Choline transport: A Research Achievement in My Early Career. (February 2024)

This is a personal recollection about a research project early in my career when I was young (early 30s) and relatively inexperienced; that is one of the reasons why I remember this successful project so well. It felt like a step forward in my career as a researcher.

An important neurotransmitter chemical in the body is acetylcholine (ACh). Neurotransmitters are chemical signals that are released from nerve terminals and produce signals in the subsequent neurons or other cells in the circuit. ACh does many things. It makes the brain and body function. Without it, for example, we cannot move, and other aspects of our body become dysfunctional. ACh is essential for life.

ACh is made in nerve cells by combining choline and acetylcoA. You can see how it got its name – from the precursors. Choline must get into the nerve cells that make and release ACh, and it does so by being transported into the cell by a special transporter/carrier molecule, the choline transporter (see figure below). Animals engineered to be born without the choline transporter, die within an hour or so of birth. The choline transporter is essential for life because it is needed to make ACh.

The existence of the choline transporter was demonstrated by some very good scientists in the early 1970s, Tatsuya Haga, Sol Snyder, Hank Yamamura, and others. The Snyder group especially was noted for brilliant work on transporters in nerves. They found a choline transporter and named it a "high affinity" transporter (because it bound choline very tightly). Yamamura and Snyder in their 1972 Science paper said, "The high-affinity choline uptake appears to represent selective choline accumulation by cholinergic neurons."

They showed that when choline was incubated with brain tissue, choline was taken up by the tissue and converted to ACh (at least a large portion of it). This close link between choline uptake and conversion to ACh suggested that the choline transporter was perhaps "rate limiting" in the formation of ACh. In other words, to get ACh, the choline had to be delivered by the choline transporter. The capacity of the transporter (or rate of choline uptake) limited the amount of ACh that could be made. Hence the phrase "rate limiting" as it is used here.

The work of my group started with the idea that the choline transporter was critical for the formation of ACh. I was familiar with transporters because I had trained earlier with the Snyder group on transporters in brain tissue. From the work of those mentioned above, we knew that it was important. But I and my group wanted to see if the transporter had different speeds. Did the firing of ACh-containing nerves (and release of Ach) result in an increase of choline uptake/transport? Did the choline transporter change in response to demand for ACh? The experiment was a relatively simple one: release ACh by stimulating or by depolarizing the nerves containing ACh, and then see if the transport of choline was activated or increased. Because one single type of experiment cannot always provide stand alone and totally

convincing results, we used multiple approaches or different ways to release ACh. This is common in science; use multiple ways to test your hypothesis.

First, we electrically stimulated the brain in a region thought to have ACh-containing nerves. This did result in an increase in choline transport activity – Wow, very exciting. Because the electrical stimulation was not focused to only ACh-containing nerves, and because electrical stimulation can do many, even unknown things, we used additional approaches to release ACh. We did this by incubating nerve tissue with depolarizing agents, namely high potassium concentrations or a sodium ionophore, veratridine. Both approaches resulted in increased choline transport, and this really excited us. Taking our several results together provided strong evidence that the choline transporter was regulated by depolarization and the release of ACh. When ACh was released, the choline transporter was activated, i.e., more choline was transported to help replace the ACh that was released. The transporter was not only closely linked and limiting in the formation of ACh, but now we showed that the transporter could also be regulated and sped up by a release of ACh after depolarization. This was new. No one had seen this before. The work was published in several papers in the mid to late 1970s – very early in my career. Joe Coyle and his colleagues, using a slightly different approach, found the same result. It was an important support for our idea.

The molecular mechanism for this was not known or understood for many years. There is now more recent (after the 1970s) evidence that choline transporters are re-located (because of depolarization) from inside the nerve cell to the surface of the cell so that more choline could be moved into the nerve cell to make more ACh. The transporter on the surface of the cell was the needed part; more surface transporter resulted in more choline transported to inside the cell where it could be used to make more Ach.

T Haga, an expert on this problem, wrote in 2014: “These (more recent) results suggest that the ... (choline transporter)... is translocated to the … (nerve cell surface)… in response to neuronal activity, and *provides an explanation for the depolarization-induced increase in choline uptake activity, which was demonstrated almost three decades ago* (by Kuhar and some others. – italics are mine)” (Haga, J Biochem, 2014; 156:181. This technical paper is a reasonable, thorough, up-to-2014 review of the choline transporter and its function).

This was a great accomplishment as far as I was concerned. We showed that transport could be activated or regulated by depolarization. This was the first time that such a depolarization-induced increase in transport was observed. We were very pleased. As I mentioned above, this success for me and my colleagues happened early in my career. It gave me confidence that we could reason out an approach to a problem and test it. In retrospect, it was an important part of my career and development as a researcher.

But not ALL experimental observations on ACh and choline from all labs can be explained yet. There are still experiments and explanations needed to complete the picture. The activated transporter declines in minutes. The transporter does not behave the same in all

brain regions. Why? This is how it goes in neuroscience. Nevertheless, Haga's comments in italics above were very gratifying to us and recognized the significance of our early work on transporter activation.

I cannot leave this topic without pointing out that *some* of our experiments could not be reproduced, for various reasons. Unfortunately, this has led to some doubts about our conclusions which we do not doubt. For one thing, some of our experimental conditions were extreme, although appropriate for the time; for example, our stimulating techniques affected more tissue and greater areas than we thought at that time. Also, we allowed ourselves to be misled about the existence of certain septal/nerve pathways in the brain. This was confusing at that time and even now, but still showed that stimulations influenced choline transport. Oddly, the mistake led to a correct conclusion. Similarly, our lesion studies had greater widespread effects than we intended. But even if certain papers are left out of the argument, the body of work from our lab and others showed that the choline transporter can respond to neuronal activation and Ach release.

I am most grateful for my colleagues who worked diligently with me on this project: Hans Rommelspacher, Jay Simon, Charles Murrin, Samir Atweh, Alan Goldberg, and Bob DeHaven. I am close to almost all of them to this day.

The following are some of the relevant publications from my group on choline transport.

Rommelspacher, H., Goldberg, A.M., and Kuhar, M.J. Action of Hemicholinium-3 on Cholinergic Nerve Terminals After Alteration of Neuronal Impulse Flow. Neuropharmacology 13: 1015-1023, 1974.

Simon, J.R. and Kuhar, M.J. Impulse-Flow Regulation of High Affinity Choline Uptake in Brain Cholinergic Nerve Terminals. Nature (London.) 255: 162-163, 1975.

Atweh, S., Simon, J.R., and Kuhar, M.J. Utilization of Sodium-Dependent High Affinity Choline Uptake *in vitro* as a Measure of the Activity of Cholinergic Neurons *in vivo*. Life Sci. 17: 1535-1544, 1975.

Simon, J.R., Atweh, S., and Kuhar, M.J. Sodium-Dependent High Affinity Choline Uptake: A Regulatory Step in the Synthesis of Acetylcholine. J. Neurochem. 26: 909-922, 1976.

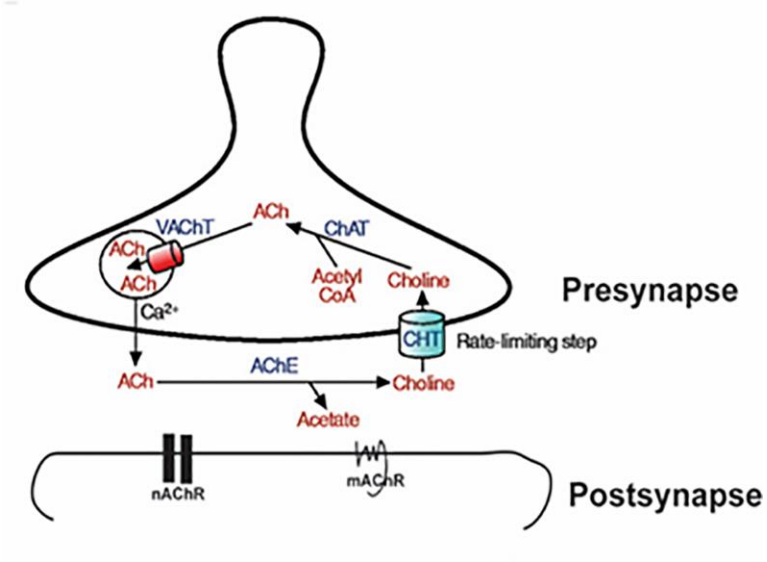
Simon, J.R. and Kuhar, M.J. High Affinity Choline Uptake: Ionic and Energy Requirements. J. Neurochem. 27: 93-99, 1976.

Murrin, L.C. and Kuhar, M.J. Activation of High Affinity Choline Uptake *in vitro* by Depolarizing Agents. Mol. Pharmacol. 12: 1082-1090, 1976.

Murrin, L.C., DeHaven, R.N., and Kuhar, M.J. On the Relationship between ^{3}H-Choline Uptake Activation and ^{3}H-Acetylcholine Release. J. Neurochem. 29: 681-687, 1977.

Klemm, N. and Kuhar, M.J. Post-mortem Changes in High Affinity Choline Uptake. J. Neurochem. 32: 1487-1494, 1979.

Figure: Neurotransmission using acetylcholine (ACh) and the choline transporter (CHT).



This schematic describes an ACh-containing nerve terminal. ACh is stored in the presynapse (nerve terminal) and released by depolarization or nerve impulses onto the next neuron in the circuit (postsynapse). This produces an important change in the postsynaptic nerve.

ACh is formed from AcetylCoA and choline, which is transported by the (CHT) choline transporter, the rate-limiting step. The enzyme that combines the precursors to form ACh is choline acetyltransferase (ChAT). The newly formed ACh is then stored in vesicles by the vesicular an ACh transporter (VAChT). When Calcium (Ca2++) enters the nerve terminal during depolarization, ACh is released from the nerve and the choline transporter is activated. The position and importance of the choline transporter (CHT) is shown.

Figure is Modified From:
https://www.google.com/search?q=choline+uptake+images&espv=2&biw=1255&bih=553&tbm=isch&tbo=u&source=univ&sa=X&ved=0CCIQsARqFQoTCOP3tpjqxMcCFUmKDQodQ6YEkA&dpr=1.5#imgrc=vQRPSDCAOk75gM%3A (on Aug 25, 2015)