Antimuscarinic drugs elevate neurite outgrowth in isolectin B4 subpopulation of adult sensory neurons

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Background

a) Peripheral Neuropathy

(1) A neurodegenerative disorder characterized by one of two issues:
- loss of sensation in the peripheries.
- increased painful sensations when no pain stimulus exists.

(2) Due to the degeneration of sensory neurons, specifically dorsal root ganglia (DRGs).
(3) Associated with diabetes and other illnesses.
(4) Currently studying antimuscarinic drugs that can prevent or reverse the degeneration of neurons and promote neurite outgrowth, hence mitigating peripheral neuropathy.

b) Dorsal Root Ganglia (DRG) and Isolectin B4 cells (IB4)

(1) DRGs
- Sensory neurons that send messages from the peripheries to the brain.
- There are several subpopulations of DRG neurons.
- All sub-populations of DRG neurons express beta-tubulin protein and respond to a variety of growth factors and antimuscarinic drugs.

(2) IB4 cells
- One of the subpopulations of DRGs
- respond exclusively to the growth factor glial cell line-derived neurotrophic factor (GDNF).

c) Growth Factors and Antimuscarinics

Dissociated DRG neurons were treated with two families of growth factors
(1) Neurotrophin family (nerve growth factor = NGF, neurotrophin – 3 = NT3).
(2) GDNF family.
Antimuscarinic drugs are antagonists to the muscarinic GPCR receptor, M1 receptor, of the neurotransmitter acetylcholine (ACh). When ACh saturates receptors, neurite outgrowth is inhibited. When antimuscarinic drugs outcompete ACh and saturate the receptors, neurite outgrowth is promoted. The cells were treated with one of two antimuscarinics:
(1) pirenzepine (PZ) or
(2) muscarinic toxin 7 (MT7).

Hypothesis

The IB4 subpopulation of DRG neurons respond to antimuscarinic drugs.

Research Aims

(1) Develop staining technique for studying neurite outgrowth of the IB4 subpopulation. This will allow us to compare the neurite outgrowth of IB4 neurons to the response of all DRG neurons (stained with beta tubulin).
(2) Confirm that the IB4 subpopulation responds exclusively to GDNF (not neurotrophin).
(3) Study the response of the IB4 subpopulation to antimuscarinics.

Methods

(1) The DRG were treated enzymatically with collagenase and trypsin. Dissociated neurons were centrifuged and then a bovine serum albumin (BSA) column was performed to purify neurons. Neurons were cultured in Ham's F12 media.
(2) Neurons were fixed and stained with fluorescent antibody that detected IB4.
(3) Zeiss Axiovision microscope with the FITC filter visualized the neurons on the computer and images were saved for analysis.
(4) Microscope pictures were converted to black and white using ImageJ. The total axon area was measured in pixels. This pixel measurement was adjusted for cell number and generated the level of total neurite outgrowth.

Statistical analysis

One way ANOVAs with post hoc comparisons by Dunnett's or Tukey's and Welch's t-test were performed on Graph Pad.

Results

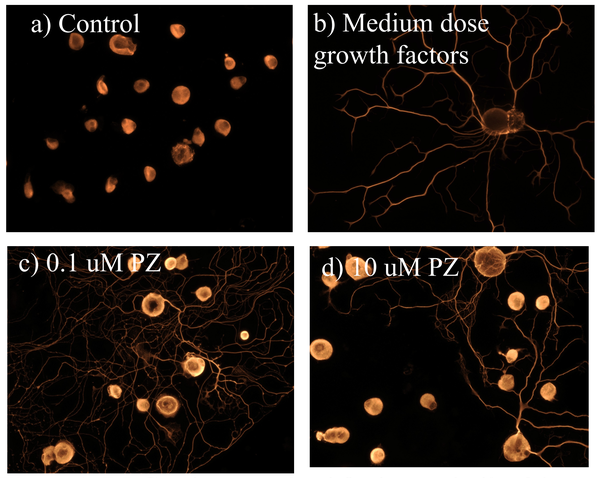


Figure 1. DRG cultures stained for beta-tubulin. 24 hour culture. Control (a) compared with growth factors (b) and 0.1 μM and 10 μ M pirenzepine (PZ) (c and d).

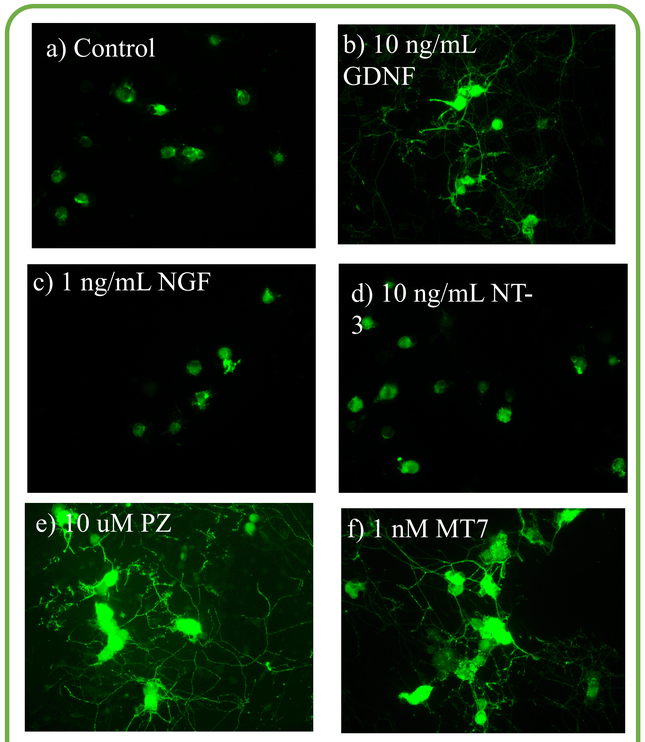
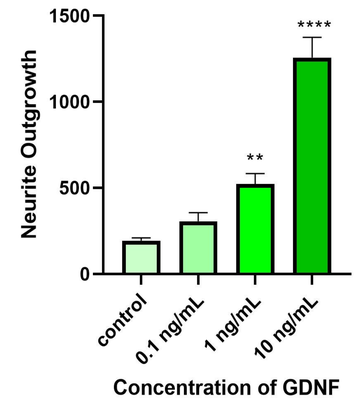
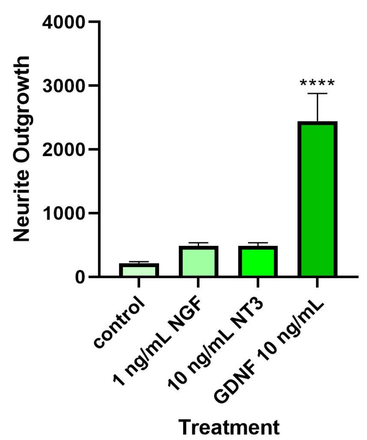


Figure 2. DRG cultures stained for IB4. 48 hour culture. Control (a) compared to growth factors: GDNF (b), NGF (c), and NT-3 (d). Control compared to antimuscarinics: pirenzepine (e) and MT7 (f).



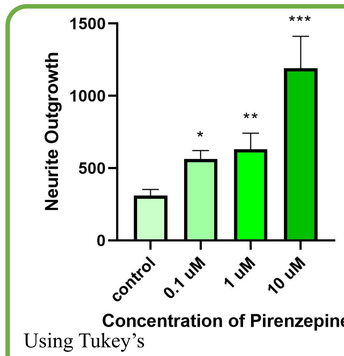
Using Dunnett's
**** significant p value < 0.0001 between control and 10 ng/mL
** significant p value < 0.01 between control and 1 ng/mL
n=7 for control, n=9 for 0.1 and 1 ng/mL, n=8 for 10 ng/mL

Figure 3. 48 hour control rat DRG culture with GDNF stained for IB4.



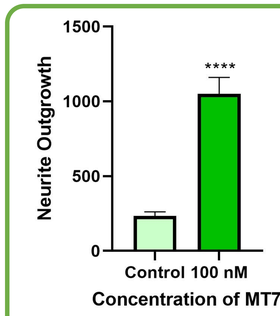
Using Dunnett's
**** significant p value < 0.0001 between control and GDNF
n=9 for all treatment groups

Figure 4. 48 hour control rat DRG culture with NGF, NT3, and GDNF stained for IB4.



Using Tukey's
*** significant p value < 0.001 between control and 10 μM
** significant p value < 0.01 between 0.1 μM and 10 μM
*significant p value < 0.05 between 1 μM and 10 μM
n=9 for all treatment group

Figure 5. 48 hour control rat DRG culture with pirenzepine stained for IB4.



Using a parametric, unpaired t test and not assuming the same standard deviation (Welch's t test)
**** significant p value < 0.0001
n=7 for control and n=9 for MT7

Figure 6. 48 hour control rat DRG culture with MT7 stained for IB4.

Conclusions

Knowing more about DRG subpopulations and their response to specific growth factors and antimuscarinics will allow researchers to develop treatment for peripheral neuropathy. This research supports that the IB4 subpopulation:
(1) Responds significantly to GDNF, pirenzepine, and MT7.
(2) Does not respond to neurotrophins (NGF and NT-3).
More research is needed to find out if IB4 cells responds substantially more to antimuscarinics than other subpopulations.

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