Analgesic Activity of Systemic Tetrodotoxin: an Electrophysiological Study in Anesthetized Mice

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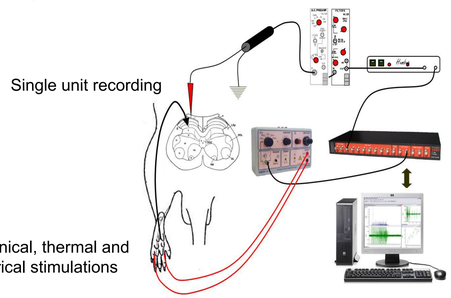
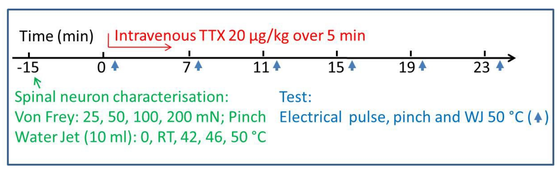
Introduction

- The aim of the present work was to explore the analgesic activity of systemic tetrodotoxin (TTX, selective nanomolar blocker of the voltage gated channels (Nav) 1.1-1.4, 1.6, 1.7).
- Upon systemic delivery, the blockade of Nav1.4 in skeletal muscles, together with Nav1.6 and/or Nav1.7 in autonomic nerves result in respiratory depression and other dysfunctions described upon TTX intoxication.
- The analgesic effect of TTX should be due to blockade of Nav1.7, the main Nav contributor of action potential in non-myelinated fibre, shown to be essential for pain sensation, and may be also to blockade of Nav1.6[1,2].
- Here, the response of nociceptive specific polymodal spinal neurons to noxious mechanical, thermal and electrical stimuli was recorded upon systemic i.v. injection of TTX.

Set up and method

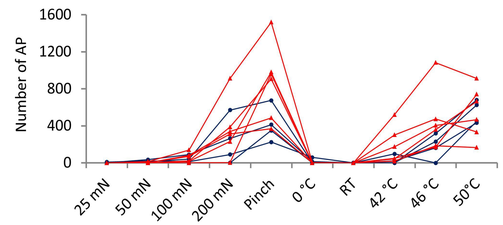




- Male Swiss mice anesthetized with isoflurane, artificially ventilated, with continuous measure of end tidal CO2, blood pressure and body temperature.
- Preliminary studies showed that 10 µg/kg TTX (i.v. over 5 min) was sufficient to induce respiratory failure in anaesthetized mice (n=2). Thirty µg/kg TTX (i.v. over 5 min) abolished responses to noxious stimuli within less than 10 min (n=2).
- In the present experiment, 20 µg/kg TTX or the corresponding vehicle was delivered i.v. over 5 min from T0 to T5.

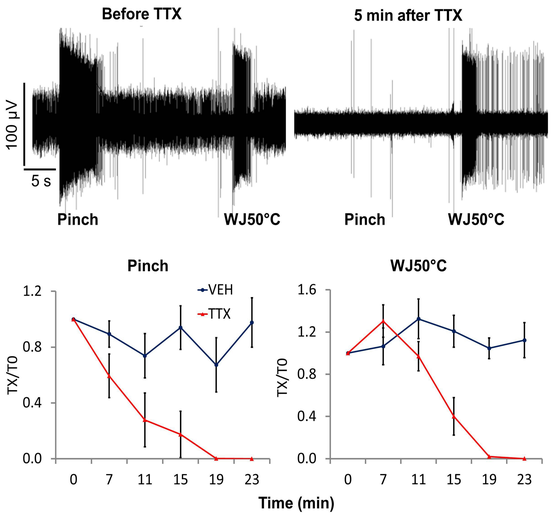
Results

1 Responses of neurons at baseline before TTX injection



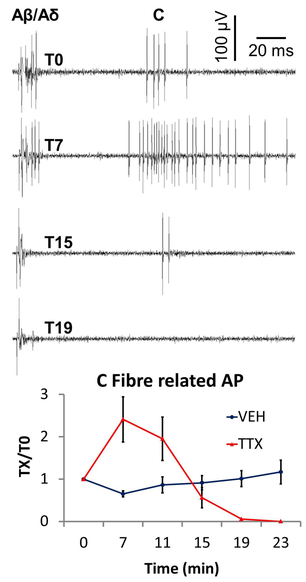
- Responses to Von Frey application (force in mN), pinch with mini haemostat clamp, and water jet at different temperatures (RT, room temperature) were quantified as number of action potentials (AP) over 5 s. Red, TTX group; blue, vehicle group.

2 Responses to pinch and WJ at 50 °C are abolished after TTX injection



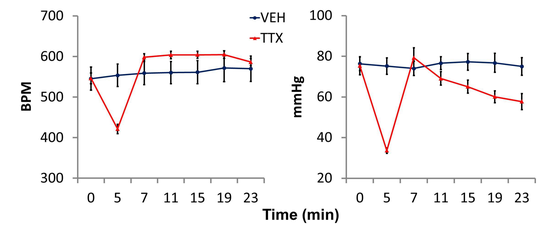
- Recordings: example of responses to mechanical and thermal stimuli before and 5 min after TTX injection. Note that TTX abolished the background activity present at T0.
- Graphs: quantification of the effect of TTX on responses to pinch and WJ at 50 °C. TTX, n=6; VEH, n=4. Mean ± s.e.m.

3 C fibre related AP are transiently increased after TTX injection



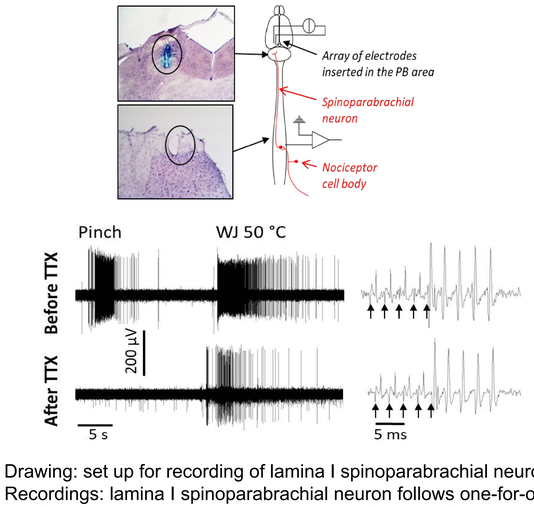
- Recordings: response to electrical stimulation of the receptive field.
- Each sweep corresponds to the response to a single electrical pulse (2.5 mA, 2 ms) before (T0) and 7, 15 and 19 min (T7/15/19) after the start of systemic TTX injection. Note the transient increase of C fibre related AP at T7 before their complete disappearance.
- Graph: effect of TTX on C fibre related response to electrical stimulation of the receptive field.
- TTX, n=5; VEH, n=4.

4 TTX injection induces a dramatic drop in heart rate and blood pressure



- I.v. injection of TTX resulted in a drop in heart rate and blood pressure. Cardiovascular parameters were restored by the i.v. injection of dopamine (2-4 mg/kg followed by 0.6-1.2 mg/kg/h).
- Note the rapid cardiovascular collapse compared to the inhibition of spinal neuron responses. TTX, n=6; VEH, n=4.

5 Loss of responses to noxious stimuli after TTX injection is not due to inhibition of neural activity in the CNS



- Drawing: set up for recording of lamina I spinoparabrachial neuron.
- Recordings: lamina I spinoparabrachial neuron follows one-for-one a train of 5 antidromic stimulations at 400 Hz before and after injection of TTX when responses to pinch have been abolished and responses to heat markedly decreased (n=2).

Conclusion

- Systemic TTX abolished responses of polymodal spinal neurons to noxious stimuli. This was accompanied by a transient increase of C fibre related responses and a drop in blood pressure and heart rate.
- The kinetic of inhibition of responses to noxious stimuli was slow and suggests progressive diffusion of TTX from the vascular compartment and/or restricted access to its targets within peripheral nerves.
- The transient increase of C fibre related AP is explained by the initial preferential inhibition of A fibre compared with C fibre, resulting in relief of presynaptic inhibition from A fibre on C fibre terminals[3].
- Assessment of cardiovascular and light touch function is mandatory in future studies exploring the analgesic activity of systemic TTX in awake rodents.

References

1 Gingras et al, PLoS One, 9:e105895, 2014.
2 Wilson et al, PNAS, 108: 10302-10307, 2011.
3 Lambertz et al, Neuroscience Letters, 409: 14–18, 2006.