinergic and glutamatergic hyperactivity and finally a predominantly glutamatergic phase after about 40 min (McDonough and Shih 1997).

The current management regimen (pyridostigmine, oxime, and atropine) can enhance the survival rate significantly, but it does not effectively reduce nerve agent-induced seizure activity resulting in brain injury. The search for more effective countermeasures has aimed at drugs exerting cholinergic and glutamatergic antagonism along with GABAergic (γ-aminobutyric acid) agonism (McDonough and Shih, 1997). However, such relatively simple search criteria will probably lead to a vast number of drugs with potential anticonvulsant properties. In order to narrow down the number of candidate drugs an alternative approach may be to determine critical subreceptors in seizure controlling brain regions and thus obtain more specific criteria for selection of drugs.

In experimental epilepsy, seizure controlling regions have been identified by means of lesion studies and microinfusion studies (Löscher and Ebert, 1996). Among the latter structures are the substantia nigra, area tempestas, perirhinal cortex, and posterior piriform cortex (Gale, 1988; Halonen et al., 1994). The anterior perirhinal cortex and posterior piriform cortex act as critical links in the propagation of epileptiform activity in limbic structures generated from microinfusion of bicuculline into the area tempestas (Halonen et al, 1994). The results from a recent study of microinfusions show that in the perirhinal cortex anticonvulsant efficacy against seizures induced systemically by soman, is achieved by procyclidine or NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzoquinoxaline), whereas a corresponding effect is obtained by scopolamine or muscimol in the posterior piriform cortex (Myhrer et al., 2010). The findings from the perirhinal cortex suggest that both AMPA (α-amino-2, 3-dihydro-5-methyl-3-oxo-4-isoxazolepropionic acid) and kainate receptors were antagonized by NBQX, although the selectivity for kainate receptors is weaker than for AMPA receptors (Fornai et al., 2005). Furthermore, the anticonvulsant efficacy of procyclidine implies that blocking of NMDA (*N*-Methyl-D-Aspartate) receptors had to be supported by antimuscarinic impact, because ketamine is without anticonvulsant effect in the perirhinal cortex (Myhrer et al., 2010). These findings seem to indicate that there is an increase of glutamatergic activity in the perirhinal cortex already during the cholinergic phase,

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and that the perirhinal cortex is a potential site for recruiting the glutamatergic phase of the 3-phase model. Hence, metabotropic glutamate receptors may also be involved in the initial stages of nerve agent-induced seizure activity in the perirhinal cortex. Evidence has been provided for the presence of functional group I, II, and III metabotropic glutamate receptors in the rat perirhinal cortex (McCaffery et al., 1999).

Through a growing body of studies, metabotropic glutamate (mGlu) receptors have been shown to fulfill unique presynaptic and postsynaptic roles (Alexander and Godwin, 2006). In contrast to ionotropic glutamate receptors, which mediate fast synaptic transmission, mGlu receptors often modulate ongoing activity. When located postsynaptically mGlu receptors may modulate membrane properties by second messenger interactions, whereas presynaptic mGlu receptors have been shown to control synaptic release (Alexander and Godwin, 2006). These modulatory aspects appear to have attracted attention in experimental epilepsy. Group I mGlu receptor antagonists and Group II mGlu receptor agonists have been demonstrated to exert anticonvulsant efficacy, whereas Group III mGlu receptor agonists show mixed responses in an animal model of epilepsy (Alexander and Godwin, 2006).

The purpose of the present study was to examine potential anticonvulsant impact of Group I and II mGlu receptor modulators microinfused into the perirhinal cortex of rats that subsequently received systemically a convulsant dose of soman. Because too high dose of mGlu receptor modulators may generate proconvulsant effects in epilepsy research (Folbergrová et al., 2001; Miyamoto et al., 1997), the rats in the first part of this study (Experiment 1) received doses that have been shown to produce anticonvulsant efficacy in microinfusion studies in experimental epilepsy. In the second part (Experiment 2), the rats received doses shown to cause anticonvulsant effects in systemic studies of the same mGlu receptor modulators in experimental epilepsy. The modulators selected were LY367385 ((S)-(+)-α-Amino-4-carboxy-2-methylbenzeneacetic acid) acting at mGlu1 receptors, MPEP (2-Methyl-6-(phenylethynyl) pyridine hydrochloride) acting at mGlu5 receptors, and DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine) acting at mGlu2/3 receptors that all have been shown to assure anticonvulsant efficacy in rats exposed to kindling or chemoconvulsants (Folbergrová et al., 2001; Nagaraja et al., 2004). The drugs were infused into the target area 20 min before soman was administered systemically. Prevention of seizures/convulsions or increased latency to onset of seizures was used as a measure of anticonvulsant effects.

2. Materials and methods

2.1. Subjects

A total of 68 male Wistar rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 300–330 g (about 90 days old) at the time of surgery were used as subjects. The experiments were approved by the National Animal Research Authority. In Experiment 1, 4 groups of rats ($N = 7$–8) received bilateral microinfusion of low doses of drugs or vehicle into the perirhinal cortex. In Experiment 2, 5 groups of rats ($N = 6$–8) received microinfusion of high doses of drugs or vehicle into the perirhinal cortex. The animals were housed individually and had free access to commercial rat pellets and water. The rats were handled individually 4 days preoperatively and 4 days postoperatively, being allowed to explore a table top (80 × 60 cm) for 3 min per day. The climatized vivarium (21 °C) was illuminated from 0700 to 1900 h.

2.2. Surgery

The rats were anesthetized i.p. with diazepam (10 mg/kg) and fentanyl fluanisone (2 mg/kg). Lidocain liniment was applied to the periost. The rats were implanted sterotaxically (flat skull) with guide cannulas aimed at the perirhinal cortex in both hemispheres. The guide cannula (25 gauge) was 0.5 mm in diameter and cut to a length of 11 mm. The upper part of the cannula was roughened in order to improve the grip of the dental cement (Durelon; ESPE, Seefeldt, Germany), which was anchored to the skull by steel screws. The point of insertion was 4.5 mm behind the bregma and 6 mm lateral to the midline. The cannula was lowered in an angle of 15° (end pointing laterally) 6 mm from the top of the skull. A cannula 0.3 mm in diameter and 12 mm long (30 gauge) was fitted into the guide cannula and protruded 1 mm beyond the latter one. The infusions were made by means of a microinjection pump (Model CMA 100, Carnegie Medicine AB, Stockholm, Sweden). To prevent plugging of the indwelling cannulas, smaller cannulas (30 gauge) with a cut and bent top were inserted to a depth of 10 mm. The rats were allowed to recover 8 days before experimentation.

2.3. Histology

The perirhinal cortex was defined as areas 35 and 36 of Brodmann (Burwell, 2001). The injection sites were marked with 1 µl of blue tissue marking dye (Triangle Biomedical Sciences, NC, USA) 1 mm beyond the tip of the implanted cannulas. This marking was carried out immediately after death due to soman poisoning or under anesthesia (as for surgery) before decapitation. The brains were removed and stored in 10% formalin and dehydrated before being embedded in paraffin. The sections were made frontally 5 µm thick and stained with hematoxylin and eosin (HE). Rats that did not convulse (survival time 7 days) plus 3 rats that convulsed in group DCG-IV 1 µg (survival time 1 h) were anesthetized as described for surgery, perfused intracardially with 10% formalin and post-fixed in 10% formalin for at least 24 h. The brains were dehydrated and embedded in paraffin and the sections (5 µm) were stained with Fluoro-Jade B (Schmued and Hopkins 2000). The anterior piriform cortex, hippocampal CA1, and basolateral amygdala were used as index areas for the assessment of neuropathology.

The tissue was examined by using an epifluorescent microscope with blue (450–490 nm) excitation light, and a fluoroscein isothiocyanate filter was applied. A digital microscope camera (AxioCam, Zeiss, Jena, Germany) was used to make photomicrographs. This technique allows processing of the photographs so that elements of particular interest can be made clearer by adjusting contrasts.

2.4. Drug administration

Because too high dose of mGlu receptor modulators can cause proconvulsant effects in experimental epilepsy (Folbergrová et al., 2001), doses used in previous microinfusion studies in preclinical epilepsy were initially applied. In Experiment 1, the doses used were LY367385 0.40 µmol, MPEP 0.06 µmol (Nagaraja et al., 2004), and DCG-IV 1 nmol (Folbergrová et al., 2001). In Experiment 2, the doses were taken from systemic studies of the same drugs in epilepsy research. The doses were MPEP 1 µg and 0.1 µg (Jesse et al., 2008) and LY367385 10 µg (Murotomi et al., 2008). The dose of 1 µg of DCG-IV was estimated on the basis of doses infused intracerebroventricularly (Folbergrová et al., 2001), because no previous systemic studies of this drug could be found. The drugs were purchased from Tocris Cookson Ltd (Bristol, UK), and they were dissolved in 0.9% saline. Saline (0.9%) was used as vehicle. All drugs were given 1 µl over 1 min while the rats were gently held, and the cannula remained in position for an additional ½ min before retraction. All bilateral injections were carried out simultaneously. Twenty minutes following microinfusions the rats received 1.3 × LD_{50} of soman (100 µg/kg) subcutaneously. This dose of soman produces seizures in all rats, and the lethality is 100% (Sterri et al., 1980). Unless treated systemically with anticonvulsants, rats poisoned with the present dose of soman will die within 2 h. The