Dynaflow® Application Note

Cell type:
Native rat
DRG neurons

Ion channel:
P2X

Chip type:
DF-Resolve

P2X currents in cultured primary neurons-characterization of fast-acting ion channels in DRGs

Introduction

P2X receptors belong to the purinergic receptor family and are membrane ion channels that open in response to the binding of extracellular ATP [1]. The receptors are characterized by the opening of a cation-conducting pathway within a few milliseconds of applying the ATP. Such responses have been described for a wide range of mammalian cells, including neurons, striated, smooth and cardiac muscles, epithelia, bone, and many different leukocytes [2].

We have developed a patch clamp-based assay for characterization of fast-acting ion channels in primary dorsal root ganglion (DRG) neurons. This assay is based on the use of primary DRG neurons in culture as a cell model for chronic pain. These neurons retain their sensory functionality and remain responsive to thermal, mechanical and functional stimuli, and, when supplemented with nerve growth factor (NGF), they can be used to mimic peripheral sensitization. The Dynaflow Resolve system was utilized to enable fast and pre-programmable drug application.

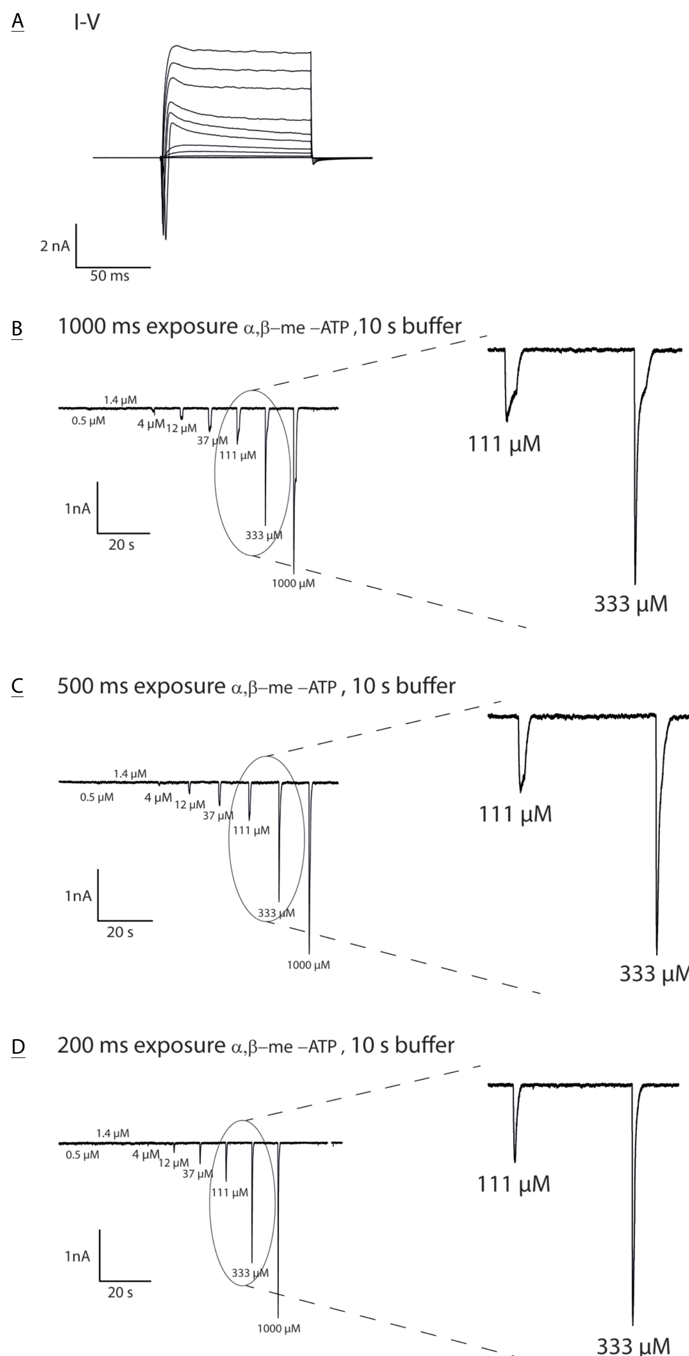
Methods

For the experiments , rat DRG neurons were dissociated with collagenase and the cells were cultured in laminin-coated plates for 24-48 h in media supplemented with NGF. The neurons were then detached by rinsing with HBS and added to the recording chamber of the Dynaflow Resolve chip. We obtained concentration-response curves by loading every second microchannel with different concentrations of the ATP-analogue α,β-methyleneATP, interdigitated with buffer solution for clearance after each agonist exposure.

Determining onset time for fast desensitization

The different members of the purinergic receptor family display vast differences in receptor kinetics. The agonist α,β-methyleneATP is strongly characterize the P2X currents we recorded several concentration-response curves from the same cell with different concentrations and application time of α,β-methyleneATP [Table 1]. Only cells having a resting membrane potential of over -40 mV and expressing sodium channels were chosen for the experiments. As shown in Figure 1A the patched cells expressed I-V curves typical for DRG neurons with distinct, fast activated sodium channels followed by slower activated potassium channels (each voltage step was +10 mV starting at -80mV to +60 mV).

Figure 1



Responses to various concentrations of the agonist α,β-methyleneATP. The agonist exposure time was varied while each agonist application was followed by 10 s of buffer.

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Determining onset time for fast desensitization (cont.)

Figure 1B-D show the responses to various concentrations (0,5-1000 μM) of α,β-methyleneATP. The agonist exposure time was varied while each agonist application was followed by 10 s of buffer wash. At 1000 and 500 ms exposure the onset of fast desensitization is clearly seen at higher concentrations of the agonist, but not for low concentrations. The current amplitude remains the same, also for 200 ms agonist exposure. However, the fast desensitization at higher concentrations of the agonist is eliminated and the onset time for fast desensitization was found to be on the time scale 200-300 ms.

This illustrates how the Dynaflow Resolve system's rapid solution exchange and stable recordings can be used to titrate, utilizing different exposure time, the onset time for fast desensitization.

Optimizing experimental

Figure 2 show the responses from one cell using two different buffer washout times. Exposure time to various concentrations (0,5-1000 μM) of the agonist α,β-methyleneATP was 500 ms, while the buffer was applied for 10 or 5 s. In this experiment the difference in wash-out time did not produce any significant change in the current amplitude of the response.

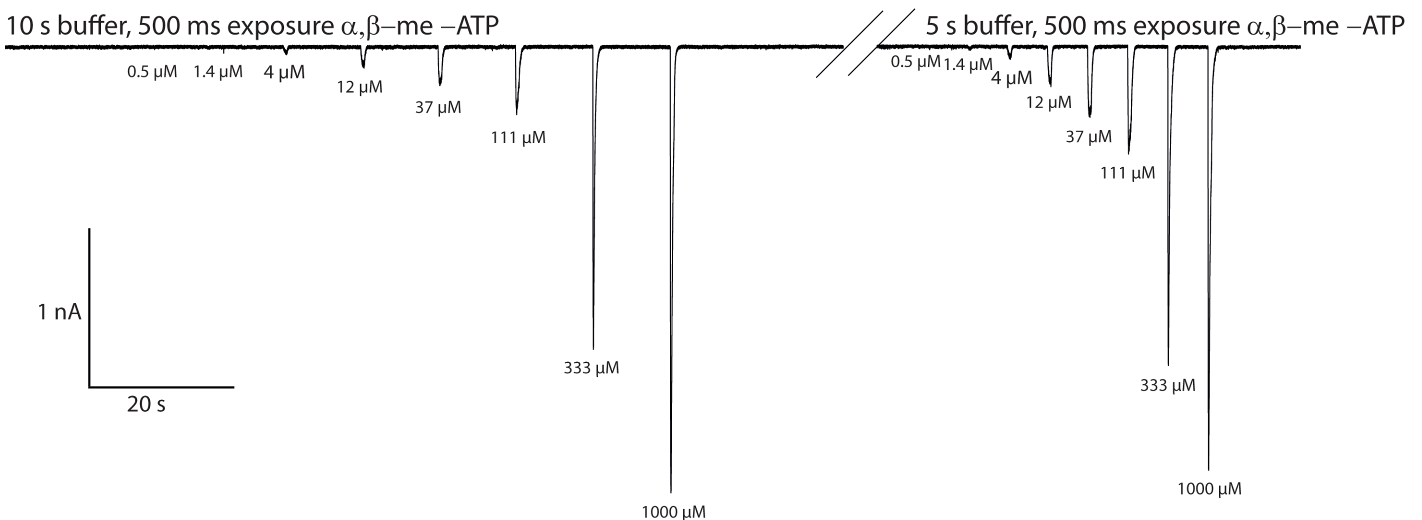
The experiment illustrates an easy way to optimize the experimental parameters, in this case the wash-out time between substance exposure, to minimize the time required for each step, thereby gaining time and increasing the likelyhood of generating more data points from each cell.

Table 1

Dynaflow Resolve chip loading pattern and exposure times for the experiment in Figure 1. Each well of the Resolve chip can be loaded with maximally 150 μl of compound, which is enough for 90 minutes recording time.

Chip channel	Solution	Application time
1.	buffer	10 s
2.	α,β-me-ATP	1000 / 500 / 200 ms
3.	buffer	10 s
4.	α,β-me-ATP	1000 / 500 / 200 ms
5.	buffer	10 s
6.	α,β-me-ATP	1000 / 500 / 200 ms
7.	buffer	10 s
8.	α,β-me-ATP	1000 / 500 / 200 ms
9.	buffer	10 s
10.	α,β-me-ATP	1000 / 500 / 200 ms
11.	buffer	10 s
12.	α,β-me-ATP	1000 / 500 / 200 ms
13.	buffer	10 s
14.	α,β-me-ATP	1000 / 500 / 200 ms
15.	buffer	10 s
16.	α,β-me-ATP	1000 / 500 / 200 ms

Figure 2



The figure shows the responses from one cell using two different washout times. Exposure time to various concentrations (0.5-1000 μM) of the agonist α,β-methyleneATP was 500 ms, while the buffer was applied for 10 or 5 s. The Dynaflow Resolve system is ideal for investigating ligand-gated ion channels since it allows for rapid and precise switching of solutions surrounding the patch-clamped cell.

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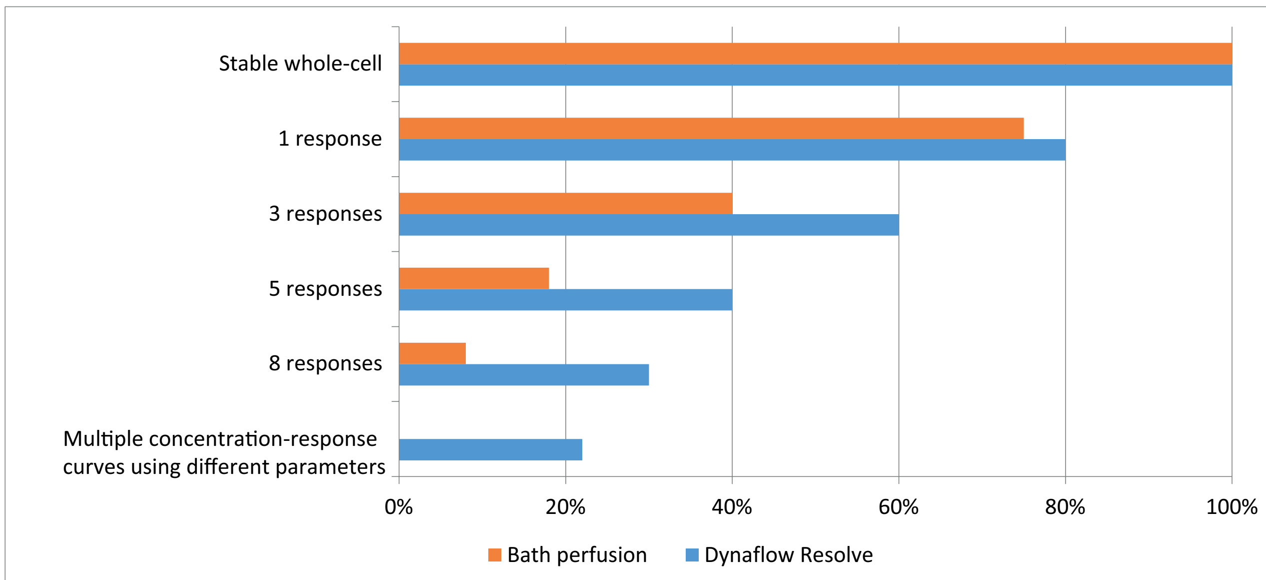
Figure 3



The diagram compares success rates for the Dynaflow Resolve system and a conventional bath perfusion system for whole-cell recordings from DRG neurons. [x-axis - percentage of succesful recordings]

Enabling complex protocols

Figure 3 shows a comparison between the Dynaflow Resolve system and a conventional bath perfusion system with approximately 120 whole cells in each group. We found that the success rates for conventional bath exchange and the Dynaflow Resolve system were comparable for one drug application per patched cell. (Note: when using the Dynaflow Resolve system, the cells must be lifted from the bottom of the Resolve chip using the patch pipette. Initially, the user may require some training in performing this specific procedure.) When the complexity of the perfusion protocol increased beyond one compound application per patched cell, the success rate for the Dynaflow Resolve system superseded the conventional system. Finally, when running a full concentration-response recording from the same cell (more than 8 applications) Dynaflow was far superior.

Conclusion

Manual patch clamp recordings offer direct insight into ion channel properties through the characterization of ion channel activity. Due to the high quality data it is considered the gold standard for ion channel research. Still, the limited throughput means that maximizing data from each cell, especially sensitive primary cells, is important.

As shown in figure 3, conventional patch clamp and the automated Dynaflow Resolve system have similar capacity when it comes to obtaining successful high resistance seals. However, thanks to the extremely rapid and accurate fluid control with the Dynaflow system we have the possibility to maximize the data extracted from each cell. This leads to a dramatically increased productivity when performing more complex pharmacological characterizations, as for example a full concentration-response curve of an antagonist before and after adding an inflammatory mediator to the same neuron.

Other application areas for the Dynaflow platform are when investigating desensitization, receptor kinectics, on- and off-rates etc., since the Dynaflow is easily programmable to perform multiple wash steps with different timing.

References

1. North RA. Molecular physiology of P2X receptors. Physiol Rev. 2002 82(4):1013-67.
2. North RA, Surprenant A. Pharmacology of cloned P2X receptors. Annu Rev Pharmacol Toxicol. 2000 40:563-80.