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Conductance-based model of subfornical organ neurons predicts integration of cardiovascular and inflammatory signals

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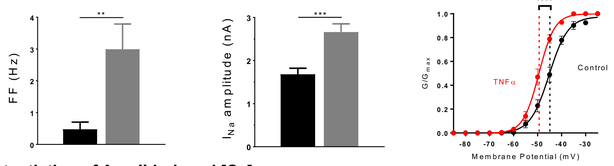
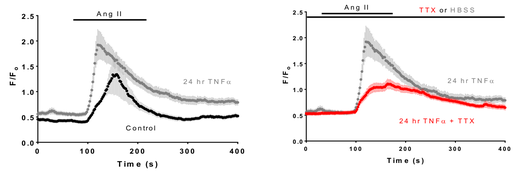
Background

- The subfornical organ (SFO) has been implicated as an important integratory site for various autonomic nervous system signals.
- Angiotensin II (Ang II) and tumor necrosis factor alpha (TNFα) are two signaling peptides implicated in hypertension via their influences on SFO neurons, but the cellular effects and ionic mechanisms remain to be investigated.
- Recent studies show that 24-hour incubation in TNFα results in:

1. Increased Neuronal Excitability



2. Potentiation of Ang II-induced $[Ca]_i$ response



Question

Does 24-hour incubation in TNFα potentiate SFO neuron excitability in response to Ang II?

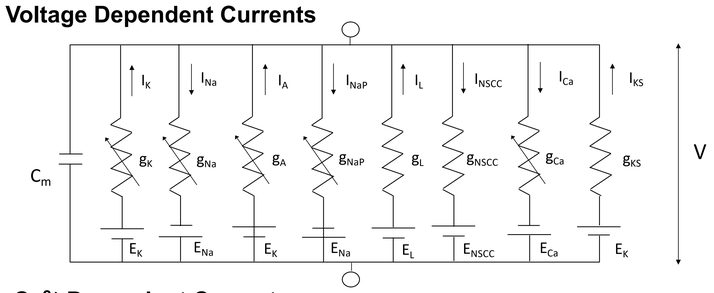
Goals

1. Combine experimental and modelling techniques to make predictions about the integration of Ang II and TNFα signals by SFO neurons.
2. Test model predictions using *in vitro* patch-clamp electrophysiology.

Methods

Hodgkin-Huxley Style SFO Neuron Model

Voltage Dependent Currents



Ca^{2+} Dependent Current

$$I_{KCa} = g_{KCa} \times z(Ca^{2+}) \times (V - EK)$$

When required, a Ca^{2+}-activated K^+ current (I_{KCa}) was added to the model to allow intracellular Ca^{2+} dynamics to modulate SFO neuron activity.

Ca^{2+} Dynamics

$$\frac{dCa^{2+}}{dt} = SAV \times \frac{I_{Ca}}{F} - \frac{Ca^{2+}}{\tau_{Ca}}$$

Electrophysiology

Step 1: Prepare dissociated SFO neurons

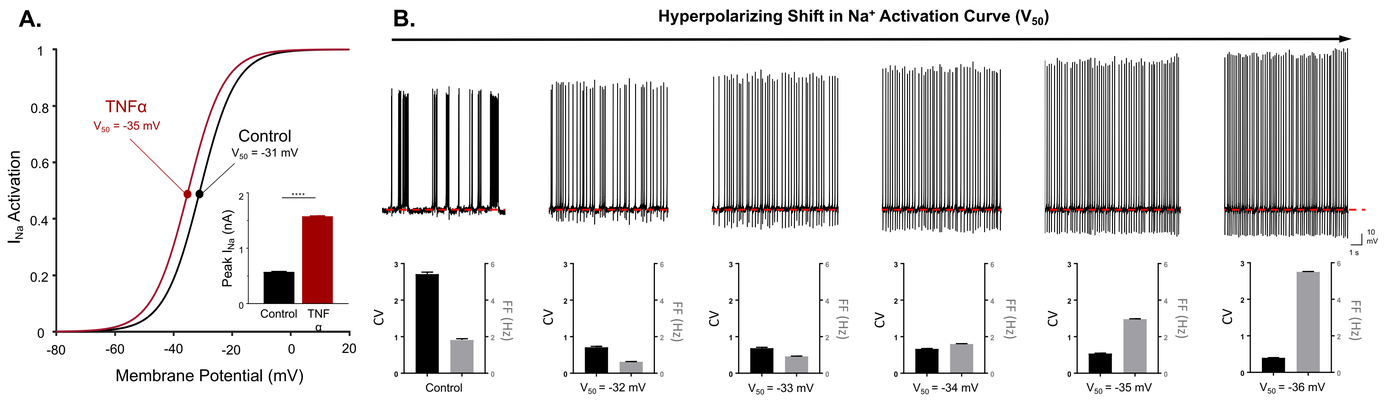
- Dissect, dissociate and plate SFOs.
- All incubation experiments were performed by incubating SFOs in 10ng/mL TNFα for 24 hours prior to recording.

Step 2: Patch-clamp electrophysiology experiments

- Experiments were performed using the perforated patch-clamp technique.
- 10nM Ang II was bath applied onto dishes containing either TNFα-incubated or non-incubated SFO neurons.

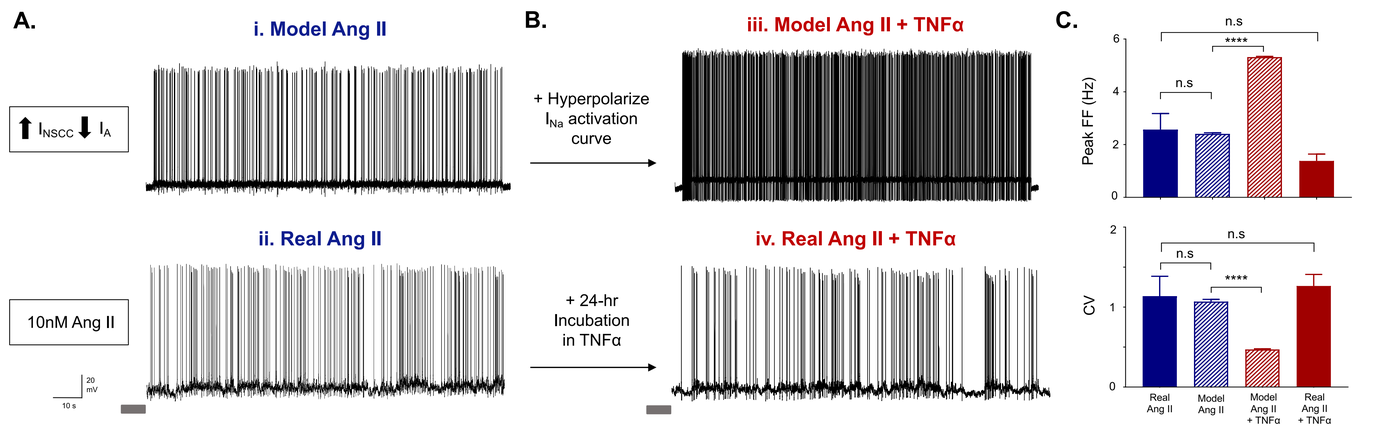
Results

A hyperpolarizing shift in the I_{Na} activation curve increases excitability in our model SFO neuron.



A. Activation curve for the Na^+ current in our SFO neuron model. To mimic the effects of 24-hour TNFα-incubation on Na^+ currents (I_{Na}) in real SFO neurons, the I_{Na} half-activation (V_{50}) in our model was hyperpolarized by 4mV. The inset shows a significant increase (n=15, p<0.0001) in peak I_{Na} following this shift in the activation curve. **B.** Representative model traces demonstrating an incremental decrease in the V_{50} and the subsequent effect on the coefficient of variation (CV) and firing frequency (FF) of the model neuron. Hyperpolarizing shifts in the Na^+ activation curve resulted in a decrease in CV and increase in FF.

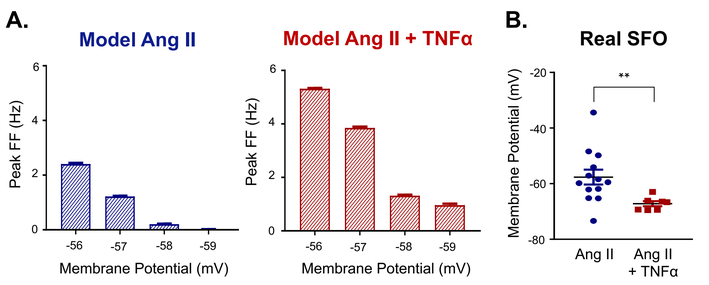
Model predicted increased excitability in response to Ang II, but is not observed in dissociated SFO neurons.



A. A representative model (i) and *in vitro* recording (ii) of a 100s peak response following Ang II application by a dissociated SFO neuron. There is no significant difference in peak firing frequency (FF) (p=0.80) or CV (p=0.79) between model (n=13) and real (n=13) Ang II responses. Grey bars represent time of Ang II application. **B.** Model prediction (iii) and real peak response (iv) of an SFO neuron to Ang II application following 24-hour incubation in TNFα. Our model (iii) predicts an increase in peak FF and decrease in CV in response to TNFα incubation (see previous section for I_{Na} activation curve shift explanation). Unexpectedly, 24-hour incubation of SFO neurons in TNFα (n=7) had no significant effect on peak FF (p=0.09) or CV (p=0.66) in response to Ang II application. **C.** Summary CV and FF data. Peak FF and CV were calculated over 100s.

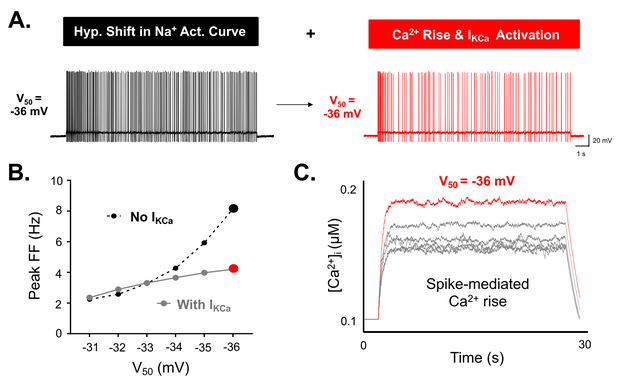
Changes in membrane potential or $[Ca2+]_i$ may account for these unexpected experimental findings.

1. Differences in Membrane Potential



A. The Ang II (i) and Ang II + TNFα (ii) models predict that hyperpolarizing the membrane potential of an SFO neuron would cause a decrease in peak firing frequency (FF) and may explain the previous unexpected experimental findings. **B.** The membrane potential of TNFα-incubated SFO neurons was significantly lower during peak FF than non-incubated SFO neurons (Ang II = -57.6 ± 2.7mV, n=13; Ang II + TNFα = -67.2 ± 0.9mV, n=7; p<0.01).

2. Increased $[Ca^{2+}]_i$ activates K_{Ca} channels



A. The Ang II + TNFα model with (red) and without (black) a Ca^{2+}-activated K^+ current (I_{KCa}). Both models are at a hyperpolarized I_{Na} half-activation (V_{50}) value of -36 mV to replicate the real shift caused by TNFα incubation. **B.** Peak firing frequency (FF) as a function of hyperpolarizing V_{50} values. The previous Ang II + TNFα model (dashed black) increases peak FF dramatically as V_{50} is hyperpolarized. The addition of I_{KCa} into the new model (solid grey), dampens this increase in FF as V_{50} shifts. The red circle indicates the peak FF of the red model trace in A. **C.** Mean internal Ca^{2+} concentration rises for models with decreasing V_{50} values from B. Consistent with previous findings, the Ca^{2+} rise in our model is spike-dependent.

Conclusions

1. Consistent with previous experimental findings, a hyperpolarizing shift in the Na^+ activation curve caused increased excitability in our model SFO neuron.
2. Our model predicted that this same shift in the Na^+ activation curve would result in potentiation of SFO neuron excitability in response to Ang II.
3. Conversely, experiments in dissociated SFO neurons showed there was no significant difference in Ang II response between TNFα-incubation and non-incubated SFO neurons.
4. Our model suggests that changes in membrane potential or $[Ca^{2+}]_i$ may explain these unexpected experimental findings.

Future Studies

1. **Predictions made by our SFO neuron model regarding membrane potential differences and Ca^{2+} dynamics need to be tested using *in vitro* patch-clamp experiments.**
2. **Further analysis of the ionic mechanisms underlying the integration of TNFα and Ang II signals are required for a more complete understanding of cardiovascular regulation at the SFO.**

Acknowledgements

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