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Long-lasting, reversible and non-neurotoxic inactivation of hippocampus activity induced by neosaxitoxin

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ABSTRACT

Background: Neosaxitoxin (NeoSTX) and related paralytics shellfish toxins has been successfully used as local anesthetic and muscle relaxants to treat a variety of ailments. The primary mechanism of action of these toxins occurs by blocking voltage-gated sodium channels with compounds such as TTX, lidocaine, or derivatives. However, most of these non-classical sodium channel blockers act with a reduced time effect as well as ensuing neurotoxicity.

New method: In this report, we show that the use of local NeoSTX injections inactivates the hippocampal neuronal activity reversibly with a by long-term dynamics, without neuronal damage.

Results: A single 10 ng/μl injection of NeoSTX in the dorsal CA1 region abolished for up to 48 h memory capacities and neuronal activity measured by the neuronal marker c-fos. After 72 h of toxin injection, the animals fully recover their memory capacities and hippocampal neuronal activity. The histological inspection of NeoSTX injected brain regions revealed no damage to the tissue or reactive gliosis, similar to vehicle injection. Acute electrophysiological recording in vivo shows, also, minimal spreading of the NeoSTX in the cerebral tissue.

Comparison with existing methods: Intracerebral NeoSTX injection showed longer effects than other voltage sodium channel blocker, with minimal spreading and no neuronal damage.

Conclusion: NeoSTX is a new useful tool that reversibly inactivates different brains region for a long time, with minimal diffusion and without neuronal damage. Moreover, NeoSTX can be used as a valuable sodium channel blocker for many studies in vivo and with potential therapeutic uses.

1. Introduction

A classical way to evaluate the function of different brain regions has involved the intervention of neural circuits by conducting permanent lesion or reversible inactivation. The techniques that reversibly inactivate a CNS region include, among others: cooling (Cooke et al., 2012), the use of pharmacological tools to block the propagation of action potentials with drugs such as lidocaine, bupivacaine or ropivacaine, among others (Gulbrandsen, Sutherland, 2014), interfering with synaptic function with a bacterial enzyme such as botulinum toxin (BoNT) (Caleo et al., 2007; Costantin et al., 2005; Restani et al., 2011), blocking synaptic transmission using agonists of inhibitory transmitters (Majchrzak, Di, 2000), or more recently, using optogenetic tools that may inhibit neural activity in a cell specific way (Knopfel et al., 2010). The blockade of the neural activity by using these manipulations usually lasts from minutes to hours, except for the case of the BoNT which displays a long-term effect, albeit it is specific only for neuromuscular synapses. Even it has been reported central effects, at high doses of this BoNT in motoneurons of injected muscle, by retrograde or transneuronal transporting from muscle to the motoneuron (Moreno-Lopez et al., 1997), this inespecific protease at high doses may produce structural and functional alterarion in the neuromuscular tissue and motoneurons (Pingel et al., 2017; Pastor et al., 1997; Moreno-Lopez et al., 1997). Overall, these techniques are particularly useful tools to study short-term functions or therapeutic interventions. Nevertheless, the short-term blockade of these manipulations may be of limited usefulness when the research interest focuses on neuronal processes with longer time course dynamics, or in therapeutics interventions that required the more prolonged time of inactivation of neuronal activity.

Some marine microalgae species produces phycotoxins that are

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responsible for massive fish kill and seafood-related poisoning in humans (Lagos, 1998, 2002). The symptoms are often neurological and gastrointestinal and linked to altered cellular excitability. Microalgae are primary producers that make up the base of both marine and freshwater food webs. Some of them also produce unusual compounds that exhibit potent biological activities. They are secondary metabolites, which are not vital to the plain metabolism and growth of the organism, but they are present in constrained taxonomic groups. Among the many algal secondary metabolites, an important number are potent biotoxins responsible for a wide array of human illnesses. One of them is Paralytic Shellfish Poison (PSP), which shown as primary clinical symptom acute paralytic illness, poses the most severe threat to public health due to its high mortality rate in mammals (Lagos, 1998).

Paralytic shellfish poisoning, is the most widespread algae-derived shellfish poisoning worldwide (Lagos, 1998). The PSP toxins are a group of over 28 structurally related imidazoline guanidinium derivatives, non-protein phycotoxins with low molecular weight ranging from 280 to 450 Daltons. They have a common chemical skeleton (3, 4, 6-trialquil tetrahidropurine) that makes them hydrophilic, so entirely soluble in water (Oshima, 1995). According to the net charge that these toxins show at pH 7.0, they can classify in three major groups: (1) saxitoxins (STXs) with a net charge of +2; (2) gonyaulatoxins (GTXs) group with net charge of +1, and (3) N-sulfocarbamoyl-11-hydroxysulfate toxins (Cs) with net charge 0. The Saxitoxin was the first PSP toxin described and its structure established by x-ray analysis (Schantz, Mold et al., 1957; Schantz, Ghazarossian et al., 1975) it is the most well know and studied. In addition, it is the most frequently found commercially available. Nevertheless, Gonyautoxins (GTXs) and specially GTX2/3 and GTX1/4 epimers are the most abundant in mollusks extract samples, and they account for the high shellfish toxicity in Chile and worldwide (Hallegraeff, 1993; Lagos, 1998, 2002; Lagos, Andrinolo, 2000).

The high toxicity of the PSP toxins is due to the reversible binding to a site receptor on the voltage-gated sodium channel on excitable cells (Catterall, 1993; Goldin, 2001), blocking neuronal transmission and causing mammals death by respiratory arrest and cardiovascular shock (Andrinolo et al., 1999; Catterall et al., 1979; Guo et al., 1987; Kao, Nishiyama, 1965; Lagos, Andrinolo, 2000; Moczydlowski et al., 1984). PSP toxins bind with high affinity (Saxitoxin Kd lower than 2 nM) to site 1 on the voltage-dependent sodium channel, inhibiting channel opening. The voltage-dependent sodium channels play a crucial role in neurotransmission at both neuronal synapses and neuromuscular junctions. Consequently, their primary physiological effect is linked to the blocking action potential at axonal level impeding both, nerve impulse propagation and neuronal transmission over the neuromuscular junction. Therefore, when they are applying locally, two clinical activities are manifested simultaneously: (i) the control of pain (anaesthetic activity) and (ii) the control of muscle hyperactivity (relaxant effect).

Here we evaluated the time curse action of NeoSTX, one of the PSP toxins, to block neuronal activity in the CNS. Various preceding manuscripts had established long-lasting relaxant and analgesic effects using Gonyautoxins and NeoSTX, both PSP toxins at the peripheral nervous system in humans (Lagos, 2014). Until now, only one study has been shown behavioral effects lasting less than a week. In that study, NeoSTX showed a reduction in the fear conditioning paradigm to inactivate the posterior insular cortex in rats (Casanova et al., 2016).

To determine more directly the loss of functionality and inactivation of a brain region, the dorsal CA3 hippocampus region was bilaterally inactivated with different NeoSTX doses. The results showed that NeoSTX inactivated that brain regions with long-lasting and reversible effects. Furthermore, NeoSTX exhibited a restricted spreading and none neuronal damage. NeoSTX showed to be a useful pharmacological tool that safely can inactivate brains regions.

2. Materials and methods

2.1. Subjects

In this study, we used 37 adult male Sprague-Dawley rats, bred at our institutional Animal Care Facility weighing 270–320 g. They were individually housed with free access to water and food pellet, in a controlled environmentally controlled room with a temperature of 23 °C and light/dark cycles of 12/12 h, ZT0 = 7:00 AM. Surgical and experimental procedures are performed according to the National Institute of Health (USA) Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996). The institutional Biosafety and Ethical Committee (CBA# 0770, FMUCH, Faculty of Medicine, University of Chile) approved these experimental protocols, designed to minimize the number of rats involved and their suffering.

2.2. Animal groups

We divided the animals in four experimental set. Group 1 included 10 rats, that were bilaterally and chronically implanted with injection cannulas as we describe below. All these rats were bilaterally injected with 0.5 µl of sterile saline (NaCl 0.9%) as vehicle in the CA3 dorsal hippocampus region. These animals were then tested for memory capacities. After one week, five of these Group 1 rats were bilaterally injected with 0.5 µl of NeoSTX (5 ng/µl), and the other five rats were bilaterally injected with 0.5 µl of NeoSTX (10 ng/µl). These 10 animals were then tested for memory performance after 24, 48 and 72 h after the drug injection. Group 2 included 15 rats that were acutely injected with 0.5 µl (10 ng/µl) of NeoSTX on one side of the hippocampus while the other side was injected with vehicle (saline) as control. Thereafter, the animals were challenging to navigate in an arena with alternating spatial configurations, as described below, at 24, 48 and 72 h after the NeoSTX and vehicle injections. One hour after the navigation was ended, the animals were euthanized, and their brains were extracted and processed for immunohistochemistry, seeking for the neuronal activity marker c-Fos to determine the effects of NeoSTX on neuronal activity in the hippocampus. Group 3 included four rats that used for acute electrophysiological recording under anesthesia to estimate the spreading of toxin action in the cerebral tissue. Group 4, four additional rats were acutely injected with the higher dose of NeoSTX on one side of the hippocampus, and another side injected with 5 ng/µl of ibotenic acid as a positive control of lesion and Nissl histological analysis of neurotoxic effects of NeoSTX.

2.3. Surgery and chronic cannula implantation for NeoSTX administration

The rats were anesthetized with isoflurane (2.5% in oxygen) for induction and 1.5% for maintenance, at 1 l/min of oxygen flow. Sedation depth was monitored by the absence of toe pinch withdraw reflex. The animals were positioned in a stereotaxic frame and implanted with two bilateral stainless-steel cannula guides of 21-gauge (Plastics one), targeting the dorsal CA3 region of the hippocampus according to the rat brain atlas (Paxinos & Watson 2007) employing the following coordinates: AP -2.5 mm from bregma; ± 3.5 mm laterality and 2,7 mm in depth. Cannulas were fixed to the skull with anchors stainless steel screws and dental acrylic. Antibiotic (Enrofloxacin 5%, 19 mg/kg i.p.; Bayer) and anti-inflammatory (Ketophen 0.2 mg/kg i.p.; Rhodia Merieux) were administered at the end of surgery and during three consecutive days. After seven days of surgery recovery, the animals were injected with NeoSTX or saline as was described before. NeoSTX was obtained from the Membrane Biochemistry Laboratory, Faculty of Medicine, University of Chile. Purification and quantification of NeoSTX were conducted as has been previously described (Lagos, 2002). The concentration of the toxin was monitored by HPLC-FLD in agreeing with previous description (Lagos, 2002; Oshima, 1995).

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