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The effect of atomoxetine, a selective norepinephrine reuptake inhibitor, on respiratory arrest and cardiorespiratory function in the DBA/1 mouse model of SUDEP

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ABSTRACT

Sudden unexpected death in epilepsy (SUDEP) is a significant public health burden. The mechanisms of SUDEP are elusive, although cardiorespiratory dysfunction is a likely contributor. Clinical and animal studies indicate that seizure-induced respiratory arrest (S-IRA) is the primary event leading to death in many SUDEP cases. Our prior studies demonstrated that intraperitoneal (IP) injection of atomoxetine, a norepinephrine reuptake inhibitor (NRI) widely used to treat attention deficit hyperactivity disorder, suppresses S-IRA in DBA/1 mice. In the current study, we injected atomoxetine intracerebroventricularly (ICV) and measured its effect on S-IRA in DBA/1 mice to determine its central effects. Additionally, to test our hypothesis that atomoxetine reduces S-IRA via altering cardiorespiratory function, we examined the effect of atomoxetine on respiratory and cardiac function using non-invasive plethysmography and ECG in anesthetized DBA/1 mice, and on blood pressure and heart rate using a tail-cuff system in conscious DBA/1 mice. ICV administration of atomoxetine at 200–250 nmol significantly reduced S-IRA evoked by acoustic stimulation in DBA/1 mice, consistent with a central atomoxetine effect on S-IRA. Peripheral atomoxetine administration at a dosage that reduces S-IRA (15 mg/kg, IP) slightly increased basal ventilation and the ventilatory response to 7% CO_2, but exerted no effect on heart rate in anesthetized DBA/1 mice. IP injection of atomoxetine produced no effect on the heart rate and blood pressures in conscious mice. These data suggest that atomoxetine suppresses S-IRA through direct effects on the CNS and potentially through enhanced lung ventilation in DBA/1 mice.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is a major burden on public health (Thurman et al., 2014), as the risk of sudden death in younger patients with epilepsy is increased more than 20-fold (Ficker et al., 1998; Tomson et al., 2016). Clinical and animal studies demonstrate that seizure-induced respiratory arrest (S-IRA) is the primary event leading to death after generalized tonic-clonic seizures in many cases (Bateman et al., 2008; Blum, 2009; Bravo et al., 2015; Buchanan et al., 2014; Faingold et al., 2010; Langan et al., 2000; Pezzella et al., 2009; Ryvlin et al., 2013; So et al., 2000; Zhang et al., 2016), although cardiac dysfunction may also contribute to seizure-induced sudden death (Frasier et al., 2016; Kalume et al., 2013). Previous studies implicate serotonergic and adenosinergic neurotransmission in the pathogenesis of S-IRA in animal models of SUDEP, including the DBA/1 mouse (Feng and Faingold, 2015; Richerson et al., 2016). Our recent data demonstrate that enhanced norepinephrine (NE) availability in the synapses by intraperitoneal (IP) administration of the NE reuptake inhibitor (NRI) atomoxetine reduces S-IRA evoked by either acoustic stimulation or pentylenetetrazole in DBA/1 mice (Zhang et al., 2017). However, it is unknown how atomoxetine suppresses S-IRA. We hypothesized that atomoxetine reduces S-IRA by altering cardiorespiratory function in the DBA/1 mouse model of SUDEP.

Abbreviations: AGSz, audiogenic seizures; DMSO, dimethyl sulfoxide; ICV, intracerebroventricular(ly); IP, intraperitoneal(ly); NE, norepinephrine; S-IRA, seizure-induced respiratory arrest; NRI, norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; SUDEP, sudden unexpected death in epilepsy

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2. Materials and methods

2.1. Animals

Experimental procedures were approved by Massachusetts General Hospital Institutional Animal Care and Use Committee, and all studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. DBA/1 mice were originally purchased from Envigo (Indianapolis, IN) and were housed and bred in the animal facility at Massachusetts General Hospital under a temperature- and humidity-controlled environment (12-h light/dark cycle), provided with rodent food and water *ad libitum*. DBA/1 mice were "primed" by daily exposure to acoustic stimulation using an electric bell (96 dB SPL, UC4-150, Zhejiang People's Electronics, China) for 3-4 days, starting from postnatal day 26–28, to establish consistent susceptibility to audiogenic seizures (AGSz) and S-IRA (Zhang et al., 2016). The same acoustic stimulus parameters were used in priming and induction of AGSz. DBA/1 mice of both sexes at approximately 2 months of age were used in the intracerebroventricular (ICV) experiments, and those at approximately 1–2 months of age were used in non-invasive plethysmography and tail-cuff experiments. Animals were randomly assigned to a treatment group. Some mice were reused in the ICV experiments (9 out of 21 mice used in the experiments), and none were reused in the other experiments. When a DBA/1 mouse was reused in the ICV experiments, the animal rested in the animal facility for at least one week to ensure the clearance of administered drugs, and the susceptibility of this mouse to S-IRA was always confirmed 24 hr prior to the next test.

2.2. The effect of ICV injection of atomoxetine on S-IRA

The guide cannula was implanted as previously described (Faingold et al., 2016; Feng et al., 2007). Briefly, a DBA/1 mouse was anesthetized using ketamine/xylazine (100/10 mg/kg, IP), and a guide cannula (26G, Plastics One, Roanoke, VA) was stereotaxically implanted into the left lateral ventricle (AP −0.4 mm; ML −1.0 mm; V −2.0 mm) (Paxinos and Franklin, 2013).

One week after the surgery, the mouse was subjected to acoustic stimulation to confirm that it was still susceptible to S-IRA and was resuscitated using a rodent ventilator (Harvard Apparatus 680, Holliston, MA). Twenty-four hr after the confirmation of S-IRA, microinjection was performed using a minipump (11 Elite Nanomite, Harvard Apparatus) and a Hamilton syringe (Harvard Apparatus) connected to the infusion cannula (33G, Plastics One) by a polyethylene tubing (PE10, Harvard Apparatus). Atomoxetine (Y0001586, Sigma-Aldrich, St. Louis, MO) (150, 200 or 250 nmol) or vehicle, 50% dimethyl sulfoxide (DMSO), at 2 µl volume was administered ICV at a rate of 0.5 µl/min in different groups of DBA/1 mice, respectively. After completion of the microinjection, the infusion cannula remained inside the guide cannula for an additional one minute and was then slowly withdrawn to avoid back flow.

AGSz and S-IRA were examined 2 h after atomoxetine or vehicle injection and videotaped for offline analysis. For those DBA/1 mice in which S-IRA was reduced by atomoxetine, susceptibility to S-IRA was tested 24 h after microinjection or at 24 h intervals thereafter until susceptibility to S-IRA returned to confirm the reversibility of the drug effect on S-IRA.

2.3. Histology

At the end of the microinjection experiment, ICV injection of fast green was performed to mark the ventricular space; guide cannula placement was verified using histology. For brain harvest, each mouse was deeply anesthetized with an overdose of ketamine/xylazine and transcardially perfused with 10 ml PBS (pH 7.4), followed by 10 ml 4% paraformaldehyde. After removal, the brain was stored in 4% paraformaldehyde at 4 °C. Each brain was sectioned into 50-µm thickness of coronal slices with a freezing microtome (CM 1850 UV, Leica, Buffalo Grove, IL), and the location of the guide cannula track was observed using a Nikon Eclipse TS100 light microscope (Nikon Instruments, Melville, NY).

2.4. The effect of atomoxetine on respiratory function and heart rate in anesthetized DBA/1 mice

Modulation of atomoxetine on respiratory function was examined using nose-only plethysmography in anesthetized DBA/1 mice, as previously described (Zeng et al., 2015). In brief, the nose of a primed DBA/1 mouse under 1.5% isoflurane (Baxter Healthcare, Deerfield, IL) anesthesia was inserted into a custom-built breathing chamber through a 0.25-in. hole in a latex diaphragm (McMaster Carr, Robbinsville, NJ). The chamber was flushed with stable flow of fresh room air (1 l/min), and the composition of gas in the chamber was continuously monitored using a Capnomac Ultima medical gas analyzer (GE Healthcare, Buckinghamshire, UK). Changes in gas pressure induced by mouse breathing inside the breathing chamber were detected by a Model 8420 pneumotachometer (Hans Rudolph, Shawnee, KS), which were converted to an analog signal by a CD15 differential pressure transducer and an MP45-14-871 demodulator (Validyne Engineering, Northridge, CA). The system was calibrated using a rodent ventilator (Harvard Apparatus 683). The body temperature of the mouse was monitored using a rectal thermistor and maintained at 37 °C by a heat lamp.

ECG recordings were performed in anesthetized DBA/1 mice as previously described (Zhang et al., 2016). Briefly, three subdermal needle electrodes (Model F-E2, Grass Instruments, Warwick, RI) were inserted into the skin across the thorax, and cardiac electrical activity was recorded using a P511 AC amplifier (Grass Technologies, Warwick, RI) with the following parameters: gain 20,000; bandpass filter 0.3-3000 Hz.

Minute ventilation (V_E), tidal volume (V_T) and respiratory frequency (f_R) ($V_E = V_T \times f_R$) as well as heart rate were recorded from the anesthetized DBA/1 mouse for 30 min in the absence of any treatment, and the data for the last 10 min were used for baseline normalization. Ventilatory response to CO_2 was recorded for 10 min by switching from room air to a 7% CO_2 gas mixture. The normalized response of each parameter was compared between the drug and vehicle groups. Drugs (i.e., atomoxetine, doxapram or atropine) or vehicle (saline) was administered IP through a 24-gauge angiocatheter inserted into the abdomen prior to data collection. Doxapram and atropine served as positive control for ventilation and heart rate in these experiments, respectively. Custom software written using LabView 2013 (National Instruments, Austin, TX) was used for data acquisition, analysis and gas flow control.

2.5. The effect of atomoxetine on blood pressures and heart rate in awake DBA/1 mice

Non-invasive measurements of heart rate and blood pressures at 37 °C were performed in conscious DBA/1 mice treated with either atomoxetine or vehicle using tail-cuff system (BP-2000 Blood Pressure Analysis System, Visitech Systems, Apex, NC) (Buys et al., 2008). DBA/1 mice were habituated to the tail-cuff system for 7 days with daily subjection to three cycles of 20 measurements of blood pressures. Atomoxetine or vehicle (saline) was administered IP, and the effect of atomoxetine or vehicle on heart rate and blood pressures was determined.

2.6. Statistical analysis

Data are reported as mean ± SEM. Statistical analyses were performed using Prism 6 software (GraphPad Software, La Jolla, CA). The incidence of S-IRA between atomoxetine and vehicle control was