Behavioural Brain Research 218 (2011) 152–157

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Substantia nigra pars reticulata is crucially involved in barbiturate and ethanol withdrawal in mice

Gang Chen[a,b], Laura B. Kozell[a,b,c], Kari J. Buck[a,b,*]

[a] Veterans Affairs Medical Center, Portland, OR 97239-3098, United States
[b] Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239-3098, United States
[c] Department of Psychiatry, Oregon Health & Science University, Portland, OR 97239-3098, United States

ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form 15 October 2010
Accepted 19 October 2010

Keywords:
Seizure
Sedative-hypnotic
Lesion
Ibotenic acid
Convulsion

ABSTRACT

Sedative-hypnotic CNS depressant drugs are widely prescribed to treat a variety of disorders, and are abused for their sedative and euphoric effects. Physiological dependence and associated withdrawal episodes are thought to constitute a motivational force that sustains their use/abuse and may contribute to relapse in dependent individuals. Although no animal model duplicates depressant dependence, models for specific factors, like withdrawal, are useful for identifying potential neural determinants of liability in humans. Recent analyses implicate the caudolateral substantia nigra pars reticulata (clSNr) in withdrawal following acute and repeated ethanol exposures in mice, but did not assess its impact on withdrawal from other sedative-hypnotics or whether intrinsic neurons or fibers of passage are involved. Here, we demonstrate that bilateral chemical (ibotenic acid) lesions of the clSNr attenuate barbiturate (pentobarbital) and ethanol withdrawal. Chemical lesions did not affect convulsions in response to pentylenetetrazole, which blocks $GABA_A$ receptor-mediated transmission. Our results demonstrate that the clSNr nucleus itself rather than fibers of passage is crucial to its effects on barbiturate and ethanol withdrawal. These findings support suggest that clSNr could be one of the shared neural substrates mediating withdrawal from sedative-hypnotic drugs.

Published by Elsevier B.V.

1. Introduction

Sedative-hypnotic (SH) drugs are commonly prescribed to treat insomnia, anxiety, and seizure disorders and are widely abused for their sedative and euphoric actions. In fact, abuse of prescription SH medications (including benzodiazepines and barbiturates) and other SH agents (including ethanol) is among the top five health problems in the U.S. [1]. SH physiological dependence and associated withdrawal episodes are thought to constitute a powerful motivational force that perpetuates SH use and abuse [2]. Unfortunately, current understanding of the neural substrates that contribute to SH withdrawal is limited, hindering treatment and resulting in a lack of alternatives for dependent individuals.

Although no animal model duplicates clinically defined SH dependence, models for specific factors, including the withdrawal syndrome, are useful for identifying potential genetic determinants of liability in humans. It is well-established that there is common genetic influence on withdrawal from a variety of SH drugs in mice [3–12]. While there are more common signs of SH withdrawal in humans, a genetic contribution to individual differences in withdrawal convulsions is apparent in humans and animal models [8,13,14]. In mice, withdrawal hyperexcitability following both acute and chronic exposure to short and long-acting SH drugs is frequently characterized using the handling-induced convulsion [4–6,12,15–18].

Previously, we identified a quantitative trait locus (QTL) on chromosome 4 with large effects on withdrawal from a barbiturate (pentobarbital [PB]) and alcohol (ethanol) in mice [10,19]. Using c-Fos as a high-resolution histological marker of neuronal stimulation [20,21], we found that animals congenic for the chromosome 4 QTLs for ethanol and PB withdrawal exhibit significantly less ethanol withdrawal-associated neuronal activation than background strain mice within the basal ganglia [22]. This was particularly evident in the substantia nigra pars reticulata (SNr). Bilateral electrolytic lesions of subregions of the SNr implicated the caudolateral SNr (clSNr) in withdrawal following acute and repeated ethanol exposure [22]. Based on our finding that the QTLs for ethanol and PB withdrawal are both located within the same small (<2 Mb) interval of chromosome 4, we tested the hypothesis that the clSNr may also influence PB withdrawal and be part of a shared neural substrate crucial for PB and ethanol withdrawal.

* Corresponding author at: VA Medical Center, Research Service (R&D40), 3710 SW US Veterans Hospital Road, Portland, OR 97239-3098, United States. Tel.: +1 530 220 8262x56659; fax: +1 503 220 3411.
E-mail address: buckk@ohsu.edu (K.J. Buck).

0166-4328/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbr.2010.10.025

Electrolytic lesions impact both intrinsic nuclei and fibers of passage [23], whereas ibotenic acid lesions produce little or no damage to fibers of passage at appropriate doses [24–29]. Here, by utilizing both approaches, we sought to dissociate the effects of intrinsic neurons from those of fibers of passage on PB and ethanol withdrawal convulsions. Additionally, to investigate whether clSNr lesions influence CNS excitability more generally, chemically lesioned mice were also tested for pentylenetetrazole-enhanced seizures. Our results indicate that the clSNr is intrinsically and crucially involved in both PB and ethanol withdrawal.

2. Materials and methods

2.1. Animals

DBA/2J inbred strain breeders were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were group-housed 2–4 per cage by strain and sex. Mouse chow (Purina #5001) and water were available *ad libitum*. Procedure and colony rooms were kept at 21 ± 1 °C and lights were on from 06:00 to 18:00. Behavioral testing was initiated between 07:00 and 09:00. All procedures were approved by the Oregon Health & Science University and VA Medical Center Care and Use Committees in accordance with USDA and USPHS guidelines. A total of 84 male DBA/2J mice were used for the lesion analyses, and were 50–70 days old at the time of surgery.

2.2. Electrolytic and ibotenic acid lesions

Mice were anesthetized by i.p. injection of anesthetic cocktail (10 mg xylazine, 100 mg ketamine, 2 mg acepromazine per kilogram body weight in 0.9% saline) and then placed in a stereotaxic instrument (Cartesian Research Inc., Sandy, Oregon). The skull surface was exposed and a burr hole was drilled. The coordinates for the clSNr were estimated based upon coordinates for the C57BL/6J strain [30] and determined empirically for DBA/2J strain mice [22], and were as follows: 3.0 mm caudal to bregma [anterior–posterior (AP) = −3.0], 1.55 mm lateral to midsagittal suture [medial–lateral (ML) = ± 1.55], and 4.5 mm deep from the skull surface [dorsal–ventral (DV) = −4.5]. For electrolytic lesions, an insulated 0.1 mm O.D. tungsten wire electrode with a conductive tip was lowered to the lesion site. Bilateral lesions were performed using a 0.4 mA current for 4 s. The procedure for sham-lesioned animals was identical except that no current was passed. For ibotenic acid lesions, a fine glass pipette (diameter = 40 μm) glued to a 1 μl Hamilton syringe was lowered to the lesion sites. Bilateral lesions were performed using 0.8 μg ibotenic acid per side (80 nl of 10 μg ibotenic acid/μl in 0.01 M phosphate buffered saline). This dose was chosen in order to lesion an area of the appropriate size without disruption of the fibers of passage. Electron microscopic analyses indicate that, at doses up to 7 μg ibotenic acid, the projecting nerve terminals as well as axons traversing the SNr remain intact [27,28]. Sham lesioned mice received bilateral injections of an equivalent volume of vehicle. An additional group of animals had no surgical procedure (unoperated control group).

2.3. PB and ethanol withdrawal

Withdrawal seizures are one of the primary characteristics of withdrawal from SH drugs including barbiturates and ethanol [31,32], and are a useful index of withdrawal because they are displayed in humans and in rodent models. In order to focus on CNS mechanisms of physiological dependence, withdrawal was assessed following an acute injection of PB or ethanol, because chronic treatment induces metabolizing enzymes resulting in metabolic tolerance [33,34]. McQuarrie and Fengl [35] and Crabbe et al. [6] first demonstrated a state of withdrawal CNS hyperexcitability after acute ethanol or PB administration, respectively. Withdrawal severity was examined by monitoring handling-induced convulsions (HICs) associated with withdrawal, which is a sensitive index of SH withdrawal severity [6]. Details of the acute PB and ethanol withdrawal procedures have been published [19,36], and the same 7-point HIC scoring procedure (Table 1) was used in the present studies. Individual baseline HICs were measured twice on the day before surgery. 7–10 Days post-lesion, baseline (pre-PB) HICs were measured immediately before PB administration (60 mg/kg, i.p., in sterile physiological saline). HIC testing continued hourly between 1 and 8 h post-PB administration. Two weeks later, the mice were tested for ethanol withdrawal. Baseline (pre-ethanol) HICs were scored immediately before ethanol (4 g/kg, i.p., 20%, v/v, in saline) administration. HIC testing continued hourly between 2 and 12 h post-ethanol administration. The last HIC test was performed at 24 h to confirm that HIC scores had returned to pre-drug baseline levels. Previous empirical observations show that prior testing for PB withdrawal does not affect severity of HICs associated with ethanol withdrawal tested 1–2 weeks later.

In order to create indices of PB and ethanol withdrawal response that are independent of individual differences in baseline HIC scores and reflect differences in withdrawal convulsion severity, post-PB and post-ethanol HIC scores were corrected for the individual's average baseline (pre-drug) HIC score as in previous work [19,36]. PB and ethanol withdrawal severity scores were calculated as the area under the curve (AUC; the summed post-PB or post-ethanol HIC scores) over 8 h post-PB and 12 h post-ethanol. Individual withdrawal severity scores correspond to these corrected AUC values.

Table 1
Handling-induced convulsion (HIC) rating scale.

Symptom	Score
No convulsion or facial grimace after gentle 180° spin	0
A facial grimace is seen after gentle 180° spin	1
No convulsion when lifted by the tail, but a tonic convulsion is elicited by a gentle 180° spin	2
Tonic–clonic convulsion after a gentle 180° spin	3
Tonic convulsion upon lifting by the tail	4
Tonic–clonic convulsion when lifted by the tail, often with the onset delayed up to 1–2 s	5
Severe, tonic–clonic convulsion when lifted by the tail, with a quick onset and long duration, often continuing for several seconds after the mouse is released	6
Severe, tonic–clonic convulsion elicited prior to lifting by the tail, with a quick onset and long duration, often continuing for several seconds after the mouse is released	7

2.4. Pentylenetetrazole (PTZ)-enhanced HICs

The mice were assessed for PTZ-enhanced HICs one week after being tested for ethanol withdrawal. Previous studies show that the severity of PTZ-enhanced HICs is not influenced by prior testing for ethanol withdrawal or surgery [22]. The mice were tested for baseline (pre-PTZ) HICs immediately before i.p. administration of 30 mg/kg PTZ. PTZ-enhanced HICs were measured 1, 3, 5, 8, 10, 15, 20, 35, 50 and 65 min post-PTZ as in our previous work [22]. This time period was selected based upon the time course of PTZ elicited convulsions in a panel of mouse strains [37]. This PTZ dose increases HIC intensity, but does not induce other types of convulsions (e.g., tonic hindlimb extensor) that are associated with higher doses of PTZ. To create an index of PTZ response that is independent of individual differences in baseline HIC scores, all PTZ-enhanced HIC scores were corrected for the individual's baseline (pre-PTZ) scores. PTZ-enhanced HIC severity scores were calculated as the area under the curve as in previous work [22].

2.5. Histology

Within 2 h after behavioral testing was completed, the animals were euthanized using an overdose of mouse anesthetic cocktail. Thionin staining was used to confirm lesion locations as well as determine the rostrocaudal extent of the lesions. Only behavioral results from animals with confirmed bilateral lesions of the clSNr are included in the statistical analyses presented.

2.6. Statistics

The behavioral data were not normally distributed based upon a Shapiro–Wilks test, and were therefore analyzed using non-parametric statistical tests. We generated a Mann–Whitney *U* statistic for a comparison of two groups, or an *H* statistic for a comparison of more than two groups. All data were analyzed using Systat 12 statistical software version 12.00.08 (Systat Statistical Inc.). Unless noted otherwise, the significance level was $p < 0.05$ (two-tailed).

3. Results

3.1. Lesions

Fig. 1 shows the extent of chemical ibotenic acid (Fig. 1A) and electrolytic (Fig. 1B) lesions of the clSNr, and representative photomicrographs of the lesion sites (Fig. 1C and D). Based upon coordinates from a mouse brain atlas [30], confirmed electrolytic lesions of clSNr extended approximately −3.1 to −4.0 mm AP from Bregma. The chemical lesions were generally smaller in size than electrolytic lesions, with the latter occasionally extending into the