Characterization of dissociated catecholamine-containing GFP-expressing mouse area postrema neurons and their response to GLP-1 receptor agonists.

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BACKGROUND

The area postrema (AP) is a sensory circumventricular organ of the hindbrain and is well-recognized to play a role in regulation of energy balance. Catecholamine-containing (CA) AP neurons are a key subpopulation acting to inhibit food intake[1]. In particular, they appear to mediate the appetite reducing effects of circulating GLP-1 acting on the AP[2,3]. Previous work has demonstrated that subpopulations of AP neurons may be identified based on K^+ current properties and the presence of a hyperpolarization activated current (I_H)[4,5].

OBJECTIVE

To investigate the electrophysiological properties of GLP-1 sensitive neurons from the rat area postrema

METHODS

Animals

All animal protocols were approved by UofM and conformed to CCAC guidelines. Experiments utilized 4-8 week old male and female RBRC02095 B6.Cg-Tg(TH-GFP) mice obtained from a colony maintained at the UofM and housed under standard conditions.

Neuron Culture

Mice were decapitated, brain slices cut and the AP dissected. The AP was incubated in 2 mg/ml papain in Hibernate media for 25 min and gently triturated to liberate single cells. Cells were plated on 35 mm glass culture dishes in Neurobasal media supplemented with B27 and Glutamax. Neurons were maintained for 5 days in culture with media changed every 1-2 days.

Electrophysiology

All experiments were carried out on days 2-5 of culture using standard solutions and protocols. Whole cell patch clamp in voltage clamp configuration was used to measure K^+ current, Na^+ current, and I_H properties. Current clamp was used to measure membrane potential. Data were acquired using a HEKA EPC 10 amplifier and PatchMaster software. All data are reported as mean ± SE.

Immunohistochemistry

Brains were removed and fixed in 4% PF for 4h at 4°C. Coronal sections through the AP were cut to 30 µM. Sections were incubated for 24h with (1:1000) rabbit polyclonal anti-TH primary antibody, followed by three washes and a 1h incubation with (1:1000) Alexa Fluor 546 goat-anti-rabbit secondary antibody.

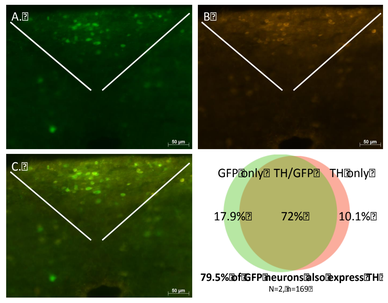

Figure 1. Immunohistochemistry for tyrosine hydroxylase (TH) in mouse AP. (A) Fluorescence from TH-GFP positive neurons. **(B)** Immunofluorescence showing expression of TH. **(C)** Overlap of GFP and TH immunofluorescence. **(D)** Venn diagram quantifying overlap between GFP-positive and TH-positive neuronal populations. White lines in A, B, and C outline AP.

RESULTS

I_K and I_{TO} properties of AP neurons

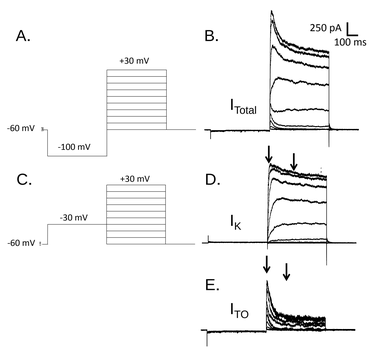

Figure 2. Two voltage gated K^+ currents in AP neurons, I_K and I_{TO}. (A) A hyperpolarizing prepulse to -100 mV followed by a series of depolarizing voltage steps from -60 mV to +30 mV. **(B)** Traces of total K^+ current elicited by A. **(C)** A depolarizing prepulse to -30 mV followed by a series of depolarizing voltage steps from -60 mV to +30 mV. **(D)** Traces of non-inactivating K^+ current (I_K) elicited by C. **(E)** Traces of transient K^+ current (I_{TO}) revealed by subtracting D from B. *Arrows indicate periods used to calculate mean peak current.*

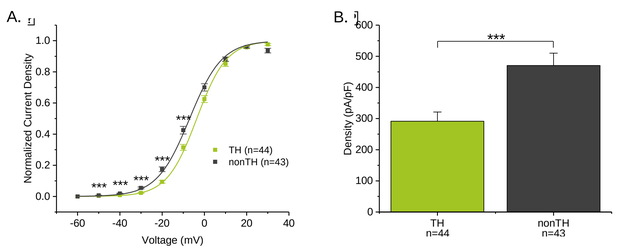

Figure 3. Analysis of peak I_K. (A) Normalized activation curve of peak I_K density. Repeated two-sample t-tests with Bonferroni correction indicated that the voltage dependence of I_K activation was significantly depolarized in TH neurons (n=44) compared to nonTH neurons (n=43) (***; (p<0.005)). **(B)** Peak current density of I_K current at 30 mV was lower (t-test, p<0.001) in TH neurons (n=44) compared to nonTH neurons (n=43).

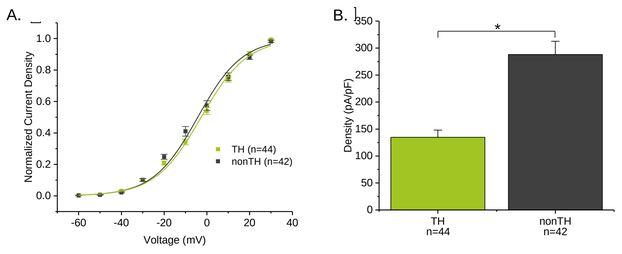

Figure 4. Analysis of peak I_{TO}. (A) Normalized activation curve of mean peak I_{TO} density. (**B**) A two-sample t-test revealed absolute peak I_{TO} density at 30 mV was significantly lower (p<0.001) in TH (n=44) neurons compared to nonTH (n=42) neurons.

I_{TO} fitting for component analysis

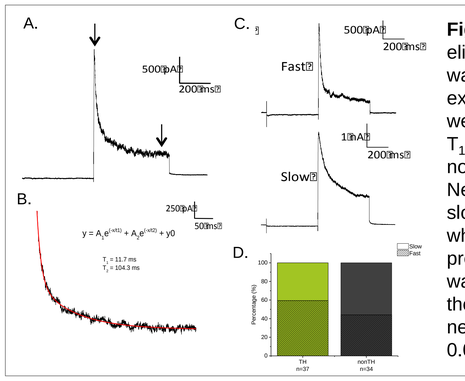

Figure 5. I_{TO} fitting. (A) I_{TO} elicited during a step to +30mV was fitted to a double exponential, shown in **(B)**. There were no significant differences in T_1, T_2, A_1 or A_2 between TH and non-TH neurons (not shown). **(C)** Neurons were classified as fast or slow, depending on whether which time constant predominated, T_1 or T_2. There was no significant difference in the proportion of fast or slow neurons (Fisher exact test, p> 0.05).

SUPPORT

NSERC CRSNG

Research Manitoba

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RESULTS

I_{Na} properties of AP neurons

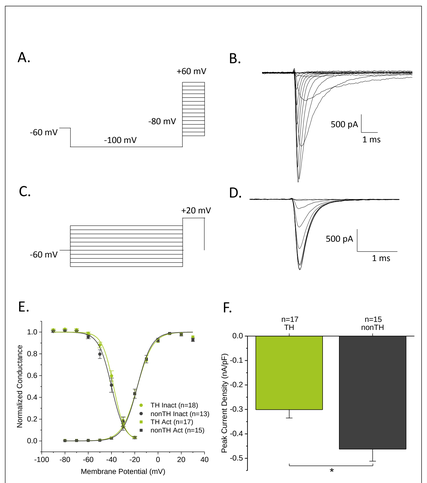

Figure 6. Analysis of transient Na^+ current. (A,C) Commands used to elicit Na^+ voltage gated Na^+ currents from AP neurons. **(B,D)** Representative currents. **(E)** Analysis of the currents did not reveal significant differences in voltage dependence of activation and inactivation. **(F)** Experiments did reveal that TH neurons expressed significantly lower peak current density than non-TH neurons (p< 0.05, Student's t-test).

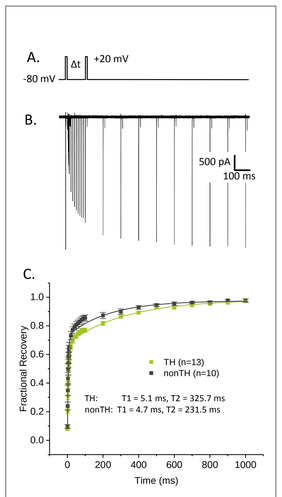

Figure 7. Time dependent recovery from inactivation. (A) Two pulse protocol used to investigate time dependent recovery. **(B)** Series of Na^+ currents from AP neurons **(C)**. Fractional recovery was plotted and fitted to a double exponential. TH neurons recovered significantly more slowly than non-TH neurons (p<0.05, Student's t-test).

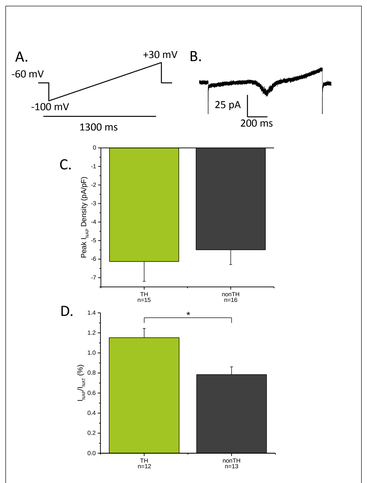

Figure 8. Persistent Na^+ current. (A) Persistent current was elicited using a voltage ramp from -100 to +30 mV over 1300 ms. **(B)** Representative persistent Na^+ current, I_{NaP} . **(C)** There was no significant difference in the peak density. **(D)** There was a significant difference in the ratio of I_{NaP} to I_{NaT} (p<0.05, Student's t-test).

Fewer TH neurons have I_H

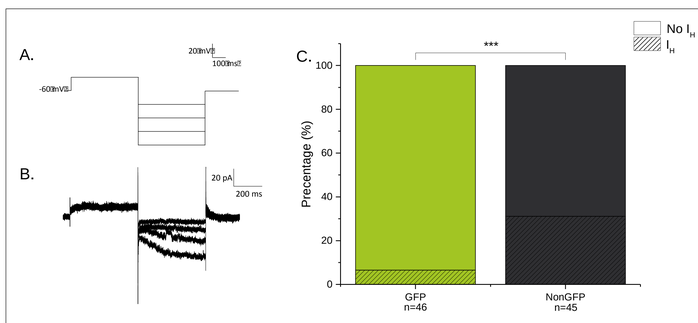

Figure 9. Comparison of proportion of neurons with I_H. (A) I_H was activated with a -40 mV depolarizing prepulse followed by a series of hyperpolarizing voltage steps from -80 mV to -140 mV. **(B)** Traces of current elicited by A. I_H current is indicated by the arrow. **(C)** Neurons were categorized as either having I_H present or lacking I_H. Significantly fewer TH neurons were found to have I_H current than nonTH neurons (two-sample t-test p = 0.003).

TH neurons are depolarized by GLP-1 receptor agonists

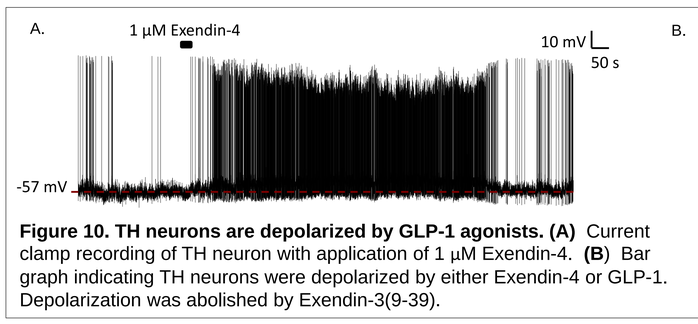

Figure 10. TH neurons are depolarized by GLP-1 agonists. (A) Current clamp recording of TH neuron with application of 1 µM Exendin-4. **(B)** Bar graph indicating TH neurons were depolarized by either Exendin-4 or GLP-1. Depolarization was abolished by Exendin-3(9-39).

CONCLUSIONS

1. **There are subtle differences in voltage-gated ion currents between TH and non-TH neurons of the AP.**
2. **In spite of the differences, TH neurons cannot be electrophysiologically distinguished from non-TH neurons.**
3. **GLP-1 and agonists activate TH neurons.**

LITERATURE

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2. Parker, J. A. *et al. Int. J. Obes. (Lond).* **37,** 1391–8 (2013).
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