Definitely, my cup of tea. Focus on “Caffeine inhibits glucose transport by binding at the GLUT1 nucleotide-binding site”

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CAFFEINE AND THEOPHYLLINE are the most widely ingested substances affecting behavior. Consumption is particularly high in Scandinavia at >400 mg per person per day; this is double that of the US or UK (5). A single cup of coffee raises plasma concentration to around 4 μM. Caffeine acts as a nonspecific antagonist of brain interstitial fluid adenosine with G-type A1 and A2A adenosine receptors (IC50 ≈ 5-10 μM), thereby raising neural firing rates and brain metabolic rate. Caffeine also inhibits phosphodiesterase (IC50 ≈ 0.5 mM) and triggers Ca2+ release from sarcoplasmic reticulum (IC50 ≈ 2–4 mM).

Caffeine and theophylline inhibit glucose transport by direct action on the glucose transporter SLC2A1, GLUT1 (IC50 ≈ 2–4 mM; Refs. 3 and 8), and they also inhibit insulin-dependent GLUT4 trafficking in adipocytes (IC50 ≈ 250 μM; Ref. 1).

While concerns about xanthine-dependent inhibition of glucose uptake across the brain endothelial barrier may have been overstated (7), the likely stimulatory effects of normally ingested caffeine plausibly could, by depleting the compromised brain energy reserve in GLUT1 deficiency syndrome patients, exacerbate the tendency to epileptic seizures, as seen with exercise, or hunger (10).

Although caffeine’s inhibitory effects on glucose transport are not physiologically important, they usefully contribute to the still contentious debate regarding the mechanism of sugar transport.

The main objective of Sage et al.’s (9) paper is discovery of caffeine’s inhibition site on GLUT1. They conclude that it binds to an endofacial vestibular site overlapping both the noncontiguous neighboring ATP and cytochalasin B binding sites. This conclusion is based on four different types of study.

The apparent Ki of caffeine inhibition (obtained from Dixon plots) of 3-O-methyl-d-glucose uptake into erythrocyte ghosts increased from 0.9 ± 0.3 mM in nucleotide-free conditions to 2.6 ± 0.6 mM with 4 mM ATP in the cytosol. This competitive interaction between ATP and caffeine indicates a common endofacial binding site for these ligands.

Both caffeine and ATP displace fluorescence-labeled trinitrophenyl-ATP from purified GLUT1. Neither cytochalasin B nor glucose alters trinitrophenyl-ATP binding. This result is validated by demonstration of caffeine-dependent displacement of 3H-labeled cytochalasin B bound to GLUT1 incorporated within proteoliposomes. Cytochalasin B binding is unaltered by either ATP or d-glucose.

Previously, Carruther’s group showed that the GLUT1 COOH-terminal chain with the large endofacial linker chain between TM6 and 7 when conjoined by ATP forms a cage around the endofacial exit of GLUT1’s central pore. This retards glucose efflux and promotes glucose reflux, thereby reducing both the Km and the Vm of zero-trans net entry (2, 6). Because zero-trans net glucose exit is relatively unaffected by endofacial ATP, this binding enhances transport asymmetry (4). The bridging action of ATP masks the COOH-terminal chain and reduces antibody to COOH-terminal binding to GLUT1 (2). Caffeine is now shown also to reduce GLUT1 COOH-terminal antibody binding. Neither d-glucose nor cytochalasin B affects COOH-terminal antibody binding. These findings support the view that caffeine and ATP compete at a common endofacial site.

In accord with the expectations of the experimental results, in silico molecular docking studies simulating ligand docking to the crystal structure of GLUT1 demonstrate that highest affinity caffeine docking sites within the endofacial vestibule are partially coincident with the ATP binding site and adjacent to the cytochalasin B binding site.

Sage et al.’s results (9) apparently contradict those of Ojeda et al. (8), who deduced, on the basis of transport kinetics that “there is a methylxanthine regulatory site on the external surface of the transporter, which is close but distinguishable from the d-glucose external site.” Their most persuasive evidence for an external xanthine binding site is that, pentoxifylline, a xanthine derivative, competes with glucose binding to an external site, as deduced from assays of infinite-cis net glucose exit from human erythrocytes. Neither caffeine nor theophylline competes with glucose binding at this external site.

None of the methods used by Sage et al. (9) can exclude the possibility of additional low-affinity xanthine binding sites. Pentoxifylline [1-(5-oxohexil)] sidechain may provide van der Waals’ forces to strengthen binding to an external low-affinity binding site.

Just as there is evidence for low-affinity sugar-binding sites within GLUTs undetectable using current crystallographic methods, (4) so also there may be multiple sites of xanthine binding that leave only subtle traces, seen in their kinetic profiles.

Glucose transport is not wholly determined by high-affinity sites but also is subject to influences of a network of low-affinity sites traversing the transporter. This network is partially affected by nucleotides and other ligands within the endofacial vestibule.

The paper of Sage et al. (9) shows that deductions based on “mere” kinetics, while important, can only be tentative guides to transporter function. The advance in methodologies of...
examining transporter structures is an opportunity to consider more unconstrained ways of viewing transport function.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

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REFERENCES