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Evaluation of crotalphine effects, a rattlesnake potent analgesic toxin, on the tetrodotoxin-sensitive Na current of adult mouse dorsal root ganglion neurons

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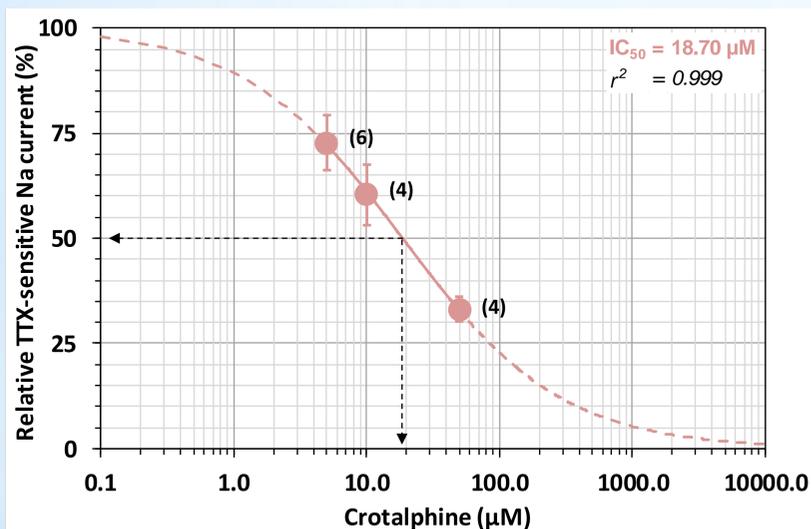
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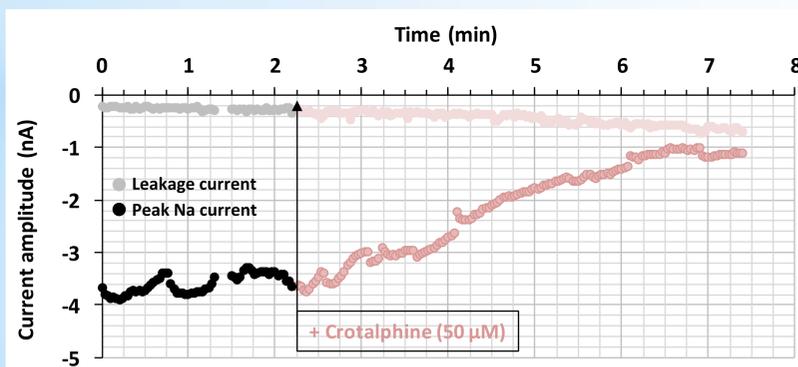
INTRODUCTION. Crotalphine is an analgesic peptide identified in the venom of the South American rattlesnake *Crotalus durissus terrificus*. Although its antinociceptive effects are well documented, its direct mechanisms of action are still under investigation. The aim of the present work was to study the action of the crotalid peptide on the sodium channel subtype Na_v1.7, a genetically validated pain target. To this purpose, the effects of crotalphine were evaluated on the tetrodotoxin-sensitive Na current of dorsal root ganglion neurons of adult mice, using the whole-cell patch-clamp configuration, and on the cell viability, using the propidium iodide fluorescence and trypan blue assays.

1. A crotalphine concentration of 18.70 μM is necessary to inhibit 50% of Na current

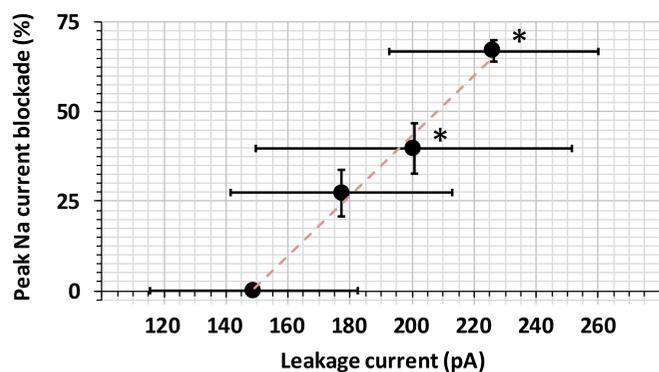


Concentration-response curve of crotalphine steady-state effects on TTX-sensitive Na current. Each value is expressed as percentage of that determined before toxin application.

4. Crotalphine induces increased leakage current amplitude

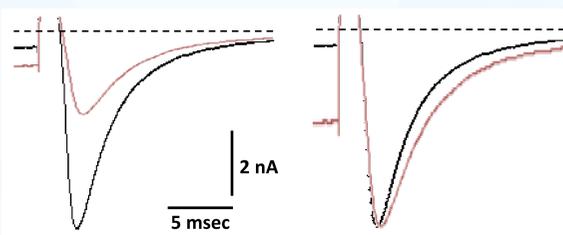


Representative experiment of the time courses of crotalphine (50 μM) effects on the leakage (light circles) and peak Na (dark circles) current amplitudes, before and after toxin addition (arrow). Note the crotalphine-induced increase in leakage current amplitude of approximately 150%.



Linear relationship ($r^2 = 0.988$) between the peak Na current blockade and the leakage current amplitude in the absence and in the presence of 5, 10 and 50 μM of toxin. Means \pm S.E.M. of data obtained from 4-9 neurons. *: $P \leq 0.049$.

2. The blocking effect occurs without any marked change in the current activation and inactivation kinetics

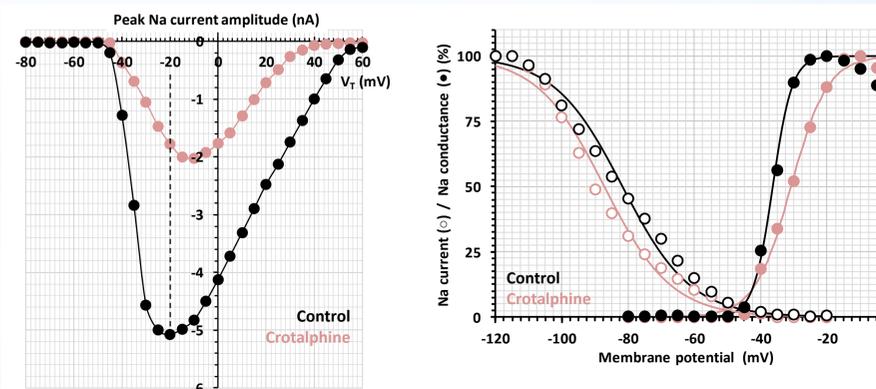


Traces of TTX-sensitive Na currents recorded before (in black) and after (in pink) application of 50 μM of crotalphine. On the right, the toxin peak current is normalized to that of control.

	Activation		Inactivation	
	Time _A (ms)	Slope _A (nA/ms)	Time _I (ms)	Slope _I (nA/ms)
Control	0.66 \pm 0.06	-3.328 \pm 0.756	6.30 \pm 0.67	0.336 \pm 0.052
Crotalphine (50 μM)	0.68 \pm 0.03	-3.144 \pm 0.335	6.67 \pm 0.62	0.307 \pm 0.019

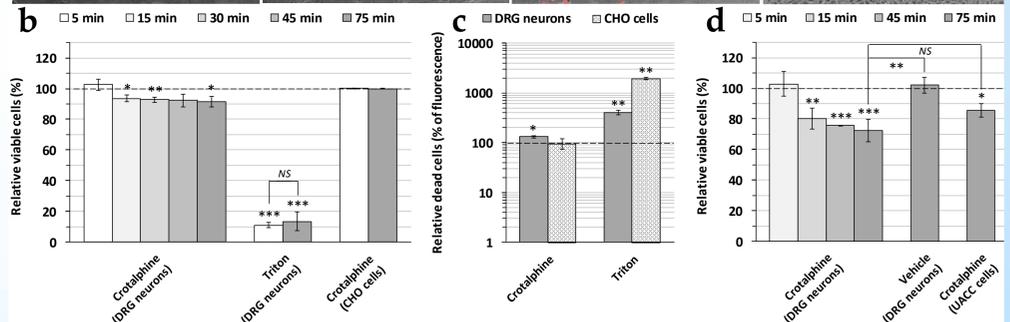
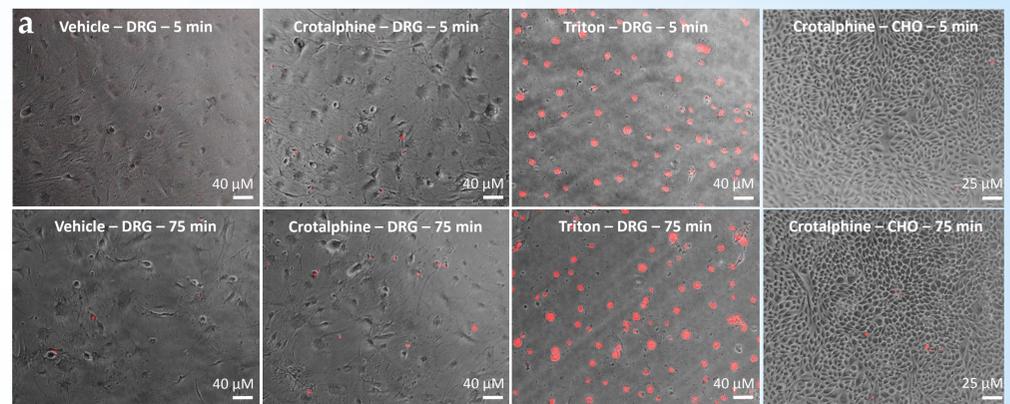
Kinetic parameters of activation (Time_A and Slope_A) and inactivation (Time_I and Slope_I) of TTX-sensitive Na current recorded under control conditions and in the presence of 50 μM of crotalphine (means \pm S.E.M. of 4 neurons).

3. The blocking effect occurs without any marked change in the current activation and inactivation voltage-dependencies



Current-voltage relationships (left panel), and steady-state inactivation- (open circles) and conductance- (filled circles) voltage relationships (right panel), before (black circles) and after (pink circles) exposure of neurons to 50 μM of crotalphine. Each value is expressed as percentage of either maximal peak amplitude of current at strongly negative pre-pulse voltages or maximal conductance calculated at strongly positive test-pulse voltages.

5. Crotalphine induces decreased DRG neuron viability



A decrease of DRG neuron, but not of CHO cell, viability is detected in the presence of crotalphine (50 μM), using the propidium iodide fluorescence assay (a,b,c). Similarly, a decrease of DRG neuron and UACC-257 cell viability is detected in the presence of crotalphine (50 μM), using the trypan blue assay (d). Note that the toxin induces a maximal decrease of 28% of cell viability. Means \pm S.E.M. of data obtained from 3-12 cell cultures. *: $P \leq 0.0500$, **: $P \leq 0.0050$ and ***: $P \leq 0.0005$ (b,c,d).

CONCLUSION. Taken together, these results point out, for the first time, the effectiveness of crotalphine to act on the Na_v1.7 channel subtype, directly and/or indirectly by affecting cell membrane integrity. This mechanism of action could contribute to the peptide analgesic properties.

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