available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Anticonvulsant impact of lesions in the ventrolateral forebrain of rats challenged with soman

Trond Myhrer, Siri Enger, Pål Aas*

Norwegian Defence Research Establishment, Protection Division, P. O. Box 25, NO-2027 Kjeller, Norway

ARTICLE INFO

Article history:
Accepted 6 June 2008
Available online 16 June 2008

Keywords:
Soman
Convulsion
Lesion
Anticonvulsant effect

ABSTRACT

Mapping of trigger sites and/or propagation pathways for soman-induced seizures may provide clues for the designing of anticonvulsant drugs. In the present study, anticonvulsant efficacy against soman intoxication ($1.3 \times LD_{50}$) was examined in rats with either lesion of the perirhinal cortex, posterior piriform cortex, entorhinal cortex, hippocampal region, or amygdala. The results showed that prevention of convulsions or increased latency to onset of convulsions was ensured in rats with perirhinal or piriform cortical lesions, whereas anticonvulsant effects were not achieved in rats with damage to the entorhinal cortex, hippocampal region, or amygdala. The results from the present study suggest that critical structures for induction of seizures after soman exposure are located in the ventrolateral aspect of the forebrain. This suggestion is in compliance with convulsant reactions to microinfusions of soman or VX into ventrolateral brain structures and increased neuronal activity in corresponding structures revealed by c-*fos* staining in response to soman. Furthermore, results from studies of kindling, lesions, and microinfusion of chemoconvulsants in experimental epilepsy also imply that the perirhinal and piriform cortices are critically involved in seizure control.

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-secretion, respiratory distress, tremor, seizures/convulsions, coma, and death (Taylor, 2001). The elevated cholinergic activity in the brain likely induces the initial phase of seizures (Lallement et al., 1992; McDonough and Shih, 1997), and sustained seizures are probably associated with increased glutamatergic activity leading to excitotoxic lesions predominantly in the piriform cortex, entorhinal cortex, amygdala, and hippocampus (Carpentier et al., 1991; McDonough et al., 1995; McDonough and Shih, 1997).

Immediate medical treatment of individuals exposed to nerve agent is important to avoid death or brain damage among survivors. Medical strategies have focused on pre-treatment with carbamate cholinesterase inhibitors, such as pyridostigmine, to shield a fraction of the acetylcholinesterase from irreversible inhibition by the nerve agent. Subsequent therapeutic treatment with an anticholinergic drug like atropine sulfate has been used along with an oxime to reactivate any unaged inhibited enzyme. Even if such treatment regimen can increase the survival rate significantly, it does not effectively diminish nerve agent-induced seizure activity leading to neuropathology. Therefore, efforts in search for countermeasures have aimed at drugs assuring cholinergic and glutamatergic antagonism along with GABAergic agonism (McDonough and Shih, 1997). This search, however, does not seem to be

* *Corresponding author*. Fax: +47 63 80 75 09.
E-mail address: trond.myhrer@ffi.no (T. Myhrer).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.06.014

based on a rational screening foundation, because any drug with the above properties appears relevant for testing. In order to shorten the list of candidate drugs, identification of critical structures involved in seizure control should be made as well as specification of receptor types in the same structures.

Electrical discharges are induced and propagated by specific anatomical routes (Gale, 1988; Löscher and Ebert, 1996). Within this network, 2 regions have been identified in experimental epilepsy as exerting seizure controlling gating function. These sites are the substantia nigra (SN) and area tempestas (AT) (Gale, 1988). AT that is located in the anterior piriform cortex, has been defined morphologically and termed the pre-endopiriform nucleus (Ekstrand et al., 2001). In the case of intoxication by nerve agents, the induction phase of seizures is related to cholinergic systems. It has been suggested that muscarinic receptors in the piriform cortex may play a pivotal role in eliciting seizures in response to soman (Zimmer et al., 1998). However, cholinoceptive cells in the septal area, hippocampal region, and entorhinal cortex have also been suggested to play a key role in the soman-induced seizures (Denoyer et al., 1992, Lallement et al., 1992).

Potential trigger sites for seizures and/or propagation may be mapped by selective removal of brain areas. If selective lesion of seizure controlling regions assures anticonvulsant effects against soman intoxication, the nature of the damaged areas may provide clues for designing more efficacious anticonvulsant drugs. In a recent study from our laboratory, we showed that bilateral aspiration lesion of AT prevented convulsions or increased latency to onset of convulsions, whereas no anticonvulsant effect was seen from SN lesions. Furthermore, lesion of the medial septum produced marked anticonvulsant efficacy, whereas lesion of the nucleus basalis magnocellularis or nucleus accumbens did not (Myhrer et al., 2007). To make the picture more complete, other relevant structures with potential proconvulsant properties in response to soman should be examined. For this reason, aspiration lesions were made in the perirhinal cortex, posterior piriform cortex, entorhinal cortex, hippocampal region (hippocampus proper, fascia dentata, subiculum), and amygdala. The anticonvulsive measure used was prevention of convulsions or prolonged latency to onset of convulsions after exposure to soman.

2. Results

2.1. Behavior

Rats with lesions showed normal behavior and had only a transient aphagia/adipsia. Onset of convulsions was never observed later than 18 min after soman exposure. Hence, the criterion for complete anticonvulsant effect was set to 19 min. Increased latency to seizure onset was seen among rats with perirhinal or piriform cortical lesions (Table 1). ANOVA revealed a reliable overall effect (H (6)=23.53, P=0.0003). The perirhinal group displayed anticonvulsant effects relative to the control group ($P<0.01$) and the groups with entorhinal or amygdaloid lesions ($P<0.05$). Within the perirhinal group, 3 rats did not convulse. The piriform group also had longer latency to onset of convulsions than the control group ($P<0.05$). Among rats with perirhinal or piriform cortical lesions, about half of the convulsing animals did not display full tonic movement with hind legs splayed out. ANOVA revealed no significant differences in body weight among the groups on the day of exposure to soman ($P>0.05$) (Table 1).

Table 1 – Anticonvulsant effects of bilateral aspiration lesions in rats intoxicated by soman (1.3 × LD_{50}) and change of preoperative body weight

Group	n	Latency (min) to convulsion/ nonconvulsion		Body weight (g) change postoperative day 9
		Median	Range	Mean (S.E.M.)
Control	8	6.3	1.2–9.0	+4.5±2.9
Perirhinal	8	15.2**	12.0–19.0	+6.3±3.6
Piriform	7	14.1*	9.0–18.0	+1.1±2.8
Entorhinal	8	6.1	3.5–13.3	+1.0±2.1
Hippocampal	7	7.4	4.3–10.2	+9.3±6.7
Amygdaloid	8	6.0	3.2–17.0	+8.1±1.8

Significantly different from control group *$P<0.05$, **$P<0.01$.

2.2. Histology

All lesions within each group appeared rather consistent. In the perirhinal group, the lesions generally comprised the cortical area aimed at (Fig. 1). The mean percentage of perirhinal damage was 74 (range 65–90). In 2 rats, the lesion site was 1 mm dorsal to the target area. In some animals, the temporal or insular cortex was slightly affected. The posterior piriform cortex was defined to make up the region from the level of the septal area to the border of the lateral entorhinal cortex. Within the posterior piriform group, the mean percentage of damage was 75 (range 65–95) (Fig. 2). The anterior portion of the lateral entorhinal cortex was slightly damaged bilaterally in most rats. None of the piriform lesions affected AT. In the entorhinal group, the mean percentage of damage was 74 (range 60–88) (Fig. 3). In 1 rat, the ventral tip of the hippocampal region was destroyed unilaterally. In the hippocampal group, the lesions comprised the dorsal 2/3 of the total extent. Within this area, the percentage damage was 75 (range 60–88) (Fig. 4). The dorsal portion of the thalamus was slightly damaged unilaterally in 3 rats. In the amygdaloid group, the mean percentage damage was 82 (range 50–100) (Fig. 5). The ablated area consisted of the basolateral and lateral nuclei. The basomedial and ventral basolateral nuclei were not affected.

Three rats in the perirhinal group did not convulse. In these rats, no neuropathology was detected in sections stained with Fluoro-Jade. Piriform cortex, hippocampal CA1, and amygdala appeared normal 48 h after the soman challenge (data not shown).

3. Discussion

The results from the present study showed that lesion of the perirhinal or posterior piriform cortices caused anticonvulsant efficacy against soman-induced convulsions, whereas damage to the entorhinal cortex, hippocampal region, or amygdala did