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Prevention of organophosphate-induced chronic epilepsy by early benzodiazepine treatment

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ABSTRACT

Poisoning with organophosphates (OPs) may induce status epilepticus (SE), leading to severe brain damage. Our objectives were to investigate whether OP-induced SE leads to the emergence of spontaneous recurrent seizures (SRSs), the hallmark of chronic epilepsy, and if so, to assess the efficacy of benzodiazepine therapy following SE onset in preventing the epileptogenesis. We also explored early changes in hippocampal pyramidal cells excitability in this model. Adult rats were poisoned with the paraoxon (450 μg/kg) and immediately treated with atropine (3 mg/kg) and obidoxime (20 mg/kg) to reduce acute mortality due to peripheral acetylcholinesterase inhibition. Electrical brain activity was assessed for two weeks during weeks 4–6 after poisoning using telemetric electrocorticographic intracranial recordings. All OP-poisoned animals developed SE, which could be suppressed by midazolam. Most (88%) rats which were not treated with midazolam developed SRSs, indicating that they have become chronically epileptic. Application of midazolam 1 min following SE onset had a significant antiepileptogenic effect (only 11% of the rats became epileptic; $p = 0.001$ compared to non-midazolam-treated rats). Applying midazolam 30 min after SE onset did not significantly prevent chronic epilepsy. The electrophysiological properties of CA1 pyramidal cells, assessed electrophysiologically in hippocampal slices, were not altered by OP-induced SE. Thus we show for the first time that a single episode of OP-induced SE in rats leads to the acquisition of chronic epilepsy, and that this epileptogenic outcome can be largely prevented by immediate, but not delayed, administration of midazolam. Extrapolating these results to humans would suggest that midazolam should be provided together with atropine and an oxime in the immediate pharmacological treatment of OP poisoning.

1. Introduction

Organophosphates are toxic compounds commonly used as pesticides in agriculture, but can also be used in chemical warfare. In some parts of the developing world, poisoning by OPs causes more deaths than infectious diseases (Eddleston et al., 2002). The warfare related OPs, such as sarine, soman and VX, also known as nerve agents, are extremely toxic and are considered to be among the deadliest agents. The main mechanism of action of OPs is irreversible inhibition of the ACh degrading enzyme,

Abbreviations: OP, organophosphate; SE, status epilepticus; SRS, spontaneous relapsing seizures; ACh, acetylcholine; BZD, benzodiazepines; ECoG, electrocorticographic.

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acetylcholinesterase, leading to ACh accumulation in muscarinic and nicotinic cholinergic synapses in both the peripheral and central nervous system. Clinically, respiratory failure is the main cause of death in severe OP poisoning and is attributed to a combination of bronchoconstriction, respiratory muscle paralysis and damage to the medullary respiratory centers (Eddleston et al., 2006).

Central nervous system effects of OPs include nonspecific symptoms, such as irritability, restlessness, disorientation and confusion, which can evolve into generalized seizures and SE (Marrs et al., 2007). If the poisoned animal is rescued by relieving peripheral symptoms with atropine and an oxime, SE may continue for several hours causing severe brain damage (Gilat et al., 2005; White et al., 2012). The level of neuronal damage was found to be in correlation with duration and intensity of the SE (McDonough and Shih, 1997). Thus, it is assumed that most of the neuronal damage can be avoided if seizures are controlled promptly. BZDs, potent $GABA_A$ receptor enhancers, are highly effective in arresting OP-induced SE when administered early after SE initiation (McDonough and Shih, 1997). However, delayed BZD treatment only temporarily impedes SE and only partially prevents brain damage (de Araujo et al., 2012; Gilat et al., 2005).

There are several animal models for studying the consequences of SE. In rodents, SE is commonly induced by chemoconvulsants, e.g. kainic acid and pilocarpine, or by electrical stimulation of the amygdala or hippocampus (Rubio et al., 2010). SE per se can cause a significant excitotoxicity associated with neuronal cell death, regardless of the initial insult. This neuropathology is due to the excessive release of excitatory amino acids from neurons and astrocytes leading to the prolonged depolarization of neurons, increased intracellular calcium and activation of a cascade of metabolic changes that cause neuronal cell death (Holmes, 2002). A high proportion of animals that survive SE develop spontaneous recurrent seizures (SRSs), i.e. chronic epilepsy after a latent period of days to weeks, i.e. a process referred to as epileptogenesis. Previous studies have demonstrated that intrinsic changes in firing characteristics of CA1 hippocampal neurons, in conjunction with changes in network synaptic function, might contribute to the development of chronic epilepsy following pilocarpine-induced SE (Su et al., 2002).

Chronic epilepsy induced by acute OP poisoning has not been fully studied nor characterized. Recently, de Araujo et al. (de Araujo et al., 2010) demonstrated that rats poisoned with the OP nerve agent soman, who had experienced SE, showed electrographic SRSs 15 days after poisoning. Here we used paraoxon, a commonly used agricultural OP, to investigate whether paraoxon-induced SE also leads to long-term SRSs and to chronic epilepsy, and if so, to characterize these SRSs and assess whether post-poisoning BZD treatment has an effect on their development. We also attempted to compare this model with the widely used pilocarpine model of chronic epilepsy (Rubio et al., 2010) with respect to changes in hippocampal pyramidal cells excitability that may contribute to the emergence of SRSs.

2. Materials and methods

2.1. Study design and animals

The study had two separate parts; (1) prolonged ECoG recordings in awake rats several weeks after poisoning, and (2) intracellular electrophysiological recordings and analysis of intrinsic properties of CA1 pyramidal neurons in control versus OP-poisoned rats. All experiments were performed according to the institute's guidelines for animal care and use. Adult Sprague-Dawley rats (300 ± 20 g) were randomly divided according to treatment into four groups (Table 1). (1) Non-poisoned rats, treated solely with antidotes (ATOX: atropine 3 mg/kg, i.m. and obidoxime 20 mg/kg, i.m.), served as controls; (2) paraoxon (450 μg/kg; ~1.4 LD_{50} i.m.) poisoned rats treated with ATOX 1 min later; (3) paraoxon (450 μg/kg), ATOX (1 min) and midazolam (MID, 1 mg/kg, i.m.) 1 min after the onset of convulsions; and (4) paraoxon (450 μg/kg), ATOX (1 min) and midazolam (MID, 1 mg/kg, i.m.) 30 min after the onset of convulsions. ATOX treatment was given to all poisoned rats in order to reduce acute mortality due to peripheral acetylcholine-esterase inhibition.

Table 1
Study groups in the two stages of the study.

Group	Treatment	Intracellular recordings		In vivo (ECoG) recording
		Number of animals	(Total number of neurons)	Number of animals
1	ATOX	4	(18)	7
2	PXN + ATOX	7	(29)	8
3	PXN + ATOX + MID 1′[a]	8	(30)	9
4	PXN + ATOX + MID 30′[a]	9	(33)	8

ATOX – atropine + obidoxime, PXN – paraoxon, MID – midazolam, ECoG – electrocorticography.
[a] Time in minutes post seizure onset until MID was injected.

2.2. Clinical evaluation

Animals were observed continuously for at least an hour after intoxication and scored for motor manifestations of seizure activity on 10, 20 and 60 min. Scores were assigned as follows: (0) no convulsions, (1) chewing and facial clonus, (2) tremor and focal convulsions, and (3) tonic-clonic generalized convulsions. Midazolam was injected in experimental groups 3 and 4, 1 and 30 min after appearance of chewing and facial clonus (score 1 according to our scale), respectively.

2.3. In vivo recordings and analysis

ECoG was recorded 4–6 weeks after poisoning using established methods (Bastlund et al., 2004; Levi et al., 2012; Timofeeva and Gordon, 2001). In short, 21 days following drug treatment, rats were deeply anesthetized with ketamine (75 mg/kg, IP) and xylazine (5 mg/kg, IP) and placed into a stereotactic frame. The skin was disinfected and a sagittal incision was made. Chronically implanted electrodes were placed in the epidural space; 3 mm caudal and ± 2.5 mm lateral to bregma. Following a recovery period of 7 days, electrical activity was acquired (1 KHz) for 2 weeks using a telemetric ECoG system (CTA-F40 transmitter and RPC-1 receiver, Data Science International, United States). Recordings were analyzed off-line using a custom seizure detection algorithm developed in house. Briefly, ECoG signals were band-pass filtered (2–100 Hz), and five features were extracted from 2 s long epochs (with 1 s overlap), representing signal properties both in the time and frequency domains. The features were then fed to an artificial neural network (ANN)-based classifier, pre-trained to distinguish between epochs corresponding to seizure and non-seizure activity. We defined "seizure like events" (SLEs) as a minimum of 6 consecutive epochs with 'positive' ANN detections. An animal was classified as "epileptic" when the algorithm detected at least 2 unprovoked SLEs over 1 h apart. Algorithm performance has been previously validated in 3 different models of epilepsy (namely, genetic (Ketzef et al., 2011), SE-induced (Becker et al., 2008) and albumin-induced epilepsy (Weissberg et al., 2011)), revealing overall sensitivity and positive predictive value over 98% (in a total of >2800 h of ECoG, $n = 15$).

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