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2-Arachidonoylglycerol (2-AG) Membrane Transport: History and Outlook

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Anita Hermann,[1] Martin Kaczocha,[1] and Dale G. Deutsch[1]

[1]Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY

Abstract

Only a few studies have addressed the transport of 2-arachidonoylglycerol (2-AG), a naturally occurring agonist for cannabinoid receptors. Based upon saturation kinetics, these early reports have proposed that 2-AG enters the cell by a specific 2-AG transporter, via the putative anandamide transporter, or by simple diffusion. In this review, the uptake of 2-AG is discussed in light of the recent advances that have been made for anandamide transport, where the mechanism appears to be rate-limited diffusion through the membrane. Endocannabinoids may be a distinct class of agonists since they are hydrophobic and neutral, exhibiting similar biophysical properties to some anesthetics that freely diffuse through the membrane.

Keywords: anandamide, 2-AG, 2-arachidonoylglycerol, cannabinoids, endocannabinoid, transport

Introduction

The discovery that 2-arachidonoylglycerol (2-AG) is an endogenous cannabinoid receptor ligand was described in 1995 by Sugiura et al[1] and Mechoulam et al.[2] In the central nervous system, the synthesis of 2-AG has been reported to occur in mature brains on postsynaptic membranes, while the main enzyme reported to degrade 2-AG is presynaptically localized in cytosol and in the intracellular membranes (for recent reviews see Piomelli,[3] Jonsson et al,[4] and Di Marzo[5]). There have been only a handful of studies addressing the mechanism by which 2-AG is transported into the cell (Figure 1), and these are reviewed here and compared with arachidonoylethanolamide (anandamide) (AEA) transport, which has been more extensively studied.

History of 2-AG Transport Studies

The cellular uptake of 2-AG in rat basophilic RBL-2H3 and mouse neuroblastoma N18TG2 cells was described in 1998 by Di Marzo et al[6] and Ben-Shabat et al.[7] Both groups independently observed accumulation of 2-AG in those cells with concurrent disappearance from the incubation media. Di Marzo et al[6] concluded that there was no evidence for a facilitated-diffusion process for the uptake of 2-AG. In addition, Ben-Shabat et al[7] studied 2-linoleoyl-glycerol and 2-palmitoyl-glycerol, 2 acyl-glycerols, which are present together with 2-AG in brain, gut, and spleen. These 2 compounds did not bind to CB1 or CB2 receptors, but they potentiated the binding of 2-AG to CB1 and CB2 receptors. Co-incubation of 2-AG with 2-linoleoyl-glycerol reduced 2-AG loss from the media, while 2-palmitoyl-glycerol was without effect.

In 1999, Piomelli et al[8] showed evidence for a possible protein transporter for 2-AG. They observed saturable 2-AG uptake in human astrocytoma cells with a Michaelis-Menten constant (Km) of 0.7 ± 0.1 μmol/L and a Vmax of 28 ± 6 pmol/min/mg of protein, comparable to that shown for AEA transport. In these assays the clearance of radioactive material from the incubation medium was followed over a course of 20 minutes. The authors concluded that 2-AG may be internalized by the AEA transporter into these cells (Figure 1).

These results were replicated in 2000 by Beltramo and Piomelli.[9] $[^3H]$2-AG accumulation was inhibited by unlabeled 2-AG with a half-maximal inhibitory concentration (IC_{50}) of 5.5 ± 1 μmol/L and 100 μmol/L 2-AG reduced $[^3H]$2-AG accumulation to $24\% \pm 1\%$ of the control. AEA inhibited $[^3H]$2-AG accumulation with an IC_{50} of 4.2 ± 0.3 μmol/L and exhibited a maximal effect of 100%. AM404, a putative AEA transport inhibitor, also interfered with $[^3H]$2-AG uptake (IC_{50} of 1.8 ± 0.1 μmol/L). Several substrates and inhibitors of lipid transport systems had no effect on 2-AG uptake, but 100 μmol/L arachidonic acid significantly reduced 2-AG uptake in astrocytoma cells as did 10 μmol/L triascin C (an acyl-CoA synthetase inhibitor). BTNP ((E)-6-(bromomethylene) tetrahydro-3-1-naphthalenyl)-2H-pyran-2-one), a nonspecific fatty acid amide hydrolase (FAAH) inhibitor, had no effect on 2-AG uptake at 5 μmol/L. The authors concluded that a common carrier-mediated transport system is responsible for the internalization of 2-AG and AEA based on 4 key observations: (1) the similar kinetic properties of 2-AG and AEA uptake; (2) 2-AG and AEA

Corresponding Author: Dale G. Deutsch, Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY 11795-5215. Tel: (631) 632-8595; Fax: (631) 632-8575; E-mail: DDeutsch@notes.sunysb.edu

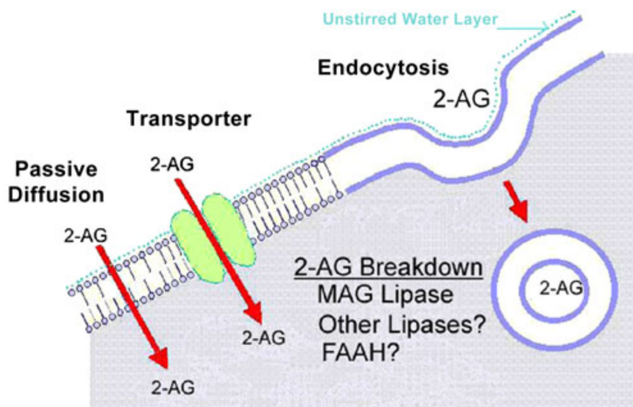

Figure 1. Possible routes for 2-AG transport.

competing with each other's uptake; (3) the blocking of [^{3}H]2-AG uptake by the putative AEA transport inhibitor AM404; and (4) the insensitivity of 2-AG and AEA transport to substrates and inhibitors of other known lipid transporters that are Na^+ and energy independent.

In 2001 Bisogno et al[10] studied the transport of AEA and 2-AG in rat C6 glioma cells at 37°C and 4°C at different time intervals and concentrations. Uptake was time- and temperature-dependent and saturable. The Km for 2-AG uptake was 15.3 ± 3.1 μmol/L and Bmax was 0.24 ± 0.04 nmol/min/mg protein. When 2-AG and AEA were co-incubated, only the uptake of 2-AG was significantly decreased. The authors suggested that if there is an AEA transporter, its efficacy with 2-AG is lower than with AEA, or that there are 2 different transporters involved. AM404 and linvanil (which is a capsaicin homolog[11]) inhibited the uptake of 2-AG with similar potency, K_i = 10.2 ± 1.7 and 6.4 ± 1.2 μmol/L, respectively. Bisogno et al[10] also found that nitric oxide donors increase the uptake of 2-AG as well as AEA. In conclusion, these authors provided evidence for a 2-AG/AEA transporter that may be identical or distinct from the putative AEA membrane transporter.

In 2004, Hajos et al[12] also observed that 2-AG uptake in primary rat cortical neurons was temperature dependent and saturable. Uptake of [^{3}H]2-AG decreased with increasing concentrations of 2-AG and AEA and also with AM404. From these data the authors concluded that there is a common transporter for the 2 endocannabinoids.

PERSPECTIVES

The neuromodulatory functions of 2-AG require regulation of its synthesis, uptake/release, and inactivation. As a retrograde transmitter, 2-AG is believed to be synthesized postsynaptically[13] and translocated to the presynaptic cell by an unknown mechanism, where it signals via CB_1 and is then inactivated by being taken into the cell and metabolized mainly by monoacylglycerol lipase (MGL).[14,15] Owing to the structural and functional similarities between AEA and 2-AG, studies examining the mechanism of 2-AG transport pose similar challenges and limitations that have been observed for AEA uptake.

Because of the lipophilicity of 2-AG, future studies should address the degree of nonspecific interactions of this compound with plastic culture dishes and tips as has been performed for AEA.[16,17] In this regard, bovine serum albumin (BSA) should be included in incubation media to reduce nonspecific interactions and stabilize 2-AG in solution. The binding affinity of AEA to BSA is known,[18,19] and its uptake as a function of free/unbound AEA has been determined.[20] Owing to its structural similarity with AEA, 2-AG may bind BSA with similar affinity, reaching unbound concentrations in the nanomolar range. Determination of the dissociation constant (K_d) of 2-AG from BSA will enable calculation of free 2-AG that is available for uptake.

By plotting the uptake rate of unbound 2-AG with increasing 2-AG concentrations, it may be possible to elucidate the processes governing its accumulation. If 2-AG uptake exhibits linear kinetics, it would suggest simple diffusion across the plasma membrane. However, apparent saturable 2-AG uptake may be interpreted in 2 ways: (1) the saturation of an endocannabinoid transporter or (2) the resistance to uptake caused by the unstirred water layer surrounding the cells.[21]

Unlike most other neuromodulators, 2-AG and AEA are hydrophobic and uncharged. Uptake of 2-AG may show saturation owing to the unstirred water layer surrounding cells, and this may limit 2-AG permeation into the membrane in a manner similar to that found for AEA (Figure 1).[20,21] Such apparent saturation of 2-AG uptake may preclude the use of transport kinetics as a criterion to define carrier-mediation because of the inability to distinguish between protein-mediated transport and rate-limited permeation of 2-AG through the unstirred water layer. Therefore, in all of the studies cited above that showed saturation of 2-AG uptake, the interpretation of the results may be confounded. Furthermore, in all experiments conducted to date, the free 2-AG concentrations are unknown because the solubility of 2-AG in aqueous buffers was not determined.

All of the 2-AG uptake studies have used incubation conditions longer than 1 minute. For AEA, it is known that such long incubation times are unable to distinguish uptake independent of downstream metabolism and sequestration.[20-24]

Since 2-AG accumulation has only been examined in the steady-state, it is not known whether the same kinetics will be observed when shorter incubation times are employed. As was found for AEA,[20,23,25,26] downstream metabolism of 2-AG by MGL may promote its accumulation in the steady-state. Consistent with this idea, competitive inhibitors of 2-AG inactivation have been found to reduce its cellular

accumulation.[7] Selective inhibitors of MGL augment 2-AG levels in brain[27,28] and may likewise reduce 2-AG uptake in the steady-state in a manner similar to AEA. This action would delay the clearance of 2-AG from the plasma membrane and prolong CB_1 signaling to produce physiological effects.[27-29]

CONCLUSION

During the last decade there has been great emphasis on AEA uptake and its putative transporter. However, as discussed above, only a few studies investigated the cellular uptake of 2-AG. Conclusions from these studies are ambiguous. Although one report suggested simple diffusion, most have shown that transport occurs by facilitated diffusion. These reports indicated either a common transporter for 2-AG and AEA or individual carriers. It has also been suggested that there are 2 transporters, one of which is solely for AEA, while the other is a cotransporter for 2-AG and AEA. Most soluble neurotransmitters such as serotonin are hydrophilic and require a transporter to pass through the hydrophobic membrane. Similarly, some hydrophobic transmitters require a membrane transporter because of their charge. An example of the latter is the prostaglandins, whose transporter has been cloned, and whose uptake displays time and concentration dependence and is blocked by specific inhibitors.[30] AEA and 2-AG may be in a special class of agonists as they are lipophilic and in terms of transport may behave like some anesthetics that freely diffuse through the membrane. To date, the identification and cloning of an endocannabinoid transporter has yielded negative results. Of course, if one were discovered it would resolve some of the recent controversies surrounding the mechanisms of AEA and 2-AG transport. Efficient uptake of these lipids may involve different mechanisms, including rate-limited simple diffusion, uptake through a putative membrane transporter, or lipid raft-mediated endocytosis depending upon the cell type (see Figure 1).[31,32]

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