Previously, we have shown that the soluble form of brain glutamic acid decarboxylase (GAD) is inhibited by ATP through protein phosphorylation and is activated by calcineurin-mediated protein dephosphorylation (Bao, J., Cheung, W. Y., and Wu, J. Y. (1995) J. Biol. Chem. 270, 6464–6467). Here we report that the membrane-associated form of GAD (MGAD) is greatly activated by ATP, whereas adenosine 5’-[(5-γ-imido)triphosphate (AMP-PNP), a non-hydrolyzable ATP analog, has no effect on MGAD activity. ATP activation of MGAD is abolished by conditions that disrupt the proton gradient of synaptic vesicles, e.g., the presence of vesicular proton pump inhibitor, bafilomycin A1, the protonophore carbonyl cyanide m-chlorophenylhydrazone or the ionophore gramicidin, indicating that the synaptic vesicle proton gradient is essential in ATP activation of MGAD. Furthermore, direct incorporation of 32P from [γ-32P]ATP into MGAD has been demonstrated. In addition, MGAD (presumably GAD65, since it is recognized by specific monoclonal antibody, GAD6, as well as specific anti-GAD65) has been reported to be associated with synaptic vesicles. Based on these results, a model linking γ-aminobutyric acid (GABA) synthesis by MGAD to GABA packaging into synaptic vesicles by proton gradient-mediated GABA transport is presented. Activation of MGAD by phosphorylation appears to be mediated by a vesicular protein kinase that is controlled by the vesicular proton gradient.

γ-Aminobutyric acid (GABA),1 the major inhibitory neurotransmitter in brain, is synthesized by a single enzymatic reaction catalyzed by γ-glutamate decarboxylase (EC 4.1.1.15; GAD) (1). Alterations in the level of GABA in the central nervous system have been linked to neurological disorders, including Huntington’s chorea (2), Parkinson’s disease (3), and epilepsy (4). In addition to its importance in regulating GABA level in the central nervous system, GAD has been implicated as an autoantigen in two human autoimmune diseases, insulin-dependent diabetes mellitus (IDDM) and Stiff-Man syndrome (5, 6). Despite its importance, the mechanism underlying regulation of GAD remains elusive.

The first comprehensive studies of regulation of GAD activity were conducted when GAD was first purified from mouse brain more than two decades ago (7). We found that GAD activity was markedly inhibited by Zn2+ ion, in contrast to its activation effect on pyridoxal kinase, an enzyme responsible for the biosynthesis of GAD’s co-factor, pyridoxal 5’-phosphate (PLP) (8). Hence it was proposed that Zn2+ has a pivotal role in the regulation of GABA biosynthesis (9). The importance of PLP in the overall regulation of GABA biosynthesis was recognized when it was found that, in rat brain, more than half of GAD is present as an apoenzyme (10, 11). Furthermore, it was reported that, in rat brain, one form of GAD is fully saturated with PLP, whereas the other form is present largely as an apoenzyme (12). This finding was later confirmed by cloning and characterization of GAD65 and GAD67, the two major forms of GAD in mammalian brain, thus showing that GAD65 is present as apoGAD in the brain to a much greater degree than that of GAD67, and hence its activity is regulated more closely by factors affecting the conversions of apoGAD to holoGAD (13–15). The interconversion between apo- and holoGAD appears to be a highly regulated mechanism and is not a simple dissociation and association of PLP (for review, see Ref. 15). The activation of GAD65 by binding of PLP to apoGAD65 appears to involve a large protein conformational change leading to increased stability (16).

In addition to Zn2+ and PLP, recently we have reported that protein phosphorylation also plays an important role in the regulation of GAD activity. Specifically, we have shown that soluble GAD (SGAD is inhibited by protein kinase A-mediated protein phosphorylation and is activated by calcineurin-mediated dephosphorylation (17, 18). We report here that MGAD is also regulated by protein phosphorylation but through a different mechanism. MGAD is activated by ATP through phosphorylation modulated by the synaptic vesicle proton gradient and is inhibited by dephosphorylation. A model linking GABA synthesis by MGAD and proton gradient-sensitive GABA transport into synaptic vesicles is also discussed.

EXPERIMENTAL PROCEDURES

Materials—Fresh porcine brains were obtained from a local abattoir. Benzethonium hydroxide (Hyamine base, 1 mM solution in methanol), PLP, 2-aminoethylisothiuronium bromide, AMP-PNP, calf intestinal phosphatase (CIP), Triton X-100, bafilomycin A1, gramicidin, and carbonyl cyanide m-chlorophenylhydrazone (CCCP) were purchased from Sigma. Okadaic acid was from Alexis Biochemicals Corp. (San Diego, CA). Protein kinase inhibitors (PMB, KN-62, H-8, and H-9) were purchased from Research Biochemical International (Natick, MA). Sodium orthovanadate was purchased from Aldrich. Protein A-Sepharose 4
Fast Flow was purchased from Amersham Pharmacia Biotech. [1-14C]Glutamate, [γ-32P]ATP, and [γ-32P]ATP were purchased from NEN Life Science Products; Western-Light Plus immunodetection kit was from Tropix Inc. (Bedford, MA).

Antibodies—Anti-GAD65 and anti-GAD67 are polyclonal rabbit antibodies raised against recombinant human GAD67 and human GAD65 expressed in separate bacterial systems. Anti-GAD65 used in this study had been preabsorbed with an excess of recombinant human GAD65 to remove GAD67-specific antibodies. Anti-GAD C38 is a polyclonal antibody directed against soluble GAD (17, 18). GAD6, a GAD65-specific monoclonal antibody, was purchased from Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). IODIM serum, which has higher titer autoantibodies against GAD65 than GAD67 in immunoblotting, as well as immunoprecipitation tests, was kindly provided by Dr. C. Y. Kuo (University of Tennessee, Memphis, TN).

Enzyme Assay—GAD was assayed by a radiometric method measuring the formation of 4°C from 1-[14C]glutamic acid as described (20). Bafilomycin A1, CCCP, and gramicidin were dissolved in ethanol in stock solution. The controls contained ethanol in concentrations not exceeding 1% of the incubation volume.

Immunoprecipitations and Immunoblotting—Five hundred microliters of GAD sample was incubated with 50 µl of anti-GAD serum in various dilutions at 4°C for 12 h. Protein A-Sepharose (20 µl) was added to each mixture and incubated at 4°C for an additional 2 h. The mixture was then centrifuged at 10,000 × g for 10 min. The pellet was then washed six times in GAD buffer, which contains 1 mM 2-aminomethylisothiocyanate, 0.2 mM PLP in 50 mM Tris/citrate (KP) buffer at pH 7.2. Both the supernatant and pellet were assayed for GAD activity. Partially purified MGAD was first separated on a 10% one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis system (SDS-PAGE) followed by immunoblotting as described (17, 18).

Preparation of Synaptosomal Membranes—Unless otherwise specified, all procedures were carried out at 4°C and all solutions contained standard GAD buffer. In a typical preparation, a 15% (w/v) porcine brain homogenate was made by use of a Teflon-glass homogenizer in ice-cold standard GAD buffer containing 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, and 1 mM theophylline. Preparation of crude synaptosomes was performed as described (17, 18). Briefly, fresh porcine brains were homogenized in 0.32% sucrose (w/v, 15 g/100 ml), and the homogenate was centrifuged at 1,000 × g for 10 min. The supernatant solution was collected and centrifuged at 12,000 × g for 30 min. The pellet thus obtained was sonicated and washed in GAD buffer solution. This was the crude synaptosomal membrane, referred to as P2M.

Phosphorylation of MGAD in Crude Synaptosomal Membranes—Phosphorylation of MGAD in P2M was carried out as described previously for SGAD (17, 18). Briefly, 0.1 mM [γ-32P]ATP or [α-32P]ATP (1 mM/Ci/ml) and P2M (3 mg) were incubated at 22°C for 1 h. The mixture was then centrifuged at 100,000 × g for 60 min. Solubilized [32P]MGAD was then cleared with preimmune rabbit serum, followed by immunoprecipitation with anti-GAD serum. The immunoprecipitates were washed six times in GAD buffer, followed by GAD assay of the supernatant and the pellet. The samples are then analyzed by SDS-PAGE and visualized by autoradiography as described (17, 18).

Purification of MGAD—Purification of MGAD was conducted, as described previously (21). In a typical experiment, a 25% P2M homogenate was made in standard GAD buffer solution containing 0.5% Triton X-100. The P2M suspension was gently rotated at 4°C for 1 h and then centrifuged at 100,000 × g for 1 h. The supernatant thus obtained was the solubilized MGAD and was further purified through conventional column chromatography consisting of an anion exchange (DEAE-52), an adsorption (hydroxyapatite), and a gel filtration column (Sephadex G-200).

Kinetic Studies—Effect of ATP on the kcat and Vmax of synaptosomal MGAD was determined as described previously for SGAD (22). Briefly, the kcat value was determined by using varying glutamate concentra-

![Image](340x605 to 522x729)

**FIG. 1.** Effects of ATP and phosphatase inhibitors on GAD activity. Aliquots of synaptosomal suspension were incubated in the presence of ATP or phosphatase inhibitors as indicated. The incubation was carried out at 22°C for 30 min with constant mixing. MGAD and SGAD were separated by centrifugation, followed by brief washing before assaying for GAD activity as described under “Experimental Procedures.” Lane 1, in standard GAD buffer; lane 2, the same as lane 1 except including 5 mM ATP; lane 3, the same as lane 1 except including 2 mM EDTA and 2 mM EGTA; lane 4, the same as lane 1 except including 2 mM EDTA; lane 5, the same as lane 1 except including phosphatase inhibitors (0.2 mM vanadate, 2 mM sodium fluoride, and 0.2 mM sodium pyrophosphate). GAD activity is expressed as percentage of activity using GAD activity in standard buffer (lane 1) as reference, 100%. Open column, SGAD activity; striped column, MGAD activity. The bar indicates the standard deviation with n = 4.

**RESULTS**

ATP Activation of MGAD—When SGAD and MGAD were assayed under conditions favoring protein phosphorylation, e.g., in the presence of ATP or protein phosphatase inhibitors, it was found that SGAD was inhibited whereas MGAD was activated (Fig. 1). In contrast to SGAD, MGAD activity was greatly enhanced by ATP, increasing by about 100% at 5 mM. Under the same conditions, SGAD activity was inhibited by 40%, similar to our earlier findings (17). The presence of divalent cation chelators, 2 mM EDTA and 2 mM EGTA, decreased SGAD activity but had no effect on the activity of MGAD, suggesting a role for calcium on SGAD regulation but not in MGAD regulation. Other phosphatase inhibitors, e.g., 0.2 mM vanadate, 2 mM sodium fluoride, and 0.2 mM sodium pyrophosphate, slightly enhanced MGAD activity and significantly inhibited SGAD activity.

Dependence of ATP Activation of MGAD on Synaptic Vesicle Proton Gradient—To determine if the energy-dependent synaptic vesicle proton gradient is involved in the activation of MGAD by ATP, we examined the effect of ATP on MGAD activity under conditions that disrupted the proton gradient. When the vesicular proton gradient was abolished by protonophore uncoupler, CCCP (100 µM), or ionophore, gramicidin (10 µM), the activation of MGAD by ATP was found to disappear as shown in Fig. 2. In addition, ATP activation of MGAD is sensitive to the specific V-ATPase inhibitor, bafilomycin A1 (23), at a concentration of 6 µM but not to the P-type ATPase inhibitor, vanadate, at a concentration of 200 µM (Fig. 2). Interestingly, CCCP, gramicidin, and bafilomycin A1 have no effect on SGAD activity, alone or in the presence of ATP.

Effect of Phosphatase Treatment on MGAD Activity—MGAD was found to be highly sensitive to the treatment with phosphatase. MGAD activity was reduced by 80% by a short incubation (10 min) with CIP, whereas SGAD activity was slightly increased (Fig. 3). This result suggested that MGAD activity is likely to be regulated by protein phosphorylation.

Effect of an ATP Analog and Kinase Inhibitors on MGAD Activity—The non-hydrolyzable ATP analog AMP-PNP was...
found to have no effect on MGAD activity up to a concentration of 5 mM while ATP activated MGAD at a concentration as low as 10 μM, suggesting that ATP is likely to exert its effect on MGAD through protein phosphorylation and not by direct binding to MGAD. At 0.1 mM ATP, MGAD activity was increased to over 200% (Fig. 4). The activation of MGAD by ATP seems to plateau at 0.1 mM since a 50-fold increase of ATP concentration to 5 mM gave a comparable effect (Fig. 4). Other nucleotides such as GTP and ADP had no effect on MGAD activity even at 5 mM (Table I). These data are compatible with the notion that ATP exerts its effect through protein phosphorylation. Lack of effect of protein kinase C inhibitors, e.g. H-8 (30 μM), H-9 (30 μM), KN-62 (5 μM), and PMB (100 μM), on ATP activation of MGAD (Table I) may indicate that protein kinase C is not involved in the regulation of MGAD activity. Furthermore, ATP activation of MGAD appears not to be due to the activation of pyridoxal kinase since theophylline, a potent inhibitor of pyridoxal kinase (K_i = 8.7 μM) (24) has no effect on ATP activation of MGAD (results not shown). Kinetic studies showed that ATP lowers the K_m of MGAD for L-glutamate from 2.1 mM to 0.9 mM and increased the V_max by about 50%. Thus, ATP gives about twice the stimulatory effect at low concentrations of glutamate (3-fold increase in V_max/K_m) than it does at saturating levels of glutamate.

**Direct Incorporation of 32P into MGAD by Protein Phosphorylation**—As shown in Table II, both SGAD and solubilized MGAD cross-react with anti-SGAD as well as serum of IDDM patients. A direct demonstration of 32P incorporation into MGAD was obtained when synaptosomal membranes were incubated in the presence of [γ-32P]ATP (Fig. 5, lane 4). This 32P-labeled protein was further identified as MGAD from immunoblotting tests using anti-SGAD serum (lane 5). No 32P incorporation was seen when [γ-32P]ATP was replaced by [α-32P]ATP (lane 3) or when anti-SGAD serum was replaced by preimmune serum (lanes 1 and 2).

**Identification of MGAD as GAD65**—Partially purified MGAD preparations were used for immunoblotting and immunoprecipitation/SDS-PAGE tests using four different antibodies, namely monoclonal anti-GAD, GAD6, which has been shown to be specific to GAD65 (25); antibodies against soluble GAD, C38, which have been shown to cross-react with both soluble and membrane-associated GAD (17, 18, 26); IDDM serum, which has been shown to have higher frequency GAD65 autoantibodies and lower frequency GAD67 autoantibodies (27, 28); and polyclonal antibodies raised against purified human recombinant GAD65 and preabsorbed with excess GAD67. In immunoblotting test, MGAD was clearly recognized by not only

**Fig. 2. Dependence of ATP activation of MGAD on synaptic vesicle proton gradient.** Aliquots of synaptosomal suspension were incubated in the presence of ATP with or without various proton gradient uncouplers or inhibitors. GAD activity in standard buffer (control) was used as reference, 100%. **Striped column**, SGAD activity; **open column**, MGAD activity. The bar indicates the standard deviation with n = 4.

**Fig. 3. Effect of phosphatase on GAD activity.** Treatment of GAD samples with CIP was conducted as described previously (17, 18). Briefly, SGAD and solubilized MGAD samples were applied to a CIP-conjugated agarose (150 units/ml) column, which had been equilibrated with 50 mM Tris-citrate buffer, pH 7.4 at 30 °C. After application of GAD sample, the column was washed for 10 min to allow sufficient time for phosphatase to interact with GAD. The column was then washed with five column volumes of 25 mM Tris-citrate buffer, pH 7.4, containing a phosphatase inhibitor mixture as described in Fig. 1, plus 1 mM phenylmethylsulfonyl fluoride and 5 mM benzamidine. The eluate was assayed for GAD activity and determined for protein concentration. As for the control, the conditions were the same, except that CIP-conjugated agarose was replaced by non-conjugated agarose beads. The CIP phosphatase activity was monitored using p-nitrophenol as substrate and measured spectrophotometrically at 405 nm. GAD assays were carried out as described under “Experimental Procedures.” GAD activity was expressed as percentage of activity using the control group as reference, 100%. **Open column**, control; **striped column**, CIP-treated. The bar indicates the standard deviation with n = 4.

**Fig. 4. A comparison of the effect of a non-hydrolyzable ATP analog AMP-PNP and ATP on MGAD activity.** The experimental conditions were the same as those described in Fig. 1 except that various concentrations (0.01–5 mM) of AMP-PNP and ATP were used. GAD activity in the standard GAD buffer was used as reference, 100%. **Open column**, in the presence of ATP; **striped column**, in the presence of AMP-PNP. The standard deviation is indicated by the vertical bar (n = 4).
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TABLE I
Activation of ATP on synaptosomal MGAD under various conditions

Washed and lysed aliquots of crude synaptosomal pellet were treated under the following conditions. GAD activity was determined by the radiometric method, as described (20). Values in percentage of MGAD activity are means ± SE.

| Treatment               | MGAD activity | n  |
|-------------------------|---------------|----|
| Control                 | 100 ± 6       | 4  |
| Calcium acetate, 200 μM | 95 ± 8        | 4  |
| GTP, 5 mM               | 103 ± 10      | 3  |
| ADP, 5 mM               | 98 ± 9        | 3  |
| ATP, 5 mM               | 220 ± 23      | 18 |
| GABA, 25 mM             | 89 ± 9        | 6  |
| ATP, 5 mM + GABA, 25 mM | 192 ± 20      | 6  |
| ATP, 5 mM + calcium     | 205 ± 19      | 4  |
| acetate, 200 μM         |               |    |
| ATP, 5 mM + PMB, 100 μM | 234 ± 18      | 3  |
| ATP, 5 mM + KN-62, 5 mM | 195 ± 16      | 3  |
| ATP, 5 mM + H-8, 30 μM  | 225 ± 17      | 3  |
| H-9, 30 μM              |               |    |

TABLE II
Immunoprecipitation of SGAD and solubilized MGAD using C38 and IDDM serum

Approximately 80 μg of protein of each GAD sample in 100 μl of standard GAD buffer was incubated with C38 and IDDM serum (50 μl) in a final volume of 800 μl for 24 h.

| GAD samples | GAD activity in the immunoprecipitate | Control serum | C38 | IDDM serum |
|-------------|---------------------------------------|---------------|-----|-----------|
|             | %                                     | %             | %   | %         |
| SGAD        | 2 ± 0.5                               | 95 ± 1.0      | 96 ± 1.5 |
| Solubilized MGAD | 3 ± 1.0                           | 93 ± 1.5      | 98 ± 2.0 |

**Fig. 5.** Direct incorporation of 32P into MGAD. Synaptosomal membranes were incubated with either [γ-32P]ATP or [α-32P]ATP, followed by solubilization with Triton X-100 and immunoprecipitation with anti-SGAD as described under “Experimental Procedures.” In addition, immunoblotting tests were also conducted with partially purified (about 10% pure) MGAD preparation to confirm the identity of 32P-labeled protein as MGAD. Lanes 1–4 are autoradiograms, and lane 5 is immunoblotting pattern of MGAD. Lane 1, incubated with [α-32P]ATP followed by precipitation with preimmune serum; lane 2, same as lane 1, except [α-32P]ATP was replaced by [γ-32P]ATP; lane 3, same as lane 1, except preimmune serum was replaced by anti-SGAD serum; lane 4, same as lane 2, except preimmune serum was replaced by anti-SGAD serum; lane 5, immunoblot of MGAD with anti-SGAD serum. Arrow indicates the position of MGAD below the indicator of molecular size marker at 67 kDa.

C38, IDDM serum (lanes 6 and 8, Fig. 6) but also by GAD65-specific antibodies, GAD6 and preabsorbed anti-GAD65 (lanes 5 and 7, Fig. 6) suggesting that MGAD is likely to be GAD65. Similar results were obtained from an immunoprecipitation/SDS-PAGE test in which a protein corresponding to GAD65 was immunoprecipitated by all three different sera used, e.g., C38, IDDM, and GAD6 (Fig. 7). These results suggest that MGAD used in the present studies is GAD65.

**DISCUSSION**

Although GABA is a major inhibitory neurotransmitter in the mammalian CNS, the mode of regulation of GABA biosynthesis in the brain remains elusive and controversial. Numerous contradicting reports have appeared in the literature regarding the effect of ATP on GAD activity. Some reports show that ATP inhibits GAD activity (24, 25, 29–31), whereas others report the activation of GAD by ATP (32, 33). These contradicting reports can now be explained by the findings reported here. MGAD is activated by ATP, and we have previously shown that SGAD is inhibited by ATP (17, 18). Hence, it is reasonable to suggest that the effect of ATP on GAD in a particular subcellular location could result in activation or inhibition and that the net effect observed would depend on the relative amounts of soluble and membrane-bound GAD present. Activation of MGAD by ATP appears to be mediated by protein phosphorylation, presumably mediated by a membrane-associated protein kinase(s) that is sensitive to vesicular proton gradient. This notion is supported by the following observations: 1) MGAD activity is greatly reduced after treatment with phosphatase (Fig. 3); 2) direct phosphorylation of MGAD has been demonstrated in the presence of [γ-32P]ATP, but not [α-32P]ATP (Fig.
5); 3) activation of MGAD by ATP could be obtained even with extensively washed membrane preparations, without addition of any soluble components; 4) ATP activation of MGAD is abolished when the proton gradient on the synaptic vesicle is disrupted such as in the presence of a vesicular proton pump (V-ATPase) inhibitor, bafilomycin A1, the ionophore gramicidin, or the protonophore uncoupler CCCP, etc. (Fig. 2); 5) MGAD has been shown to be an integral component of synaptic vesicles (34–36); 6) a non-hydrolyzable ATP analog, AMP-PNP, has no effect on MGAD (Fig. 4). Based on the above observations, together with the fact that both MGAD and GABA transporter activities depend on the functional integrity of vesicular proton gradient, the following sequence of events leading from neuronal stimulation to activation of MGAD is proposed (Fig. 8); when GABA is released by exocytosis after the arrival of an action potential (step 1), synaptic vesicles are recycled by means of coated pits (step 2). Coated vesicles are then returned to the resting state of synaptic vesicles, where the proton gradient is restored by V-ATPase (step 3) and MGAD is activated by synaptic vesicle membrane-associated protein kinase (step 4). GABA synthesized by MGAD is then transported into synaptic vesicles by the GABA transporter (step 5). These refilled GABA-containing synaptic vesicles are ready to be released upon arrival of a new action potential. This novel model provides a functional link between synthesis and packaging of GABA in the GABAergic terminals. Previously, we have reported that SGAD is activated by calcineurin-mediated dephosphorylation and is inhibited by protein kinase A-mediated protein phosphorylation (17, 18). Furthermore, we proposed that when GABA neurons are stimulated, the influx of Ca\(^{2+}\) into the terminal (step 6, Fig. 8) activates the Ca\(^{2+}\)-dependent phosphatase, calcineurin (also known as PrP2B), resulting in dephosphorylation and activation of SGAD (step 7, Fig. 8). The newly synthesized GABA can also be transported into the synaptic vesicles (step 8, Fig. 8) or be metabolized to generate ATP through the GABA shunt pathway (step 9, Fig. 8). Recently we have shown that the taurine synthesizing enzyme, cysteine sulfenic acid decarboxylase behaves similarly to MGAD, namely activation by ATP and protein phosphorylation and inhibition by dephosphorylation (37, 38). However, there is a major difference between cysteine sulfenic acid decarboxylase and MGAD in that the former is regulated by product inhibition, whereas the latter is not affected by its product, GABA, even at a concentration of 25 mM (Table I). This is also consistent with findings reported in the literature (33).

To identify the specific kinase involved in the regulation of MGAD, several inhibitors specific to serine and threonine kinases were employed. None of the inhibitors had any effect on ATP activation of MGAD (Table I), suggesting that, unlike SGAD or cysteine sulfenic acid decarboxylase, MGAD is not
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directly regulated by some common protein kinases, e.g., protein kinase A and protein kinase C. Upon examining the DNA sequences of GAD65 in porcine and rat brains, a consensus sequence site, Arg-Xaa-(Xaa)-Ser/Thr-Xaa-Ser/Thr, for an autophosphorylation-dependent serine/threonine protein kinase, which is cAMP/Ca\(^{2+}\)-independent (19), was found to exist within the open reading frame. Therefore, MGAD may be regulated by protein phosphorylation by a cAMP/Ca\(^{2+}\)-independent kinase as yet to be identified.

In summary, regulation of MGAD may be mediated through the vesicular proton gradient, perhaps by a mechanism involving protein phosphorylation. Conceivably, its regulation may be coupled to the V-ATPase, the GABA transporter, or another transmembrane protein on the synaptic vesicle. These possibilities along with a complete structural analysis of MGAD are currently under investigation.

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