NeuroToxicology 31 (2010) 147–153

Contents lists available at ScienceDirect

NeuroToxicology

Roles of perirhinal and posterior piriform cortices in control and generation of seizures: A microinfusion study in rats exposed to soman

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ARTICLE INFO

Article history:
Received 13 March 2009
Accepted 5 October 2009
Available online 13 October 2009

Keywords:
Perirhinal cortex
Posterior piriform cortex
Microinfusions
Drugs
Soman
Seizures
Anticonvulsant effects

ABSTRACT

Identification of critical receptors in seizure controlling brain regions may facilitate the development of more efficacious pharmacological therapies against nerve agent intoxication. In the present study, a number of drugs with anticonvulsant potency were microinfused into the perirhinal cortex (PRC) or posterior piriform cortex (PPC) in rats. The drugs used exert cholinergic antagonism (scopolamine), glutamatergic antagonism (ketamine, NBQX), both cholinergic and glutamatergic antagonism (procyclidine, caramiphen), or GABAergic agonism (muscimol). The results showed that in the PRC anticonvulsant efficacy against soman-induced seizures (subcutaneously administered) was achieved by procyclidine or NBQX, but not by ketamine, scopolamine, caramiphen, or muscimol (Experiment 1). Hence, both muscarinic and glutamatergic NMDA receptors had to be antagonized simultaneously or AMPA receptors alone, suggesting increased glutamatergic activation in the PRC before onset of seizures. In the PPC, anticonvulsant effects were assured by scopolamine or muscimol, but not by procyclidine, caramiphen, NBQX, or ketamine (Experiment 2). Thus, muscarinic and $GABA_A$ receptors appear to be the critical ones in the PPC. Microinfusion of soman into the PRC or PPC resulted in sustained seizure activity in the majority of the rats of both infusion categories. The rhinal structures encompassed in this study apparently have critical functions as both control and trigger sites for nerve agent-evoked seizures.

1. Introduction

The organophosphorus nerve agent soman is a highly potent inhibitor of the enzyme acetylcholinesterase. Such inhibition results in a rapid accumulation of acetylcholine and over-stimulation of muscarinic and nicotinic receptors. The toxic signs include hyper-salivation, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 2001). The progression of events after nerve agent poisoning triggering seizures/convulsions can be divided into three phases. An early phase lasting from the time of exposure to about 5 min after seizure onset is dominated by excessive cholinergic activity followed by a transitional phase of cholinergic and glutamatergic hyperactivity and finally a predominantly glutamatergic phase after about 40 min (McDonough and Shih, 1997).

Medical management of nerve agent poisoning is based on pretreatment with a carbamate cholinesterase inhibitor (pyridostigmine) to shield a fraction of the cholinesterase from irreversible inhibition by the nerve agent. Treatment after exposure to nerve agent is based on cholinergic antagonist (atropine sulfate) along with an oxime (obidoxime, 2-PAM, HI-6) to reactivate any unaged inhibited enzyme (Aas, 2003). Such treatment regimen can enhance the survival rate significantly, but it does not effectively reduce nerve agent-induced seizure activity resulting in brain injury. For this reason, efforts in search for effective countermeasures have aimed at drugs exerting cholinergic and glutamatergic antagonism along with GABAergic agonism (McDonough and Shih, 1997). However, determination of critical receptor subtypes would provide clues for the designing of more specific anticonvulsive therapeutic strategies as it has been made in epilepsy research.

Seizure activity does not spread randomly throughout the brain. The electrical discharges are induced and propagated by specific anatomical routes (Gale, 1988; Löscher and Ebert, 1996). In epilepsy models, epileptiform activity is usually recruited in structures localized in the ventrolateral forebrain of rats (McIntyre, 2006). If not terminated, such partial epileptiform activity propagates to the motor cortex by way of the perirhinal cortex and clonic convulsions are seen (McIntyre, 2006). If the activity is sufficiently intense, electrical discharges spread further to the brainstem by way of the basal ganglia, and generalized seizures accompanied by tonic-clonic convulsions occur (Browning and Nelson, 1986). The tonic extension of hind limbs likely reflects the highest level of seizure activity involving both the forebrain and brainstem (Swinyard, 1973).

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0161-813X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2009.10.002

In experimental epilepsy, seizure controlling sites 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 function as a critical link in the propagation of epileptiform activity in limbic structures evoked from microinfusion of bicuculline into the area tempestas (Halonen et al., 1994). Results from a later study suggest that seizure control in these areas is dependent on both NMDA and AMPA antagonisms in the perirhinal cortex, whereas only AMPA antagonism is effective in the piriform cortex (Tortorella et al., 1997). In a recent lesion study, we have shown that aspiration ablation of the perirhinal cortex (PRC) or posterior piriform cortex (PPC) produces anticonvulsant efficacy against soman-induced convulsions, whereas damage to the hippocampal region, entorhinal cortex, or amygdala does not (Myhrer et al., 2008a).

The purpose of the present study was to examine whether pharmacological agents microinfused into the PRC (Experiment 1) or PPC (Experiment 2) may assure anticonvulsant impact against soman-generated seizures in order to determine critical receptor types. The drugs used exert cholinergic antagonism (scopolamine), glutamatergic antagonism (ketamine, NBQX), both cholinergic and glutamatergic antagonism (procyclidine, caramiphen), or GABAergic agonism (muscimol). The drugs were infused into the target areas 20 min before soman was administered systemically. Increased latency to onset of seizures/convulsions was used as measure of anticonvulsant effects. Because seizure controlling regions also can act as trigger sites for seizures (Löscher and Ebert, 1996), the potency of soman was tested by making microinfusion of this nerve agent into the PRC or PPC.

2. Materials and methods

2.1. Animals

Male Wistar rats from a commercial supplier (Møllegaard 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, 7 groups of rats ($N = 7–8$) received bilateral microinfusions (anticonvulsant drugs or vehicle) into PRC. In 4 additional animals, soman was bilaterally infused into PRC. In Experiment 2, 7 groups ($N = 7–8$) received microinfusions (anticonvulsants or vehicle) into PPC. In 4 additional rats, soman was infused bilaterally into PPC. 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 intraperitoneally 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 target areas 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. In Experiment 1, the point of insertion for the PRC was 4.5 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 coordinates for insertion in the PPC (Experiment 2) was 4 mm behind bregma, 6 mm lateral to the midline, and the cannula was lowered in an angle of 5° (end pointing laterally) 8 mm from the top of the skull. The same coordinates were used for control infusion of soman into the somatosensory cortex by lowering the cannula 3 mm only. 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 PRC (Fig. 1B) was defined as areas 35 and 36 of Brodmann (Burwell, 2001). The rostrocaudal extent of this area is about 6 mm in the rat. The PPC (Fig. 1A) was defined as the piriform cortex from

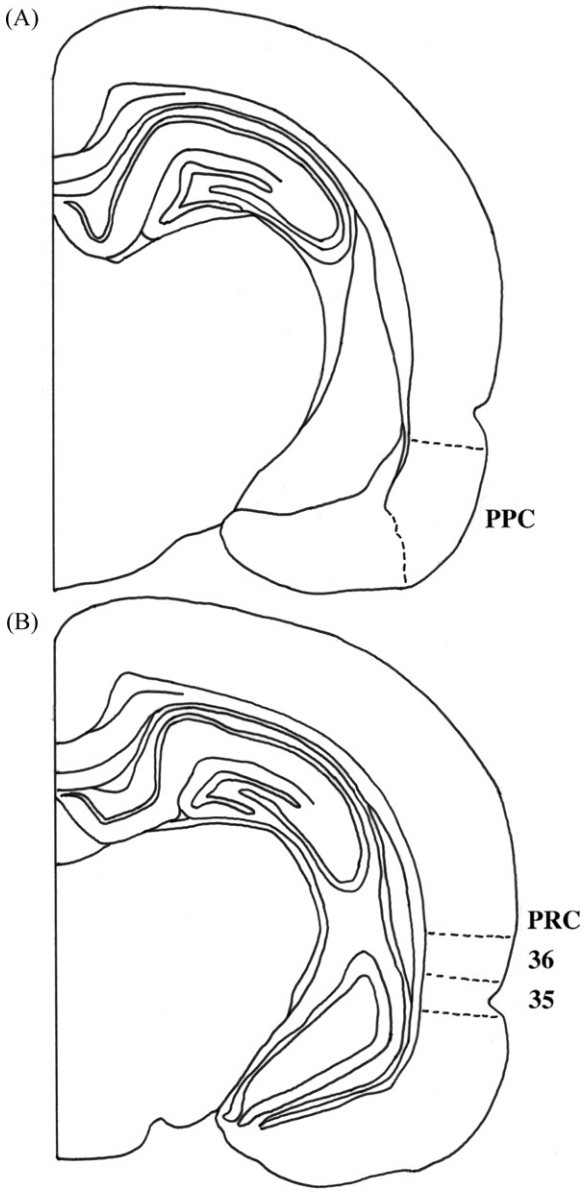

Fig. 1. Reconstruction of coronal sections showing posterior piriform cortex (PPC) (A) and perirhinal cortex (PRC) with Brodmann's areas 35 and 36 (B). Sections are adapted from the atlas of Paxinos and Watson (2005).