NeuroToxicology 34 (2013) 128–134

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

The perirhinal cortex of rats: An intricate area for microinfusion of anticonvulsants against soman-induced seizures

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ARTICLE INFO

Article history:
Received 21 June 2012
Accepted 23 October 2012
Available online 16 November 2012

Keywords:
Anticonvulsants
Microinfusions
Perirhinal cortex
Soman
Seizures

ABSTRACT

Microinfusion of anticonvulsants into the perirhinal cortex through 1 guide cannula in each hemisphere only invades a small area of this seizure controlling site in rats exposed to soman. The purpose of the present study was to examine whether infusions made through 2 cannulas in each perirhinal cortex may produce more efficacious anticonvulsant action against soman intoxication than the use of 1 cannula only in rats infused with the ionotropic antagonists procyclidine and caramiphen or the metabotropic glutamate modulators DCG-IV and MPEP. The results showed that the mere presence of indwelling double cannulas caused proconvulsant effect in response to subsequent systemic administration of soman. Both the control and caramiphen groups with double cannulas had significantly shorter latencies to seizure onset than the corresponding groups with single cannula. Procyclidine resulted in anticonvulsant efficacy, even in rats with double cannulas. In rats that received twin infusions of DCG-IV or MPEP, the anticonvulsant impact was very high, inasmuch as a majority of the rats in each group was protected against seizure activity. Drugs possessing powerful anticonvulsant potency can apparently counteract the proconvulsant effect of double cannulas, and some can even gain enhanced anticonvulsant capacity when invading a larger area of the perirhinal cortex. Perirhinal EEG recordings (electrodes in indwelling cannulas) in a separate set of rats not exposed to soman or drugs showed no differences in basal electrical activity (total power 0.5–25 Hz or the theta band 4–12 Hz) between groups with single or double cannulas. The intrinsic excitability and synaptic connectivity of the perirhinal cortex may be associated with the proconvulsant impact observed in rats with double cannulas when exposed to soman.

1. Introduction

In preclinical epilepsy studies, seizure controlling areas have been identified by means of lesion studies and microinfusion studies (Löscher and Ebert, 1996). Some of these areas 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). In nerve agent research, it has been shown that microinfusion of procyclidine or NBQX into the perirhinal cortex ensures anticonvulsant efficacy against seizures subsequently induced systemically by soman, whereas a corresponding effect is obtained by scopolamine or muscimol in the posterior piriform cortex (Myhrer et al., 2010a). In addition to ionotropic glutamate receptor antagonists, also metabotropic glutamate modulators (DCG-IV ((2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine), MPEP (2-methyl-6-(phenylethynyl)pyridine hydrochloride)) cause anticonvulsant impact against soman intoxication when microinfused into the perirhinal cortex (Myhrer et al., 2010b). These findings probably indicate that there is an increase of glutamatergic activity in the perirhinal cortex already during the cholinergic phase of nerve agent poisoning, and that the perirhinal cortex is a potential site for recruiting the glutamatergic phase of the 3-phase model of McDonough and Shih (1997).

Microinfusions made through a single cannula in each perirhinal cortex probably affect a comparatively small fraction (about 7%) of the entire structure (Myhrer et al., 2010a). Lesions of the perirhinal cortex comprising an average of 74% prevent convulsions in 38% of the rats in response to a convulsant dose of soman (Myhrer et al., 2008a). Enhancement of the anticonvulsant efficacy might have been achieved if the infusions had affected a larger area of the perirhinal cortex, because microinfusions into the area tempestas affecting almost the entire region can prevent seizures in up to 75% of the animals, whereas lesions (comprising an average of 74%) in the same area prevent convulsions in 43% of the cases (Myhrer et al., 2007, 2008b).

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0161-813X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuro.2012.10.019

In principle, pharmacological manipulation of transmitter activity may have more powerful anticonvulsant impact than mere disruption of neuronal pathways. The anterior part of the perirhinal cortex that is the site of origin of the direct projections to the frontal cortex (McIntyre et al., 1996) appears as the most strategic region to affect pharmacologically and served as the target area in the previous microinfusion studies (Myhrer et al., 2010a,b).

Procyclidine (6 μg/μl) produces anticonvulsant effect when microinfused into the perirhinal cortex, whereas caramiphen (10 μg/μl) yielding similar glutamatergic and cholinergic antagonism does not (Myhrer et al., 2010a). The latter finding may imply that higher doses of caramiphen might have resulted in anticonvulsant impact. However, variations in solubility of chemical substances and the small volume used in microinfusion studies (0.25–1 μl) limit the doses that can be applied. In our hands, procyclidine can maximally be dissolved in 0.9% saline to 6 μg/μl. Both procyclidine (6 μg/μl) and caramiphen (10 μg/μl) cause anticonvulsant effects against soman poisoning when infused into the area tempestas in which cholinergic antagonism is supposed to be the crucial action of these drugs (Myhrer et al., 2008b). Hence, 2 doses of caramiphen were used to test whether single infusion of 20 μg/μl into the perirhinal cortex may cause anticonvulsant efficacy and whether twin infusions of 10 μg/μl may produce similar effect.

The purpose of the present study was to examine whether drugs invading a larger area of the perirhinal cortex through 2 cannulas in contrast to 1 cannula may result in more powerful anticonvulsant effects against soman intoxication. The drugs used for this comparison were the ionotropic antagonists procyclidine and caramiphen and the metabotropic glutamate modulators DCG-IV (mGlu2/3 receptor agonist) and MPEP (mGlu5 receptor antagonist). Prevention of seizures/convulsions or increased latency to onset of seizure activity was used as measures of anticonvulsant efficacy. The perirhinal cortex is known to kindle faster than any other brain structure and is highly influential in the development and maintenance of temporal lobe seizure activity (Kelly and McIntyre, 1996; McIntyre and Kelly, 2000). In order to determine whether the presence of 2 versus 1 cannula in the perirhinal cortex might influence the basal intrinsic neuronal activity perirhinal EEG recordings were performed in 2 additional groups of rats.

2. Materials and methods

2.1. Animals

A total of 86 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 carried out according to EC Directive 86/609/EEC for animal experiments and approved by the National Animal Research Authority. Twelve groups of rats ($N = 6–7$) received bilateral single or double microinfusions of drugs or vehicle into the perirhinal cortex. Two groups of rats ($N = 4$) received electrodes in 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 cm × 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 stereotaxically (flat skull) with guide cannulas aimed at perirhinal cortex in both hemispheres. The guide cannula (25 gauge) was 0.5 mm in diameter and cut to a length of 11 mm. For rats provided with electrodes through implanted cannulas, the guide cannula (23 gauge) was 0.8 mm in diameter to allow penetration of the electrodes (cf., later). 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 2 steel screws. The point of insertion was for the anterior position 3.5 mm behind 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. The point of insertion for the posterior position was 5.5 mm behind bregma and 6.5 mm lateral to the midline. The cannula was lowered in an angle of 14° (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 implanted with electrodes in the perirhinal cortex were anesthetized as described above, and they were provided with single or double cannulas in the sites described above. The electrodes were made from insulated silver thread of 0.3 mm in diameter (Johnson Matthey Metals Ltd., USA) each soldered to a male golden pin component (220-PO2100 Bunker Ramo, Amphenol North America, USA). The electrodes were inserted into the cannulas localized in the anterior position with 1 mm of the end protruding the indwelling cannula. Only the tip of the electrode was bared of insulation. The golden pins were fitted into a plastic component fixed with a screw (ground) and dental cement. A female plug was used to connect the golden pins in the plastic component with the polygraph. The rats were allowed to recover 7 days before experimentation.

2.3. Histology

The perirhinal cortex was defined as areas 35 and 36 of Brodmann (Burwell, 2001). After decapitation, the brains were removed and stored in 10% formalin and dehydrated before being embedded in paraffin. Coronal sections were 5 μm thick and stained with hematoxylin and eosin.

2.4. Drug administration

Doses of drugs previously tested in microinfusion studies were applied. The doses used were: procyclidine hydrochloride 6 μg/μl, caramiphen edisylate 10 or 20 μg/μl, DCG-IV 1 μg/μl, and MPEP 0.1 μg/μl (Myhrer et al., 2010a,b). The drugs were dissolved in 0.9% saline, and they were purchased from Sigma–Aldrich. Saline (0.9%) was used as vehicle. All drugs were given in 1 μl over 1 min while the rats were gently held, and the cannula remained in position for an additional ½ min before retraction. Bilateral injections were carried out simultaneously. In the case of double infusions, they were carried out twice in rapid succession (the anterior first). Twenty min following microinfusions the rats received 1.3 × LD_{50} of soman subcutaneously that causes seizures in all rats, and the lethality is 100 percent (Sterri et al., 1980). The injection volume of 1 μl was used to ensure optimal anticonvulsant impact of the drugs. Infusion of 1 μl of 4% methylene blue in saline (0.9%) into the temporal or entorhinal cortices invades an area of about 1 mm^3 (Myhrer and Andersen, 2001) as an indication of spreading in brain tissue. The computed volume of the perirhinal cortex is about 14 mm^3 (Myhrer et al., 2010a).