

VIEWPOINT

A new role for P2 receptors: talking with calcium-activated potassium channels

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Abstract Purinergic fast synaptic transmission may play a very subtle role in regulating the excitability of enteric circuits. That is one of the important findings in a new paper by Ren and Galligan in the current issue of this Journal. They first provide compelling evidence that P2X₃ receptors (ionotropic purine receptors) are expressed by guinea-pig motor and interneurons and that these subtypes mediate the purinergic fast excitatory postsynaptic potential (EPSP). They also found that the P2X₃-mediated depolarization was often followed by a hyperpolarization. This is an intriguing finding because if the purinergic fast EPSPs are also followed by a hyperpolarization, then it could play a role in truncating bursts of synaptic potentials or in shaping periodic synaptic input. The hyperpolarization is caused by calcium entry through the P2X₃ receptor which then activates a calcium-activated potassium (K_{Ca}) channel. Surprisingly, the hyperpolarization was not affected by any of the standard blockers of calcium- or voltage-activated K⁺ channels suggesting that a novel K_{Ca} channel is present in the enteric neurons. Such a wide-spread channel could well have an important physiological role and could be an important new drug target for regulating reflex activity in the enteric nervous system.

Keywords calcium-activated potassium channels, purines, synaptic transmission.

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Abbreviations 5-HT, 5-hydroxytryptamine (serotonin); ACh, acetylcholine; ATP, adenosine triphosphate; ENS, enteric nervous system; EPSP, excitatory postsynaptic potential; K_{Ca} channel, a potassium channel that is opened by an increased concentration of intracellular calcium ions; P2X, ionotropic purine receptor with a higher affinity for ATP than for adenosine.

INTRODUCTION

Ren and Galligan in the current issue of this Journal provide compelling evidence that P2X₃ (ionotropic purine receptor with a higher affinity for ATP than for adenosine) subunit containing receptors are present on the motor neurons and interneurons of the guinea-pig myenteric plexus and that these receptors are responsible for the purinergic fast excitatory postsynaptic potential (EPSP). They also show that the P2X₃-mediated depolarization was often followed by a hyperpolarization, the result of calcium entering through the P2X₃ receptor and then activating a calcium-activated potassium (K_{Ca}) channel. Surprisingly, the hyperpolarization is not affected by any of the standard blockers of calcium- or voltage-activated K⁺ channels.

SYNAPTIC TRANSMISSION IN THE ENTERIC NERVOUS SYSTEM

The enteric nervous system (ENS) circuitry functions using both fast synaptic transmission (i.e. fast EPSPs) and slow synaptic transmission (i.e. slow EPSPs).^{1,2} That each of these is critical for the flow of information at specific classes of functional synapse has been confirmed by a variety of groups using organ bath pharmacological and electrophysiological techniques.

Slow synaptic transmission in the ENS has been studied for many years and has always had three strong

candidates vying for the title as most important transmitter – acetylcholine (ACh) acting at a muscarinic receptor, substance P at neurokinin receptors and 5-HT (5-hydroxytryptamine) at G protein coupled serotonergic receptors.³ This list has recently been expanded with the discovery that purines also participate in slow synaptic transmission as distinct slow EPSPs that mediate some descending reflexes.^{4–6}

In a similar manner, fast synaptic transmission in the ENS was, for many years, thought to be mediated by ACh acting at nicotinic receptors. Work by Galligan *et al.*⁷ has helped to show that enteric neurons utilize nicotinic receptors made up of α_3 , α_5 and β_2 subunits (all shown immunohistochemically) and of β_4 subunits (shown pharmacologically). Support for the idea that all fast EPSPs were nicotinic stemmed from the fact that many, if not all, of the complex reflexes in the ENS can be disrupted by the addition of a nicotinic receptor blocker. For example, the productive movement of fluid from one end of a segment of intestine to the other (a favourite experimental model for peristalsis) is rapidly disrupted by the nicotinic receptor antagonist hexamethonium. However, if only one functional type of synapse depends on nicotinic receptors, then blocking these receptors could well disrupt the reflex while not blocking transmission at the majority of the synapses. Thus, it is now generally accepted that the complex motor patterns seen in the ENS are composed of the coordinated activity of many neurons communicating at many different types of functional synapses.

Viewing reflexes as fundamentally complex has, in the past 15 years, supported a renewed interest in non-nicotinic fast synaptic transmission. Thus, it was that shortly after the discovery that purines were responsible for some fast EPSPs in the central nervous system,⁸ it was found that some fast EPSPs in the ENS were also mediated by P2X receptors.⁹ Subsequent work from a variety of groups has shown that purinergic fast EPSPs are common, and that they are indeed critical for some reflex pathways.⁴ ACh and adenosine triphosphate (ATP) do not act entirely alone. 5-HT acting at 5-HT₃ receptors accounts for some fast synaptic transmission, and there have been hints that other transmitters such as glutamate, γ -aminobutyric acid or glycine may also contribute.¹⁰

MOLECULAR COMPOSITION OF THE P2X RECEPTORS

Once the idea was established that P2X receptors were involved in synaptic transmission, some researchers began to investigate their molecular composition. Work by North and others has shown that there are

seven different types of subunit that make up a P2X receptor in mammalian systems.¹¹ Not all of these subunits mix with the others, and many have quite distinct properties. The present challenge has been to determine which subunits make up the native receptors. There has been great success in showing that P2X_{2/3} heteromeric receptors are the native receptors in dorsal root ganglion.¹² Using some of the same tools, previous work by Ren, Galligan and colleagues was able to show that in mouse myenteric plexus fast synaptic transmission is via P2X₂ receptors.¹³

Surprisingly then, Ren and Galligan in this issue of the Journal report that P2X₃ subunit containing receptors are the primary receptor through which fast synaptic transmission occurs in the guinea-pig myenteric plexus. Ren and Galligan did not utilize KO animals; the present study was primarily a pharmacological one with some immunohistochemical data providing support. Receptor agonists and antagonists were used to pinpoint which one (or more) of the seven P2X subunits was responsible for the fast EPSPs seen in the native tissue. When they used α,β -methylene ATP, an agonist at P2X₁, P2X₃ and P2X_{2/3} receptors, they found that it was as effective as ATP in evoking a depolarization. They used the selective receptor antagonist TNP-ATP (effective at these same receptors) and found that it blocked α,β -methylene ATP evoked depolarizations and some native fast EPSPs. Alone, these data do not discriminate between P2X₁, P2X₃ or P2X_{2/3} receptors. However, over half of the myenteric neurons stained positively for nitric oxide synthase. Previous immunohistochemical studies have shown that these neurons are predominately inhibitory motor neurons that contain the P2X₃ subunit but not the P2X₁ subunit. Thus, P2X₃ or P2X_{2/3} subunit containing receptors are those most probably mediating the fast EPSP in guinea-pig myenteric inhibitory motor neurons.

On one hand, these results suggest that the differences in the properties of P2X₂ and P2X₃ receptors are not important for the functioning of enteric circuitry. After all, guinea pig and mouse appear to get along just fine using either receptor. On the other hand, these results serve to focus attention on P2X₂ and P2X₃ subunit containing receptors as the main subtypes responsible for fast EPSPs in the native enteric circuitry.

THE P2X₃ MEDIATED DEPOLARIZATION CAN BE FOLLOWED BY A HYPERPOLARIZATION

A second important observation by Ren and Galligan is that the P2X₃-mediated depolarization evoked by

exogenous P2 agonists can be followed by a hyperpolarization. Until now, activation of P2X receptors was thought to be a simple excitatory event. They showed that the hyperpolarization reversed at the potassium equilibrium and was sensitive to changes in extracellular calcium, thus implicating a K_{Ca} channel. Voltage clamp experiments suggested that calcium entered the neuron through the P2X₃ receptor and then activated the K_{Ca} channel. Opening of the K_{Ca} channel allows the positively charged potassium ions to exit the cell causing a hyperpolarization and a reduced neuronal excitability. Up to half of the motor neurons and interneurons found in the myenteric plexus of the guinea pig showed such a hyperpolarization.

A big question left unanswered is whether purinergic fast EPSPs also show the pronounced hyperpolarization seen with exogenous application of agonist. Neither Ren and Galligan nor the previous studies observed such a hyperpolarization. On the other hand, a study by Tokimasa, Cherubini and North in the early 1980s showed that after exogenous application of ACh, or following some fast EPSPs, there was a hyperpolarization that was due to a K_{Ca} channel.¹⁴ They did not go on to identify the type of neuron involved, although they were all S neurons electrophysiologically. This suggests that while hyperpolarizations following fast EPSPs are not common, they can occur under some conditions. One idea is that the P2X receptors at the synapse are not located as closely to the K_{Ca} channels as are those P2X receptors located on the cell body. Under normal conditions, synaptic transmission would only activate synaptically located receptors while those receptors on the cell body would be activated by exogenous application of an agonist. A conceptually similar relationship between junctional and non-junctional muscarinic receptors at intestinal smooth muscle cells has been noted by Cousins *et al.*¹⁵ Extra-synaptic P2X receptors could well become a target for synaptically released purines under abnormal conditions. For example, there is an increased release of purines during fast synaptic transmission in submucosal and myenteric neurons from inflamed tissue.^{16,17}

It is worth considering how regulation of postsynaptic excitability by the fast EPSP driven hyperpolarization might occur. Clues come from computer modelling studies that have looked at many enteric neurons and at their synaptic interactions. We can suppose that a larger amount of calcium entering the cell will activate a larger K_{Ca} current and, thus, a larger reduction in the excitability of the membrane. A train of fast EPSPs would be expected to produce a larger hyperpolarization than would a single fast EPSP. Similarly, a higher frequency train would produce a

larger hyperpolarization than a lower frequency train. This relationship is a kind of filtering, one outcome of which is that a high frequency train of fast EPSPs will be progressively shortened as the train passes from interneuron to interneuron through multiple synapses. This would cause a focusing of the train into a distinct burst; a process that is similar to the progressive synchronization seen between enteric neurons in large scale computer models. Thus, the hyperpolarization following the fast EPSP would contribute to the coordinated firing seen in large networks of enteric neurons.¹⁸ From a network standpoint, the hyperpolarization following the fast EPSP could play the same role as inhibitory cotransmission.¹⁹ It has always been a mystery why there are not more inhibitory postsynaptic potentials recorded from neurons within the myenteric circuitry. Submucosal neurons generate robust inhibitory postsynaptic potentials from sympathetic and submucosal sources. It may be that this missing feature of the myenteric circuitry is actually embodied in the subtle shaping of periodic synaptic input or truncation of bursts of fast EPSPs by a K_{Ca} -mediated hyperpolarization.

A NOVEL K_{Ca} IN MYENTERIC MOTOR NEURONS

A final observation made by Ren and Galligan was that the K_{Ca} channel responsible for the hyperpolarization was not affected either by blockers for any of the three major groups of K_{Ca} channel (small, large and intermediate conductance channels) or by blockers of voltage sensitive K^+ channels. The simplest interpretation is that there is a novel K_{Ca} channel in the guinea-pig myenteric motor neurons and interneurons. Whether this K_{Ca} channel is truly different will have to await more detailed pharmacological studies at the channel level or identification of the gene product involved.

Recently K_{Ca} channels in the enteric AH/sensory neurons have received some attention. These neurons have a pronounced after-hyperpolarizing potential following the action potential (the AHP) that is due to the opening of an intermediate conductance K_{Ca} (IK) channel.²⁰ Conversely, closure of IK is thought to mediate the depolarization seen during slow EPSP-like events. For the AH/sensory neurons, these IK channels can increase or decrease neuronal excitability. That a novel kind of K_{Ca} channel is found in the myenteric motor neurons and interneurons suggests that these channels might also have control over neuronal excitability. Such a channel could become an important target for regulating the motor activity in the myenteric plexus.

CONCLUSION

The present work of Ren and Galligan provides several important observations about how fast synaptic transmission operates within the enteric circuitry. A main finding was that P2X₃ subunit containing receptors are responsible for the fast EPSP in many guinea-pig myenteric neurons. Equally important was that many fast EPSPs were followed by a hyperpolarization mediated by a novel K_{Ca} channel. The identification of such a wide-spread channel could become the basis for new drug target for gastrointestinal therapeutics.

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