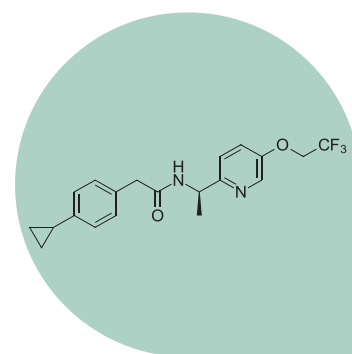


# TTA-P2 and TTA-A2: Specific and Potent $Ca_v3$ Channel Blockers

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Genes encoding T-type  $Ca_v$  channels yield three pore-forming subunits:  $Ca_v3.1$ ,  $Ca_v3.2$  and  $Ca_v3.3$  which give rise to currents that are pharmacologically and electrophysiologically different from the high voltage  $Ca_v1$  and  $Ca_v2$  channel currents. Due to their important physiological roles, and to the increasing number of channelopathies associated with T-type channels, the need of specific and potent pharmacology is rising. In this piece, we highlight the use of TTA-A2 and TTA-P2, two specific and potent T-type channel blockers exclusively available at Alomone Labs.

Right: TTA-A2 is a selective and potent T-type  $Ca_v$  channel blocker.



## Introduction

T-type  $Ca_v$  channels regulate neuronal excitability, hormone secretion, and neurotransmitter release. They also play important roles in the circadian cycle, cardiovascular and rennin-angiotensin systems.

Knockout of  $Ca_v3.1$ ,  $Ca_v3.2$  or  $Ca_v3.3$  channels in mice yields viable phenotypes, albeit each with various problems. Channelopathies associated with T-type channels include autism, epilepsy, hypertension, hyperaldosteronism, chronic pain, and neuropathic pain. Due to their important physiological roles, and to the increasing number of channelopathies associated with the channels, the need of specific and potent pharmacology is rising<sup>9</sup>.

## TTA-P2

TTA-P2, a derivative of 4-aminomethyl-4-fluoropiperidine, was first discovered to potently and reversibly block T-type currents from rat acutely dissociated DRGs, with an  $IC_{50}$  of 100 nM and stabilize T-type channels in their inactivated states. L-type  $Ca_v$  channels and  $Na_v$  channels are 100-1000 fold less sensitive to the compound's blocking actions<sup>1</sup>.

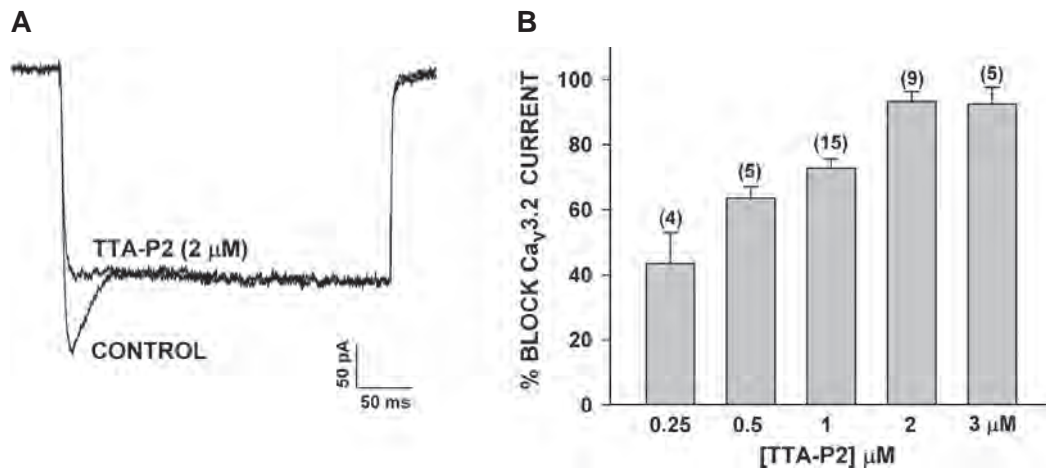
**TTA-P2** (#T-155) was used to gain more insight on the specific role and identity of  $Ca_v$  channels expressed in adrenal fasciculate cells. Indeed, TTA-P2 inhibited  $Ca_v3.2$  currents expressed in the cells (Figure 1) and

inhibited adrenocorticotrophic hormone (ACTH) and Angiotensin II-stimulated cortisol secretion<sup>2</sup>. Under hypoxia, catecholamine secretion by the adrenal gland increases in a BDNF/TrkB-dependent manner. TrkB receptor is highly expressed in the medulla as shown in immunohistochemical staining using Alomone Labs **Anti-TrkB (extracellular) Antibody** (#ANT-019), and its expression increased under hypoxic conditions. In addition to catecholamine secretion, activation of TrkB also lead to an increase in  $[Ca^{2+}]_i$  caused by T-type  $Ca_v$  currents which was inhibited by the application of TTA-P2, thus showing a linkage between BDNF/TrkB signaling and T-type currents<sup>7</sup>.

The transmission of pain begins at the spinal dorsal horn which then transmits the information onward. Rebound depolarization following hyperpolarization in dorsal horn neurons is an important feature in those cells and was characterized in part by the use of TTA-P2. Data show that rebound depolarization and firing by T-type  $Ca_v$  channels and their currents are important for integrating somatosensory information in the spinal cord<sup>6</sup>.

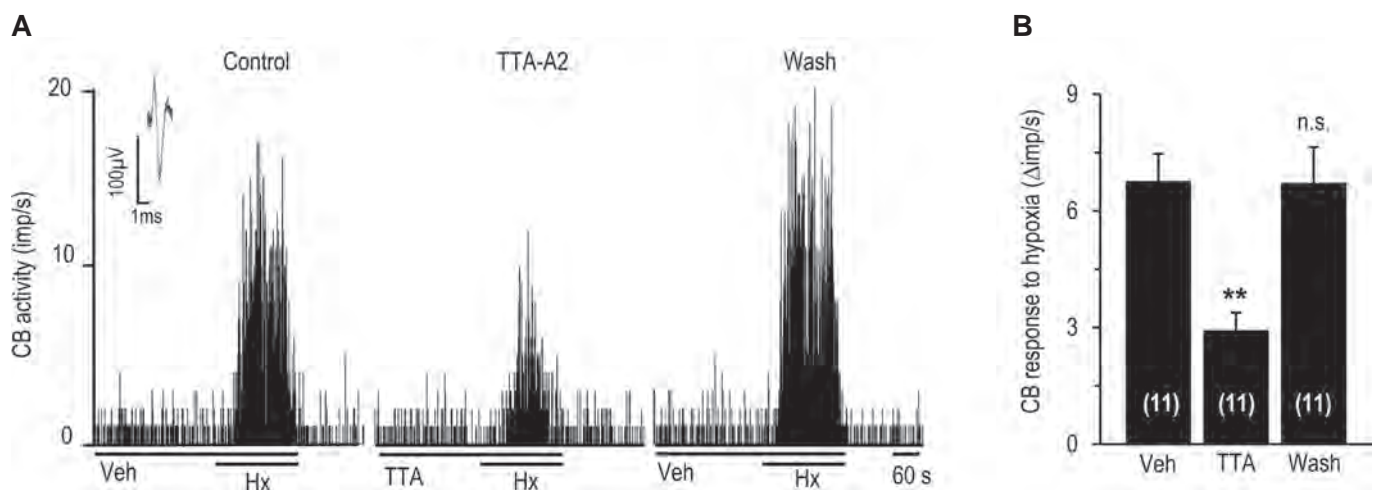
The thalamus plays an important role in integrating inputs from the cortex. Once the thalamus receives these inputs, it sends them back to the cortex creating a cortico-thalamo-cortical loop. These synapses and their contribution to higher brain functions were studied. Barrel cortex layer 5B neurons and those of the posteromedial nucleus (POM) were used to study the thalamo-cortical synapse formation. Using conditional knockout of GluA4, shRNA targeting  $Ca_v3.1$  channel and the T-type specific blocker TTA-P2, data show that GluA4 and  $Ca_v3.1$  control important aspects of the synaptic transmission at L5-POM synapse<sup>8</sup>.

Figure 1. T-Type  $\text{Ca}_v$  Channel Inhibition by TTA-P2 in AZF Cells



A. Bovine adrenal zona fasciculata (AZF) whole cell recordings.  $\text{Ca}^{2+}$  currents were recorded in 10 mM  $\text{Ba}^{2+}$  in response to voltage steps to -5 mV, applied from a holding potential of -80 mV, before and after superfusion of the cell with 2  $\mu\text{M}$  TTA-P2 (#T-155). B. Concentration-dependent inhibition of  $\text{Ca}_v3.2$  currents by TTA-P2. Values are means  $\pm$  SE for number of determinations shown in parentheses. Adapted from reference 2 with permission of the American Physiological Society.

Figure 2. T-Type  $\text{Ca}_v$  Channel Response to Hypoxia Is Inhibited by TTA-A2



A. Representative example of sensory nerve response (impulses (imp)/s) to hypoxia in the presence of vehicle or 25  $\mu\text{M}$  TTA-A2 (#T-140), and 5 min after washout. B. Effect of TTA-A2 on sensory nerve response to hypoxia. Adapted from reference 5 with permission of the American Physiological Society.

## TTA-A2

TTA-A2 was discovered in an effort to identify T-type specific and potent blockers. The compound was characterized on transfected HEK 293 cells and on native T-type currents. Data show that TTA-A2 has an  $IC_{50}$  value of 100 nM, and like TTA-P2, it binds preferentially to  $Ca_v3$  channels in their inactive state. TTA-A2 is 300-fold selective for  $Ca_v3$  channels. *in vivo*, TTA-A2 administration suppresses active wake and promotes slow-wave sleep in wild-type but not in mice lacking  $Ca_v3.1$  and  $Ca_v3.3^4$ .

Oxygen levels in the blood are detected by carotid bodies. Voltage-gated  $Ca^{2+}$  channels play an important role in detecting  $O_2$  levels.  $Ca_v3.2$  channel is the major T-type  $Ca_v$  channel expressed in glomus cells, the cells important for sensing  $O_2$ . Indeed, RT-PCR and immunohistochemistry using **Anti- $Ca_v3.2$  (CACNA1H) Antibody** (#ACC-025) shows that  $Ca_v3.2$  is highly expressed in the rat carotid body. Western blot analysis of rat DRG lysates using the same antibody shows that the  $Ca^{2+}$  channel is also expressed in DRGs. Importantly, use of the control peptide antigen completely obliterated the signal obtained with the antibody. In addition,  $Ca_v3.2$  was found to be involved in mediating the carotid body's response to hypoxia, an effect inhibited by the application of **TTA-A2** (#T-140) (Figure 2)<sup>5</sup>.

The contribution of T-type channels in myogenic reactivity of retinal arterial vessels was studied in arterioles of the rat retinal microcirculation. Immunohistochemical staining of rat retinal arterioles using **Anti-CACNA1G ( $Ca_v3.1$ ) Antibody** (#ACC-021) and Anti- $Ca_v3.2$  (CACNA1H) Antibody showed that while  $Ca_v3.2$  was not detected, strong  $Ca_v3.1$  staining was observed on the plasma membrane of retinal arteriole smooth muscle cells.  $Ca_v3.2$  was detected on glial cell end-feet surrounding the vessels. Application of TTA-A2 or **ML 218** (#M-165) dilated isolated, myogenically active retinal arterioles, demonstrating that  $Ca_v3.1$  channels are functionally expressed on arteriole smooth muscle cells or retinal arterioles and play an important role in myogenic signaling<sup>3</sup>.

### References

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## Related Products

Product name \_\_\_\_\_ Cat. # \_\_\_\_\_

### T-Type $Ca_v$ Channel Antibodies

|  |            |
|--|------------|
| Anti-CACNA1G ( $Ca_v3.1$ ) Antibody          | ACC-021    |
| Anti-CACNA1G ( $Ca_v3.1$ )-ATTO-594 Antibody | ACC-021-AR |
| Anti- $Ca_v3.2$ (CACNA1H) Antibody           | ACC-025    |
| Anti- $Ca_v3.3$ (CACNA1I) Antibody           | ACC-009    |

### T-Type $Ca_v$ Channel Blockers

|                                     |         |
|-------------------------------------|---------|
| Amlodipine                          | A-110   |
| Amlodipine besylate                 | A-120   |
| Benidipine hydrochloride            | B-120   |
| Flunarizine dihydrochloride         | F-110   |
| GTX1-15                             | STT-300 |
| L-Ascorbic acid                     | L-140   |
| Mibefradil dihydrochloride hydrate  | M-150   |
| ML 218                              | M-165   |
| ML 218 hydrochloride                | M-166   |
| Nicardipine HCl                     | N-125   |
| NNC 55-0396 dihydrochloride         | N-205   |
| NNC 55-0396 dihydrochloride hydrate | N-206   |
| Penfluridol                         | P-135   |
| ProTx-I                             | STP-400 |
| ProTx-II                            | STP-100 |
| Roscovitine                         | R-300   |
| SKF 96365 hydrochloride             | S-175   |
| TTA-A2                              | T-140   |
| TTA-P2                              | T-155   |
| TTA-P2 (S-enantiomer)               | T-210   |

### TrkB Antibody

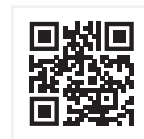
|                                    |         |
|------------------------------------|---------|
| Anti-TrkB (extracellular) Antibody | ANT-019 |
|------------------------------------|---------|

### Explorer Kits

|   |        |
|---|--------|
| Cardiac $Ca_v$ Channel Antibody Explorer Kit                  | AK-310 |
| Cardiac Channel Blocker Explorer Kit                          | EK-345 |
| $Ca_v$ Channel Antibodies for Pain Research Explorer Kit      | AK-360 |
| Classical Ion Channel Blockers for Pain Research Explorer Kit | EK-355 |
| Non-L-Type $Ca_v$ Channel Antibody Explorer Kit               | AK-216 |
| T-Type $Ca_v$ Channel Blocker Explorer Kit                    | EK-111 |

### Research Packs

|   |         |
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| T-Type $Ca_v$ Channel Basic Research Pack   | ESB-101 |
| T-Type $Ca_v$ Channel Premium Research Pack | ESP-101 |
| T-Type $Ca_v$ Channel Deluxe Research Pack  | ESD-101 |



$Ca_v3$  Channel product listing

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