Case study

Developing new therapies targeting P2X receptors

Tools:
Port-a-Patch

Prof. Samuel Fountain
featured by Nanion Technologies

Samuel Fountain is chair of Pharmacology and Associate Pro-Vice-Chancellor at the University of East Anglia. His research focuses on the function and pharmacology of P2X receptors, a family of ligand-gated cation channels activated by extracellular ATP. Known for his contribution to the pre-clinical development of Gefapixant, a $P2X_3$ receptor antagonist, recently approved as a new medicine.

ATP is widely recognized as the "universal energy currency" of cells, providing a readily accessible source of energy for all cellular processes. However, ATP is not only present inside cells. Many cell types release ATP into the extracellular space. Although the mechanisms and physiological reasons for such release are sometimes not very well understood, it is known that, for example, under inflammatory conditions, ATP can be released both passively from cells undergoing lysis and actively via vesicular and channel-mediated processes. ATP is also known to be released alongside a range of other neuromediators, such as acetylcholine, norepinephrine, glutamate, GABA, neuropeptide Y, and thus, ATP is currently recognized as an important autocrine and paracrine transmitter.

Extracellular ATP exerts its signaling function via specialized cell surface receptors, called P2 purinoceptors (for reference, P1 receptors are metabotropic adenosine receptors). These P2 receptors are of two types – metabotropic P2Y receptors and ionotropic P2X receptors. P2Y receptors typically mediate slow responses to ATP or other nucleotides, whereas P2X receptors mediate fast responses solely to ATP.

P2X receptors are ligand-gated non-selective cation channels (permeable to Na^+, K^+, and Ca^{2+}) that open within milliseconds of ATP binding. They have a widespread tissue distribution and play roles in a variety of essential cellular functions. Numerous studies have shown the involvement of different members of the P2X receptor family in pain, irritation, hypersensitivity, and inflammation, and thus, they are now regarded as promising drug targets.

The human genome encodes seven P2X receptor subtypes ($P2X_1$ – $P2X_7$) capable of forming both homomeric and heteromeric trimers. Native P2X receptors are characterized by significant heterogeneity in functional properties. In most cell types, under standard physiological conditions, P2X receptors carry a depolarizing cationic current, although the kinetics of this current, as well as the involvement of different cations, can vary greatly depending on the cell type. In some cells however, P2X channels are also significantly permeable to anions.

Nanion's Port-a-Patch platform
is a turn-key miniaturized patch clamp system enabling the user to rapidly generate high quality data, regardless of experience. Behind its small and compact appearance lies sophisticated technology, producing high-quality measurements with giga-seals and high success rates.

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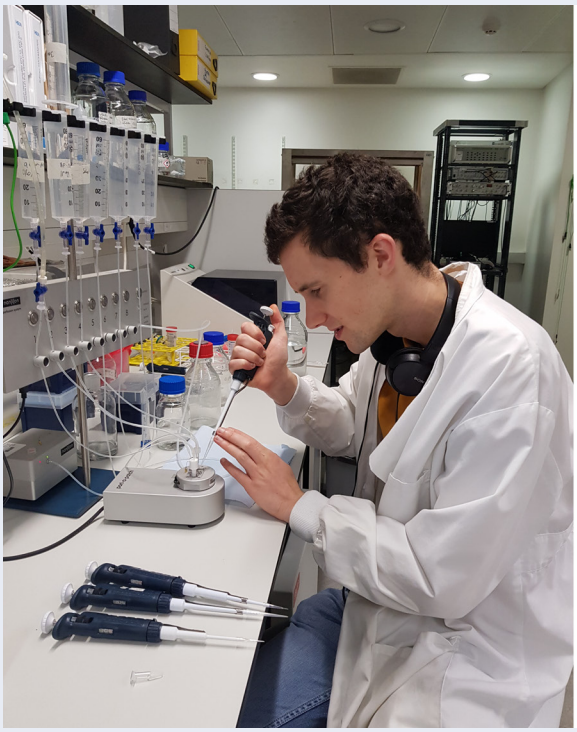
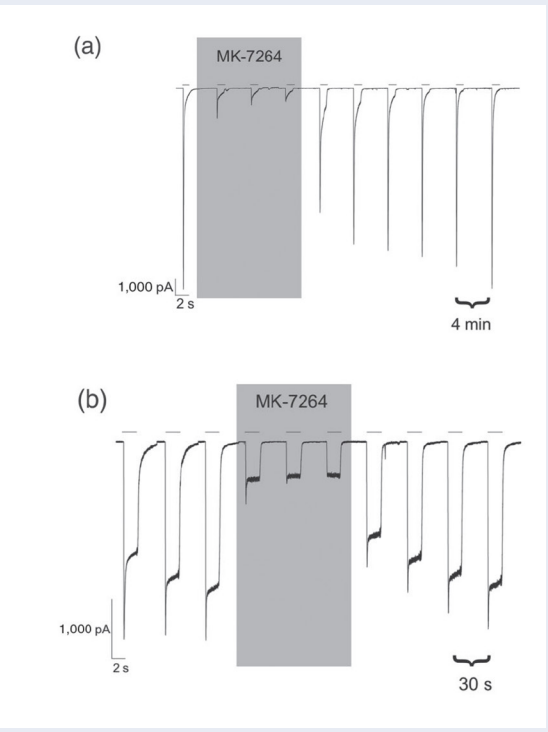
Prof. Samuel Fontain, University of East Anglia

Typically, the biophysical properties of P2X receptors, like other ion channels, are studied using the patch clamp technique. This powerful Nobel Prize-winning technique allows for the measurement of ion channel currents. However, despite its advantages, the patch clamp technique is often viewed as a complex, time-consuming, labor-intensive, and low-throughput approach. It demands significant training and technical expertise and involves the use of large, complex, and expensive equipment. Nevertheless, despite these limitations, manual patch clamp has remained the gold standard for studying ion channels, with little alternative to gather the same information-rich data content.

Thankfully, over the last two decades, several companies have developed a technique to automate patch clamp. Recent advancements in automated patch clamp (APC) instrumentation have mitigated many of the limitations associated with the manual patch clamp technique. Modern APC systems can measure currents from 1 to 384 cells simultaneously. These systems are designed for ease-of-use and frequently offer automated data analysis, saving researchers a significant amount of time.

Professor Samuel Fountain is one of those visionary group leaders who employs the latest APC technologies in his research, which focuses on the function and pharmacology of P2X receptors. He has equipped his lab with the Port-a-Patch system, a compact and user-friendly semi-automated patch clamp setup from Nanion. The Port-a-Patch enables users to rapidly generate high quality data, regardless of experience. This makes it ideal as an easy-to-use tool for learning patch clamp electrophysiology and it can be implemented in many aspects of drug discovery and basic ion channel research.





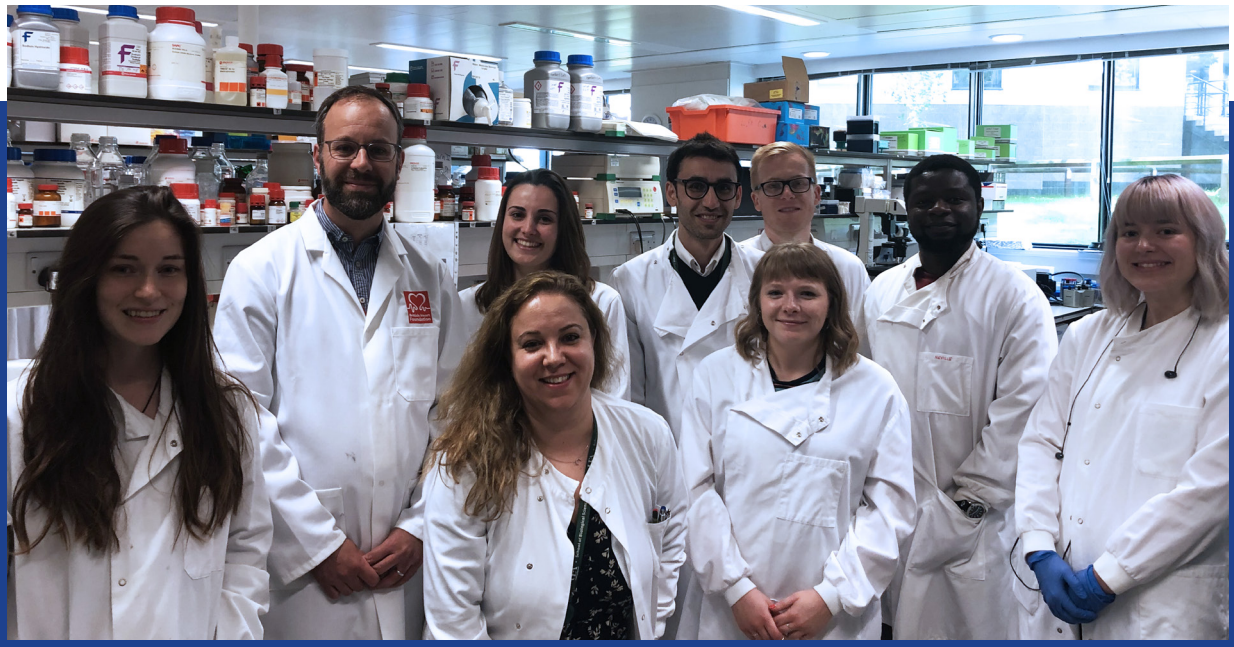
David Richards performs patch clamp experiments using Port-a-Patch. *The adapted figure shows MK-7264 activity with representative whole cell patch clamp recordings for human $P2X_3$ (a), $P2X_{2/3}$ (b) currents in 1312N1 stably transfected cells. Fully recovered $P2X_3$ currents were evoked by 10 µM α,β-meATP applied for 1 s every 4 min and 10 µM α,β-meATP applied for 2 s at 30 s intervals for $P2X_{2/3}$ currents.*[1]

Since 2016, Prof. Fountain and colleagues have utilized the Port-a-Patch to study various P2X receptors such as $P2X_2$, $P2X_3$, $P2X_{2/3}$, and $P2X_4$. Speaking about the Port-a-Patch, he states, "The Port-a-Patch has been a highly reliable instrument for us. We have used it to record from cell lines, but also primary cells from human tissue. The instrument is used by those in my group whose main focus is electrophysiology, but also those who need to undertake shorter-term electrophysiological studies. The latter is enabled by the instrument's accessibility and limited training requirement compared to manual patch-clamp."

In a recent project funded by Afferent Pharmaceuticals and Merck Sharp & Dohme Corp, the team made significant contributions to the development of Gefapixant, an oral $P2X_3$ receptor antagonist currently approved in Japan and Switzerland for treatment of adults with refractory or unexplained chronic cough. Utilizing the Port-a-Patch, they examined Gefapixant's action, potency, and kinetics, discovering its nanomolar potency for $P2X_3$ and $P2X_{2/3}$ receptors, with marginal selectivity for $P2X_3$ over recombinant $P2X_{2/3}$ receptors[1].

In two additional recent studies, Prof. Fountain and his colleagues employed the Port-a-Patch to examine the mechanism of action of various compounds on $P2X_4$ receptors, which are known to be implicated in neuropathic pain, inflammation, and arterial tone regulation. One study identified taspine as a unique natural product that inhibits $P2X_4$ activity through phosphoinositide 3-kinase suppression rather than receptor antagonism[2]. In another study, they delved deeply into the mechanism by which the small molecule 5-BDBD inhibits $P2X_4$[3]. By combining Port-a-Patch-based electrophysiology with molecular modelling and other methods, they pinpointed the essential residues for 5-BDBD binding. Great studies… and beautiful currents recorded with the Port-a-Patch.

The Port-a-Patch is easy to use and the solutions can be exchanged manually, reducing the amount of compound needed for the experiment. Alternatively, for fast and accurate exchange of solutions, the temperature-controlled External Perfusion System or the Internal Perfusion System, can be used, key add-ons for Prof. Fountain's experiments.



Prof. Samuel Fountain and his group at University of East Anglia. *Their main research interest is the role ion channels play in the cellular and molecular mechanisms that underlie cardiometabolic physiology and disease with an emphasis of blood vessels, adipose tissue, and the autonomic and sensory nerves that control these tissues. For more information, visit https://research-portal.uea.ac.uk/en/persons/samuel-fountain/projects/.*

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"The rapid perfusion system has been key for us studying the mode of action of drug-like molecules at ligand-gated ion channels. The internal perfusion system also allows for rapid exchange of intracellular components for the studies of regulator and signalling pathways. Working with small volumes is also useful when working small scales of experimental compounds, natural products, animal toxins and antibodies", says Samuel Fountain.

Samuel Fountain's lab is currently using the Port-a-Patch to explore the heteromerization of P2X receptors and to characterize the action of some new drug-like molecules targeting $P2X_4$. We're looking forward to exciting new results.

References

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2. Nadzirin IB, Fortuny-Gomez A, Ngum N, Richards D, Ali S, Searcey M, Fountain SJ. Taspine is a natural product that suppresses P2X4 receptor activity via phosphoinositide 3-kinase inhibition. Br J Pharmacol. 2021 Dec;178(24):4859-4872.
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