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ATP metabolizing enzymes ENPP1, 2 and 3 are localized in sensory neurons of rat dorsal root ganglion

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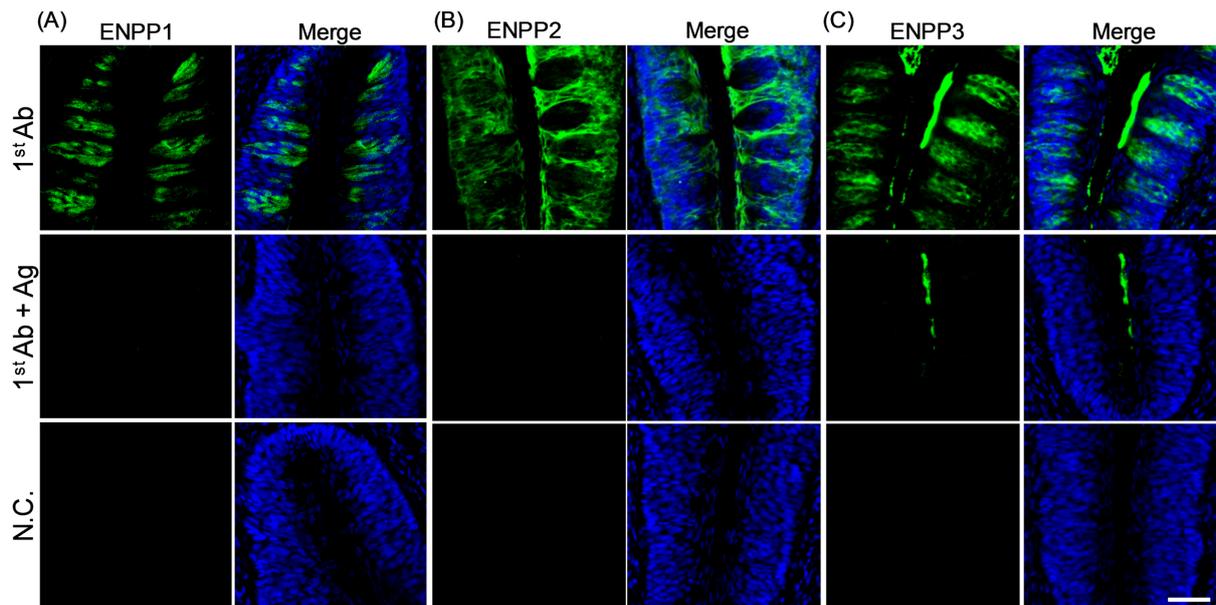
Key words: Ecto-nucleotide pyrophosphatase/phosphodiesterase; dorsal root ganglion; ATP, ectoenzyme; clearance system; sensory neuron.

Methods

Samples were prepared and applied to sodium dodecyl sulfate-polyacrylamide gels at 10 µg protein/lane, and then transferred to polyvinylidene difluoride membranes (Immobilon; Merck Millipore, Billerica, MA). After blocking with 25 mM Tris-buffered saline containing 0.05% Tween 20, 0.5 M NaCl and 3% bovine serum albumin, the membranes were incubated overnight at 4°C with a sheep anti-NTPDase2 antibody (1:500 or 1000, #AF5797; R&D Systems) or a mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:1000, #016-25523; Wako). After washing, the membranes were incubated with horseradish peroxidase-conjugated anti-sheep IgG (1:1000) or anti-mouse IgG (1:10000) antibodies. NTPDase2 or GAPDH was detected using a chemiluminescent substrate (ECL; PerkinElmer Japan, Kanagawa, Japan). Protein concentrations were determined by the method of Bradford with bovine serum albumin as the standard.

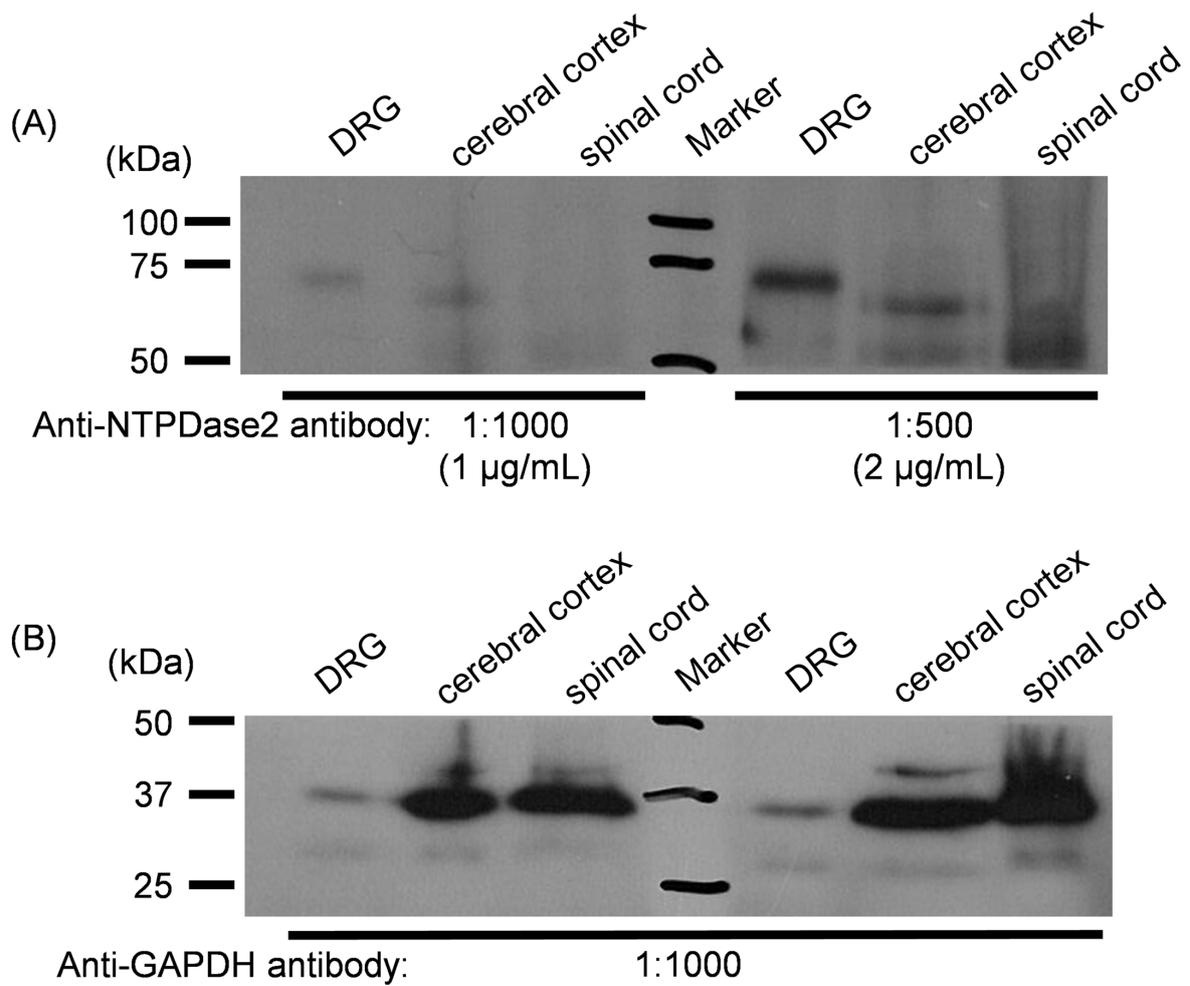
Supplementary Table 1. Antigens used for the adsorption test.

Target	Antibody	Antigens for adsorption test
ENPP1	Rabbit anti-ENPP1 Ab (10 µg/mL; #bs-1760R, Bioss)	Recombinant human ENPP1 (30 µg/mL; #6136-EN-010, R&D systems, MN)
ENPP2	Rabbit anti-ENPP2 Ab (10 µg/mL; #LS-C37249, LifeSpan Biosciences)	Recombinant mouse ENPP2 (30 µg/mL; #50663-M07H, Sino Biological, Beijing, China)
ENPP3	Rabbit anti-ENPP3 Ab (20 µg/L #bs-1568R, Bioss)	Recombinant cynomolgus ENPP3 (60 µg/mL; #90242-C07B, Sino Biological)



Supplementary Figure 1.

Antigen adsorption tests of anti ENPP1-, 2- and 3-antibodies. Representative images of immunohistochemistry with ENPP1 (A), 2 (B) and 3 (C) in rat circumvallate papillae are shown (primary antibody, green). The nuclei were counterstained with Hoechst 33258 (blue). Antibodies were preadsorbed with recombinant antigens (Ag), and then cryosections were treated with the preadsorbed antibodies, as shown for primary Ab + Ag. Cryosections were treated with the first antibody-free solution, and their immunoreactivity due to the second antibodies only was used as a negative control (N.C.). Scale bar: 50 μ m.



Supplementary Figure 2.

Antigen specificity of anti-NTPDase2 antibody in rat DRGs. The immunoreactivity of anti-NTPDase2 antibody was confirmed by Western blot analysis. Lysates from rat cerebral cortex and spinal cord were used as a control. GAPDH was the loading control.