A little bit of ammonium may be good for your brain

Païkan Marcaggi, Jonathan A. Coles

► To cite this version:

Païkan Marcaggi, Jonathan A. Coles. A little bit of ammonium may be good for your brain. *Physiology News*, 2008, 73, pp.15-17. hal-03501565

HAL Id: hal-03501565

<https://hal.science/hal-03501565>

Submitted on 7 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

PHYSIOLOGY NEWS

winter 2008 | number 73

The HoD delusion

A Christmas party trick with an intriguing physiology

A new organisation for the biosciences

Ion channel techniques in Shanghai

A little bit of ammonium may be good for your brain

Although specific ammonium-transporting proteins are well known in bacteria and plants, only recently has molecular and functional evidence begun to accumulate for ammonium transporters in animals. We describe how an ammonium transporter on the glial cells of bee retina plays an essential role in energy metabolism, and suggest an update of an old idea of a neuron-astrocyte flux of ammonium at synapses in mammalian brain

Ammonium transporters are widespread

It is convenient to use the word 'ammonium' to include NH_4^+ (the 'ammonium' of chemists), NH_3 ('ammonia') and the mixture of the two that spontaneously forms in water. At physiological pHs the equilibrium mixture contains about 98% NH_4^+ and 2% NH_3 (see Marcaggi & Coles, 2001). NH_3 can diffuse through lipid membranes (although not nearly as well as oxygen does). As shown by measuring intracellular pH, if you add NH_4Cl outside a cell, NH_3 entering the cell tends to alkalize the cytoplasm because about 98% of the NH_3 combines with H^+ (Fig. 1). In practice, some ammonium goes in as NH_4^+ , not NH_3 . A small fraction (about 2%) of this entering NH_4^+ dissociates to form NH_3 and some H^+ ions: the entering flux of NH_4^+ would have to be more than about 50 times that of NH_3 to produce a *net* acid shift. In most cells there is an initial alkalisation caused by NH_3 entry, then NH_4^+ entry produces a slow secondary acidification, but in some cells there is a primary acid shift, indicating that they take up overwhelmingly the NH_4^+ form¹. The NH_4^+ ion is about the same size as a K^+ ion and the conservative suggestion was that NH_4^+ was just leaking in via K^+ channels or transporters. However, proteins of the Rh family (notorious for the immune reactions of Rh(-) foetuses to the blood of a Rh(+) mother) which are expressed not only in erythrocytes but also in kidney, liver, testes and parts of the brain, have homology with the Amt family of ammonium transporters,

Figure 1 (left). Ammonium entry into a cell. Entry of NH_3 produces a large alkalisation. Entry of a similar amount of NH_4^+ produces a much smaller acidification.

Figure 2. A, A section through a drone retina. Each rosette is composed of six, large, identical photoreceptors, 450 μm long, which receive light passing through one corneal facette. Each rosette is surrounded by about 35 glial cells. There are no blood vessels or chemical synapses (micrograph by PM Orkand). B, Application of ammonium (green rectangles) acidifies an isolated bundle of glial cells, and this acidification is blocked when the concentration of Cl^- is reduced. This suggests $\text{NH}_4^+-\text{Cl}^-$ cotransport (from Marcaggi *et al* [1999]. *Eur J Neurosci* 11, 167–177). C, An electron micrograph showing part of two glial cells, packed with glycogen granules, and part of a photoreceptor containing many mitochondria (from A Perrelet). D, PO_2 in a bee retinal slice measured with an O_2 microelectrode. DAB, present throughout this recording, reduces supply of fuel from glial glycogen so consumption of O_2 (QO_2) falls and PO_2 rises. Application of alanine restores consumption. E, $[\text{NH}_4^+]$ in extracellular clefts. In normal Ringer solution, $[\text{NH}_4^+]$ was hardly detected in the dark or during light stimulation of the photoreceptors ("with Cl^- "). When glial uptake was reduced by removing Cl^- ("0 Cl^- ") a much greater $[\text{NH}_4^+]$ signal was measured. F, Scheme of metabolic exchange in drone retina. Methylaminoisobutyrate is thought to reduce alanine uptake (red dot), and pyruvate bypasses the alanine flux (red arrow). (Figs. D, E, F adapted from Coles *et al.* 2008).

¹ It is easy to get the textbook alkaline response: you simply use a damaged cell with an unphysiologically low intracellular pH.

Figure 3. The glutamate/glutamine shuttle at a vertebrate glutamatergic synapse showing the ammonium flux suggested by Benjamin & Quastel (1975), and the accumulation of ammonium together with glutamate in the synaptic vesicles suggested by Marcaggi (2006).

found in apparently all organisms up to and including invertebrates (Huang & Peng, 2005).

There is general agreement that Amt and Rh proteins have an external binding site for NH_4^+ , but lively debate about what happens next. One possibility is that a proton is stripped off NH_4^+ to leave NH_3 which passes through a channel, but there are also reports of electrogenic transport, i.e. transport of NH_4^+ or cotransport of NH_3 and H^+ (Javelle *et al.* 2008). Surprisingly, although Amt/Rh proteins are so widespread, the first ammonium transport selective for NH_4^+ over K^+ to be demonstrated in an animal cell appears to be by another, molecularly different variety of ammonium transporter found in the honeybee retina.

² 1,4-Dideoxy-1,4-imino-D-arabinitol.

A NH_4^+ -selective cation- Cl^- cotransporter on glial cells of the bee retina

The drone (male) honeybee has a large eye, which is useful for spotting high-flying queens, and whose retina is composed almost exclusively of photoreceptor neurons and a homogeneous population of glial cells, arranged with impressive regularity (Fig. 2A). When the intracellular pH of retinal glial cells is measured with a pH-sensitive fluorescent dye, application of ammonium is found to produce a robust intracellular acidification that is abolished in the absence of Cl^- (Fig. 2B). This suggests co-transport with Cl^- . The transporter is selective for NH_4^+ over K^+ (Marcaggi & Coles, 2000), but it shares with a wide range of K^+ - Cl^- cotransporters the presence of inhibitory binding sites for bumetanide and piretanide.

These results suggest a NH_4^+ - Cl^- cotransporter which may be molecularly related to K^+ - Cl^- cotransporters, but which is different from ammonium transporters of the Amt/Rh superfamily. The bee genome includes three Amt/Rh genes, thus making a minimum possible total of four ammonium transporters in this species.

NH_4^+ - Cl^- cotransport is central to energy metabolism in bee retina

A long series of papers have shown that, in drone retina, glucose is taken up exclusively by the glial cells, where it is converted to pyruvate then alanine. Tsacopoulos *et al.* (1994) proposed that the alanine is transferred to the photoreceptor neurons where it is deaminated to provide pyruvate for the numerous mitochondria (Fig. 2C), and that ammonium is returned to the glia, to support sustained production of alanine from glucose or glycogen. Two experimental tools not available to the earlier workers have recently been used to test and refine this model (Coles *et al.* 2008). First, it was not known whether these neurons can actually use alanine as a fuel. Normally, in a retinal slice, the huge stocks of glycogen in the glia provide a supply of fuel to the neurons that lasts for many hours. This supply can be reduced by DAB^{22} , an inhibitor of glycogen phosphorylase, as can be seen by a fall in O_2 consumption by the mitochondria (present only in the neurons). It was found that application of alanine restored O_2 consumption, as predicted (Fig. 2D). Second question: do the photoreceptors release ammonium? Instead of trying to guess ammonium movements from measurements of pH, the authors used a triple-barrelled ammonium-sensitive microelectrode to directly measure $[\text{NH}_4^+]$ in the extracellular clefts. In normal Ringer solution, extracellular $[\text{NH}_4^+]$ was below the detection limit of these electrodes. However, when NH_4^+ uptake by the glia was blocked by removing Cl^- from the Ringer solution,

extracellular $[\text{NH}_4^+]$ was measurable in the dark and shown to increase during light stimulation (Fig. 2E). Extracellular $[\text{NH}_4^+]$ was greatly reduced by methylaminoisobutyrate, a blocker of amino acid transport, and also by pyruvate, which was expected to provide a direct substrate for the mitochondria, bypassing alanine (Fig. 2F). Hence, in this preparation, we now have very strong evidence for the neurons being fuelled by alanine, with ammonium being returned to the glial cells and taken up by a specific transporter.

A neuron-astrocyte ammonium-amino acid shuttle in mammalian brain?

Mammalian astrocytes (at least in culture) avidly take up NH_4^+ (see Marcaggi & Coles, 2001). Most of the glutamate released at glutamatergic synapses in the mammalian brain is taken up into astrocytes by glutamate transporters where, in the astrocytes, it reacts with ammonium to form glutamine. Glutamine, which does not bind to synaptic receptors, is transferred back to the pre-synaptic terminals and deaminated to glutamate. By analogy with bee retina, the shuttle might be completed by transfer of ammonium from the neurons to the astrocytes, as originally proposed by Benjamin & Quastel (1975). Fig. 3, is an update of this proposal which shows uptake of NH_4^+ rather than NH_3 , and the co-release of ammonium with neurotransmitters (Marcaggi, 2006).

Within the astrocyte, ammonium might act as a signal as well as a

reactant. For example, enzymes of the glycolytic pathway are stimulated by ammonium and exogenous ammonium does increase lactate production in rat brain *in vivo* (Provent *et al.* 2007). What would really help this field is a good membrane permeable ammonium-sensitive fluorescent indicator. Please tell your chemist friends.

Païkan Marcaggi

Department of Neuroscience,
Physiology and Pharmacology,
University College London

Jonathan A Coles

Grenoble Institute of Neuroscience,
France (present address: Centre for
Biophotonics, University of
Strathclyde, UK)

References

Benjamin A & Quastel J (1975). Metabolism of amino acids and ammonia in rat brain cortex slices *in vitro*: a possible role of ammonia in brain function. *J Neurochem* **25**, 197–206.

Coles JA, Martiel JL & Laskowska K (2008). A glia-neuron alanine/ammonium shuttle is central to energy metabolism in bee retina. *J Physiol* **586**, 2077–2091.

Huang CH & Peng J (2005). Evolutionary conservation and diversification of Rh family genes and proteins. *Proc Natl Acad Sci U S A* **102**, 15512–15517.

Javelle A, Lupo D, Ripoché P, Fulford T, Merrick M & Winkler FK (2008). Substrate binding, deprotonation, and selectivity at the periplasmic entrance of the *Escherichia coli* ammonia channel AmtB. *Proc Natl Acad Sci U S A* **105**, 5040–5045.

Marcaggi P (2006) An ammonium flux from neurons to glial cells. *Proc Physiol Soc* **3**:SA16.

Marcaggi P & Coles JA (2000). A Cl^- cotransporter selective for NH_4^+ over K^+ in glial cells of bee retina. *J Gen Physiol* **116**, 125–141.

Marcaggi P & Coles JA (2001). Ammonium in nervous tissue: transport across cell membranes and fluxes from neurons to glial cells. *Prog Neurobiol* **64**, 157–183.

Provent P, Kickler N, Barbier EL, Bergerot A, Farion R, Goury S, Marcaggi P, Segebarth C & Coles JA (2007). The ammonium-induced increase in rat brain lactate concentration is rapid and reversible and is compatible with trafficking and signaling roles for ammonium. *J Cereb Blood Flow Metab* **27**, 1830–1840.

Tsacopoulos M, Veuthey AL, Saravelos G, Perrottet P & Tsoupras G (1994). Glial cells transform glucose to alanine which fuels the neurons in the honeybee retina. *J Neurosci* **14**, 1339–1351.

Editor-in-Chief Elect

The Physiological Society is seeking to appoint an Editor-in-Chief Elect for its prestigious publication *The Journal of Physiology*. The Editor-in-Chief Elect will work alongside the current Editor-in-Chief from mid-2009 and then take over full editorial control in July 2010 when the present Editor-in-Chief, William Large, finishes his term of office.

Since 1878 *The Journal of Physiology* has published original research papers in all areas of physiology illustrating new physiological principles or mechanisms. It also publishes commissioned reviews and commentaries, and organises sponsored symposia, the proceedings of which are published as reports in *The Journal*.

The Editor-in-Chief works closely with the Managing Editor, Carol Huxley, and her staff at The Society's Publications Office based in Cambridge, UK. The Editor-in-Chief chairs regular meetings of *The Journal's* Executive Committee, comprising Senior Editors and representatives of The Society and the publishers (Wiley-Blackwell). This Committee is responsible for strategic planning and operational matters relating to *The Journal*. Serving under the Senior Editors is an international Editorial Board of over 50 members, which meets annually.

Candidates should submit a CV together with a letter of no more than three pages of A4 by 1 December 2008 summarising the following:

- a statement describing the reasons for their interest in the position;
- an assessment of the current state of *The Journal*;
- a vision for the future of *The Journal* and how it could be achieved;
- an opinion on the organisation and composition of the Editorial Board;
- any other comments that the candidate feels are relevant.

For full details go to
<http://www.physoc.org>

Going to the US next year?

If you are travelling to the USA after 12 January 2009 under the Visa Waiver Program you will need to apply for advance authorization using the online process available at <https://esta.cbp.dhs.gov>.

Jonathan Coles (left) and Païkan Marcaggi.