

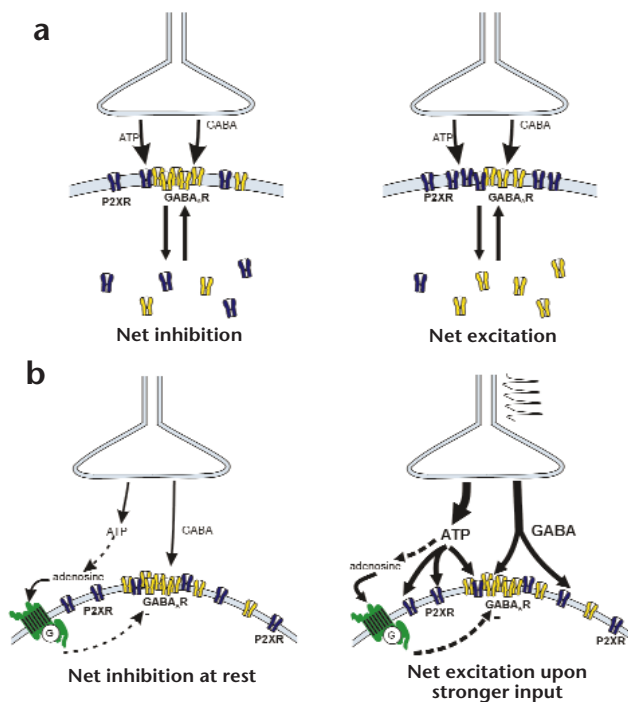
# An ambiguous fast synapse: a new twist in the tale of two transmitters

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**Jo and Schlichter describe GABA and ATP corelease from the same presynaptic cell, which suggests that a dorsal horn synapse could be excitatory or inhibitory depending on its postsynaptic receptors or the amount of transmitter released.**

Science, at least the kind of science that makes us sit up and take notice, is revolution—controlled and hopefully nonviolent, but revolution nonetheless. For example, it was not until just after the midpoint of this century that the idea of chemical transmission, in a coup d'état, usurped the main competing idea of electrical transmission as the dominant form of neuron–neuron communication in the CNS<sup>1</sup>. With this insurrection came the concept, termed Dale's principle, that each neuron releases the same transmitter from all of its terminals. Sir Henry Dale never explicitly discussed the possibility of a neuron releasing more than one transmitter<sup>2–5</sup>, and debate has raged about 'what is' versus 'what should have been' Dale's principle. In any event, the concept of 'one neuron, one transmitter' became entrenched only long enough for several transmitters to be identified before it was found that neurons may release more than one transmitter<sup>3</sup>. Semantic clashes then erupted as to what was a 'transmitter', 'modulator' or 'mediator' as it became apparent that, as an endpoint, synaptic transmission may have diverse time courses, mediation and modulation.

Through an enormous amount of work on cotransmission (see ref. 6), two



**Fig. 1.** Possible mechanisms to explain how the balance of P2X-receptor-mediated excitation versus GABA<sub>A</sub>-receptor-mediated inhibition could be altered to set the net 'sign' of this ambiguous synapse, which releases both ATP and GABA as fast neurotransmitters. **(a)** The postsynaptic cell may control what it hears by altering receptor expression at the synapse. **(b)** Alternatively, as transmitter release increases, more ATP is able to reach its distant receptor before being intercepted by extracellular nucleotidase. Thus the balance may shift to convert an inhibitory synapse to an excitatory one.

themes emerged. First, there are differences in the time domain of responses, such that a given neuron may release one fast transmitter and then one or more slower transmitter(s); second, different firing patterns may cause differential release, particularly of the slowly acting transmitters like peptides. As the peptide phenotype of a neuron is highly modifiable, the idea 'one neuron, one transmitter' became in essence 'one neuron, one fast transmitter'—until the finding that two fast transmitters, GABA and glycine, were coreleased<sup>7</sup>. In that case, however, both transmitters are inhibitory, and thus one could preserve the concept of 'one synapse, one fast synaptic action'. Now even this concept comes under attack in a compelling series of experiments reported by Jo and Schlichter on page 241 of this issue.

Fundamental to the common under-

standing of fast synaptic transmission in the CNS is the concept that a given synapse is either excitatory or inhibitory. So firmly entrenched is the idea that the sign of a synapse—excitation or inhibition—is a basic property that most of us never even give it a second thought. This concept is consistent with the apparently sensible and world-simplifying notion that a synapse should transduce information unambiguously. In cases where two opposing transmitters are released, the two components of the response are separated temporally. So a synapse where there is corelease of fast transmitters with opposing actions of similar kinetics would seem illogical.

The results of Jo and Schlichter, however, are quite clear. Using cultures of superficial spinal cord dorsal horn, they stimulated individual neurons and

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recorded postsynaptic currents (PSCs) from neighboring cells with monosynaptic connections. When GABA, glycine and glutamate receptors were blocked pharmacologically, nearly half of the presynaptic neurons released ATP, an excitatory transmitter, as inferred from PSCs mediated by ionotropic P2X receptors in the postsynaptic cells. Washing out CNQX and AP-5, drugs that block ionotropic glutamate receptors, did not reveal an additional component of the synaptic current. Surprisingly, though, when bicuculline, a GABA<sub>A</sub> receptor antagonist, was washed out, all of the neurons releasing ATP also elicited GABA<sub>A</sub>-receptor-mediated PSCs, and importantly the latency and stimulation thresholds for the GABA-mediated responses were the same as those for the ATP-mediated responses. Thus, the logical conclusion is that ATP and GABA are released from the same presynaptic neuron and possibly at the same synapses.

As it happens, ATP was one of the first culprits implicated as a cotransmitter<sup>3</sup>. In the peripheral nervous system, ATP is released together with classical transmitters like noradrenaline or acetylcholine. In these cases, however, ATP acts in the same direction as the other transmitter. In the dorsal horn, however, P2X and GABA<sub>A</sub> receptor PSCs have opposite polarities. The kinetics of the PSCs are virtually identical and therefore tend to cancel each other at the resting membrane potential.

What could be the point of generating two such similar yet opposing signals? There are many potential implications, and we will limit our discussion to some possible consequences for this apparently heretical synapse. Jo and Schlichter show that ATP and GABA are coreleased from the same cell, and for simplicity our speculative models (Fig. 1) show ATP and GABA coreleased from the same presynaptic terminal. Although this could not be determined directly, the functional implications would be much the same if ATP and GABA are released from separate terminals on the same postsynaptic cell. Indeed, in such a case, the results would be even more provocative and would drive the final nail into the cherished concept that the transmitter repertoire is the same at all of a cell's synapses.

Given the corelease of ATP and GABA, the sign of the synapse may be ambiguous. This arrangement provides

the opportunity to switch between excitation and inhibition simply by changing one of the dominant parameters, either the balance of the responsiveness of the postsynaptic receptors or how much transmitter is released. In other words, this synaptic arrangement depends not only on how loudly the presynaptic cell speaks but also on what the postsynaptic cell wants to hear.

One plausible model for switching sign is that the postsynaptic cell controls what it hears by altering the cohort of receptors expressed at the synapse (Fig. 1a). This could occur, for example, via rapid translocation of receptors to the membrane<sup>8</sup> and/or their clustering and declustering at synapses, or by post-translational modifications of the receptors<sup>9</sup> that would render cell-surface receptors either more or less responsive to released transmitter.

Another way of changing the sign of this synapse stems from the peculiar behavior of synapses that use ATP, due to its rapid extracellular degradation to adenosine<sup>10</sup>. A change in sign is conceivable based on the amount of ATP being released under given conditions. Interestingly, in the dorsal horn neurons, P2X-receptor-mediated miniature excitatory PSCs (mEPSCs), attributed to spontaneous release of single vesicles, were not observed, whereas action potentials in the presynaptic neurons did elicit P2X-mediated EPSCs. The lack of P2X mEPSCs could be explained if the amount of ATP released by a single vesicle was sufficiently small that most of it was intercepted by degradation enzymes before reaching the receptors. This would be especially plausible if P2X receptors are located at a distance from the release site. On the other hand, raising the amount of ATP released (for example through synchronous release of multiple vesicles, which could be evoked by action potentials) may allow ATP to escape and reach the receptors. From that, we envisage that increasing the frequency of action potentials may allow progressively more ATP to escape, and eventually the balance may shift, effectively converting an inhibitory synapse to an excitatory one (Fig. 1b).

Another interesting observation was that GABA<sub>A</sub> receptors are inhibited postsynaptically by adenosine generated from ATP, whereas P2X receptors were unaffected. Thus, the balance of sensitivity of the postsynaptic cell to ATP or to GABA could be set by adenosine, presumably acting through

metabotropic adenosine receptors. Thus, increasing firing frequency could increase extracellular generation of adenosine, which could feedforward to accentuate switching from excitation to inhibition. This seemingly paradoxical effect of adenosine might be further complicated if adenosine were to activate postsynaptic potassium channels<sup>11</sup>. Thus, the sign of ATP-GABA synapses may have a complex relationship to the level of activity, but this remains to be shown experimentally.

Could there be other functions of ATP and GABA corelease? One possibility is that P2X receptor activation may provide a local means to regulate GABA<sub>A</sub> receptors, for example by allowing influx of calcium, which has been shown to modulate the function of GABA<sub>A</sub> receptors<sup>12</sup>. Alternatively, ATP might act on other P2 receptor subtypes<sup>14</sup> or even modulate GABA<sub>A</sub> receptors directly.

In summary, the past year has seen two striking examples of the corelease of fast synaptic transmitters at central synapses. These will undoubtedly provoke further exciting investigations and perhaps a renewed revolution in some basic views on synaptic transmission. What would Sir Henry Dale have thought of all of this? We guess that, like us, he would remain astonished by the richness, subtlety and diversity of communication between neurons.

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