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Neuropharmacological specificity of brain structures involved in soman-induced seizures☆

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ABSTRACT

Pharmacological control of seizure activity following nerve agent exposure is critical in reducing neuropathology and improving survival in casualties. Three classes of drugs, anticholinergics, benzodiazepines and excitatory amino acid (EAA) antagonists, have been shown to be effective at moderating nerve agent-induced seizures. However, little is known about which brain structures are involved in producing the anticonvulsant response. This study evaluated drugs from each class, injected directly into one of three specific brain structures, the perirhinal cortex, the entorhinal cortex, or the mediodorsal thalamus, for their ability to modulate seizures induced by the nerve agent soman. The drugs evaluated were the anticholinergic scopolamine, the benzodiazepine midazolam, and the EAA antagonist MK-801. For each drug treatment in each brain area, anticonvulsant ED_{50} values were calculated using an up-down dosing procedure over successive animals. There was no statistical difference in the anticonvulsant ED_{50} values for scopolamine and MK-801 in the perirhinal and entorhinal cortices. MK-801 pretreatment in the mediodorsal thalamus had a significantly lower anticonvulsant ED_{50} value than any other treatment/injection site combination. Midazolam required significantly higher doses than scopolamine and MK-801 in the perirhinal and entorhinal cortices to produce an anticonvulsant response and was ineffective in the mediodorsal thalamus. These findings support the contention that specific neuroanatomical pathways are activated during nerve agent-induced seizures and that the discrete brain structures involved have unique pharmacological thresholds for producing an anticonvulsant response. This study is also the first to show the involvement of the mediodorsal thalamus in the control of nerve agent-induced seizures.

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1. Introduction

Seizures are one of a myriad of toxic signs that can occur following exposure to organophosphorus nerve agents such as sarin, soman and VX. A result of excessive cholinergic neurotransmission within the central nervous system (CNS), seizures not only complicate the treatment of nerve agent poisoning, but can also lead to extensive brain damage if uncontrolled. Neuropathology has been shown to develop after as little as 20 min of nerve agent-induced seizure activity with the extent and severity of damage increasing rapidly as time progresses (Lallement et al., 1994; McDonough et al., 1995). Pharmacological control of nerve agent-induced seizures is critical to survival following exposure and has been strongly associated with protection against the brain damage caused by these agents (Shih et al., 2003). An understanding of the brain structures and mechanisms that initiate, propagate, and modulate these seizures would, therefore, be beneficial in identifying targets for therapeutic intervention to increase survival and reduce brain damage.

The perirhinal cortex, entorhinal cortex, and mediodorsal thalamus are three brain structures that have been consistently associated with seizure activity in epilepsy. The perirhinal cortex (PRC) is the fastest structure in the brain to produce convulsive seizures from electric kindling (McIntyre et al., 1993) and it has been suggested to serve as a relay station for the generalization of seizure activity from limbic structures to the sensorimotor cortex (Kelly and McIntyre, 1996; Fukumoto et al., 2002). Lesions of this structure have also been shown to have an anticonvulsant effect against soman-induced seizures (Myhrer et al., 2008). The entorhinal cortex (EC) has also been implicated as a structure

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involved early in the initiation or propagation of nerve agent-induced seizures and shows intense labeling for the proto-oncogene c-Fos, a marker of activated neuronal cell bodies, following soman exposure (Denoyer et al., 1992; Zimmer et al., 1997). The mediodorsal thalamus (MDT) is a structure that may not only propagate and synchronize seizure activity, but also be capable of initiating seizures (Bertram et al., 1998, 2008). Additionally, this structure exhibits significant damage following prolonged seizure activity (Bertram and Scott, 2000; Bertram et al., 2001).

In a recent study, we began to map the anticonvulsant response of several brain structures to drugs from three classes: scopolamine, an anticholinergic, midazolam, a benzodiazepine, and MK-801, an EAA antagonist (Skovira et al., 2010). The anticonvulsant effect of these drugs against nerve agent-induced seizures is thought to occur primarily through their action on the M_1 muscarinic receptor, $GABA_A$ receptor, and NMDA receptor, respectively (McDonough and Shih, 1997). Although each drug was capable of eliciting an anticonvulsant response, the effectiveness of a given drug was differentially dependent upon the brain structure into which it was injected. The present study was designed to continue to map the anticonvulsant response of brain structures known to be involved in seizure activity, PRC, EC, and MDT, to treatments acting on different receptor systems against soman-induced seizures.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Charles Rivers Labs., Kingston, NY), weighing 250–300 g before surgery, were used for this experiment. Animals were individually housed in an environmentally controlled room (temperature 21 ± 2 °C, 12 h light–dark cycle) with food and water ad libitum except during experimentation.

2.2. Materials

Atropine methylnitrate (AMN), scopolamine hydrobromide, midazolam hydrochloride, and MK-801 hydrogen maleate were purchased from Sigma–Aldrich (St. Louis, MO). HI-6 (Lot#BN44621) was obtained from Walter Reed Army Institute of Research (Silver Spring, MD). Buprenorphine HCl (Buprenex Injectable, 0.3 mg/ml) was purchased from Reckitt Benckiser Pharmaceuticals Inc. (Richmond, VA). Isoflurane (Attane®) was purchased from Minrad Inc., Bethlehem, PA. Soman (pinacolyl methylphosphonofluoridate) was obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). Soman was diluted in ice-cold saline to a concentration of 360 μg/ml. AMN, HI-6, scopolamine, midazolam, and MK-801 were prepared in 0.9% normal saline. Soman, AMN, and HI-6 were administered subcutaneously (SC), intramuscularly (IM), and intraperitoneally (IP), respectively, with a 0.5 ml/kg injection volume. Scopolamine, MK-801, and midazolam were delivered via microinjection.

2.3. Surgery

Rats were anesthetized with isoflurane (3% induction, 1.5–2% maintenance; with oxygen) and placed in a stereotaxic frame. To record brain electroencephalographic (EEG) activity two stainless steel cortical screw electrodes were placed equidistant between bregma and lambda 2–3 mm from the midline in each hemisphere and a third was placed over the cerebellum. For drug administration two 22 gauge guide cannula (Plastics One, Inc., Roanoke, VA) were implanted bilaterally toward a designated area of the brain (PRC, EC, or MDT).

Targeting of brain structures was done using the atlas of Paxinos and Watson (2005). The following coordinates were used relative to bregma: PRC–A-P −3.5, L ±6.2, V −7.3; EC–A-P −6.0, L +6.9, V −8.0; and MDT – (15° off vertical) A-P −3.25, L ±2.5, V −6.13. Guide cannula were lowered 1 mm dorsal to the targeted brain site. To prevent plugging of the guide cannula dummy stylets of matching length were inserted. Cortical screw electrodes were covered with dental acrylic cement to electrically isolate the electrodes from the overlying tissue and to mechanically fix the electrodes, plug, and cannula as a unit. Buprenorphine HCl (0.05 mg/kg) was administered SC upon recovery from anesthesia. Animals were given 7 days rest before experimentation.

2.4. Experimental procedure

2.4.1. EEG recording

To monitor EEG, animals were connected to recording leads and placed in individual 25 cm × 25 cm × 45 cm plastic recording cages. EEG recording was done using CDE Model 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK). EEG activity was monitored for 30 min to establish baseline brain activity prior to any treatments. After baseline recording, animals were pretreated with a test drug through bilateral microinjection into the guide cannulae. In order to inject the pretreatment test drugs, animals were removed from the cage and EEG monitoring, and restrained gently in a wrapped towel.

2.4.2. Microinjections

The dummy stylets were removed, and 28 gauge injection cannula were inserted into the bilateral guide cannula. The injection cannula projected 1 mm beyond the tip of the guide cannula. Treatment was delivered manually at a rate of 0.2 μl/min for 5 min (total injection volume was 1 μl/cannula) using DMP electronic micrometers (World Precision Instruments Inc., Sarasota, FL). Injection cannula remained in place 1 min after completion of the injections to allow for drug diffusion. The dummy stylets were then replaced, and the animal was pretreated with the oxime HI-6 (125 mg/kg, IP) to antagonize the peripheral effects of the nerve agent. Animals were then returned to a recording cage for continued EEG monitoring.

Dosing followed the up-down procedure of Dixon and Massey (1983). Using this procedure, animals were given a starting pretreatment dose of a drug, and if that dose prevented or significantly increased the latency to onset of seizures, the next animal received a lower dose; if the initial dose did not prevent or significantly increase the latency to onset of seizures, the next animal received a higher dose. Dosing proceeded using this rule until five reversals occurred (i.e., seizure to no seizure, no seizure to seizure). Pretreatment doses ranged between 0.15–81.54 μg/μl per cannula with 1.45 μg/μl as the starting dose for scopolamine and MK-801 and 14.50 μg/μl for midazolam treatment based on our previous data (Skovira et al., 2010). A 0.25 $\log_{10}$ interval was used between successive doses.

2.4.3. Nerve agent exposure

Thirty minutes following drug pretreatment, animals were injected with the nerve agent soman (180 μg/kg, SC). The peripheral muscarinic receptor antagonist AMN (2.0 mg/kg, IM) was given 1 min after soman. Animals were monitored for seizure activity for at least 4 h following exposure and again at 24 h. Seizure onset was operationally defined as the appearance of ≥10 s of rhythmic high amplitude spikes or sharp wave activity in the EEG tracing. For each animal, treatment was categorized as successful (prevention or significant increase in the latency to