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L-homocysteine sulfinic acid and other acidic homocysteine derivatives are potent and selective metabotropic glutamate receptor agonists

QI SHI, JASON E. SAVAGE, SANDRA J. HUFEISEN, LAURA RAUSER, EWA GRAJKOWSKA, PAUL ERNSBERGER, JARDA T. WROBLEWSKI, JOSEPH H. NADEAU AND BRYAN L. ROTH[1]

Departments of Biochemistry (Q.S., B.L.R.), Genetics (Q.S., J.H.N.), and Nutrition (P.E.), National Institute of Mental Health Psychoactive Drug Screening Program (J.E.S., S.J.H., L.R., B.L.R.), School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106; Department of Pharmacology (J.T.W., E.G), Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, D.C. 20007

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To whom correspondence should be addressed:
Bryan L. Roth MD, PhD
Biochemistry; RM W438
Case Western Reserve University Medical School
Cleveland, OH 44106
roth@biocserver.cwru.edu (email)
216-368-2730 (Office); 216-368-3419 (Fax)

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Abstract

Moderate hyperhomocysteinemia is associated with several diseases including coronary artery disease, stroke, Alzheimer's disease, schizophrenia and spina bifida. However, the mechanisms for their pathogenesis are unknown but could involve the interaction of homocysteine or its metabolites with molecular targets such as neurotransmitter receptors, channels or transporters. We discovered that L-homocysteine sulfinic acid (L-HCSA), L-homocysteic acid (L-HCA), L-cysteine sulfinic acid (L-CSA) and L-cysteic acid (L-CA) were potent and effective agonists at several rat metabotropic glutamate receptors (mGluRs). These acidic homocysteine derivatives (1) stimulated phosphoinositide hydrolysis in the cells stably expressing the mGluR1, mGluR5, or mGluR8 (plus $G_{\alpha qi9}$), and (2) inhibited the forskolin-induced cAMP accumulation in the cells stably expressing mGluR2, mGluR4 or mGluR6, with different potencies and efficacies depending on receptor subtypes. Of the four compounds, L-HCSA is the most potent agonist at mGluR1, mGluR2, mGluR4, mGluR5, mGluR6 and mGluR8. The effects of the four agonists were selective for mGluRs because activity was not discovered when L-HCSA and several other homocysteine derivatives were screened against a large panel of cloned neurotransmitter receptors, channels, and transporters. These findings imply that mGluRs are candidate G-protein coupled receptors for mediating the intracellular signaling events induced by acidic homocysteine derivatives. The relevance of these findings for the role of mGluRs in the pathogenesis of homocysteine-mediated phenomena is discussed.

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Introduction

Homocysteine, a sulfur-containing amino acid, is involved in many metabolic pathways including trans-sulfuration in cysteine synthesis, re-methylation in methionine synthesis, trans-methylation of DNA, proteins and lipids, and biosynthesis of small hormonal and neuronal signaling molecules. The normal range for total plasma homocysteine in adults is 5-15 μM with an average of 10 μM (Kang et al., 1992). Abnormal elevation of total plasma homocysteine is classified as moderate (16~30 μM), intermediate (31~100 μM), or severe (>100 μM) hyperhomocysteinemia (Kang et al., 1992). Numerous epidemiological studies have reported that as a risk factor, moderate hyperhomocysteinemia is associated with (1) cardiovascular diseases such as atherosclerotic (Durand et al., 2001; McCully, 1996) and ischemic cardiovascular diseases (Brattstrom and Wilcken, 2000; Ueland et al., 2000), stroke (Hankey and Eikelboom, 2001), and venous thrombosis (Makris, 2000), (2) neuropsychiatrical disorders such as Alzheimer's disease (Miller, 2000), schizophrenia (Levine et al., 2002) and cognitive dysfunction (Morris et al., 2001), (3) developmental disorders such as neural tube defects (van der Put et al., 2001), and (4) complications of pregnancy (Aubard et al., 2000). However, the pathogenic mechanisms underlying the moderate-hyperhomocysteinemia-associated diseases are not fully understood. Major impediments hindering our understanding of the mechanisms by which hyperhomocysteinemia influences these diseases include (1) moderate hyperhomocysteinemia, as a risk factor, co-exists with other conventional risk factors in these diseases, (2) all the diseases for which hyperhomocysteinemia is a risk factor are polygenetic traits that are also affected by environmental factors, and (3) because homocysteine is also

involved in normal metabolic pathways of many biologically functional molecules, abnormalities in homocysteine metabolism may adversely affect many related and unrelated pathways which in turn cause diseases and disorders.

Considerable effort has been made to characterize the adverse effects of homocysteine on cells or tissues since McCully's initial report regarding pathogenic homocysteine (McCully, 1969). Homocysteine-induced oxidant stress (HIOS) is a major finding from studies that attempt to discover how homocysteine elevations contribute to disease (Loscalzo, 1996). According to the HIOS model, the highly reactive thiol-group of homocysteine is readily oxidized to generate reactive oxygen species that in turn cause damage to proteins and lipids (Loscalzo, 1996). The HIOS hypothesis is established mainly on the results from *in vitro* studies using high concentrations of homocysteine (Kokame et al., 1996; Lang et al., 2000; Outinen et al., 1999; White et al., 2001). Other studies (Weiss et al, 2001) suggest that HIOS might be important *in vivo* when homocysteine levels are not greatly elevated. It is likely that there are additional, unknown, molecular mechanisms that control moderate hyperhomocysteinemia-associated diseases in common cases (Miller, 2000; Spence et al., 1999; Vollset et al., 2001). Given that moderate hyperhomocysteinemia can act as a risk factor for various diseases at low levels in comparison to the high doses *in vitro* studies, we wondered if a specific interaction between homocysteine and some unidentified functional proteins, such as receptors, might be required to link the moderately elevated level of homocysteine to the pathogenesis of the associated diseases.

Certain non-essential amino acids and derivatives, such as L-glutamate, L-aspartate and γ-aminobutyric acid (GABA), have long been known to act as neurotransmitters (Bennett and Balcar, 1999). A group of sulfur-containing amino acids were previously found to exhibit effects similar to L-glutamic acid and L-aspartic acid (Thompson and Kilpatrick, 1996). These sulfur-containing amino acid analogues include (1) the L-glutamate analogues, L-homocysteine sulfinic acid (L-HCSA) and L-homocysteic acid (L-HCA), and (2) the L-aspartate analogues, L-cysteine sulfinic acid (L-CSA) and L-cysteic acid (L-CA). Kingston and colleagues demonstrated that L-HCSA, L-CSA, and L-CA, but not L-HCA, were capable of stimulating phosphoinositide hydrolysis in the cells transfected with human metabotropic glutamate receptor subtype 1 (mGluR1) or subtype 5 (mGluR5) which belong to Group I mGluRs. The potencies of these analogues were similar that of glutamate (Kingston et al., 1998). These findings suggest that homocysteine derivatives may interact with Group I mGluRs and regulate their signaling function. However, it remains unclear whether these L-glutamate analogues act as full- or partial-agonists at Group I mGluRs, and whether they can interact with other mGluRs (Group II and III) that are negatively coupled to adenylate cyclase (De Blasi et al., 2001) or ionotropic glutamate receptors (iGluRs). Recent studies of homocysteine metabolism revealed that homocysteine thiolactone, a homocysteine derivative, is synthesized by methionyl-tRNA synthetase under physiological conditions (Jakubowski et al., 2000). Therefore, other homocysteine derivatives are also candidates for interaction with cell-surface receptors, which may link the effects of homocysteine to its final cellular targets.

The goal of this study is to identify the cell-surface receptors, ion channels and transporters that act as biological sensors for homocysteine and its derivatives. We screened 60 different membrane-bound receptors, ion channels, and transporters from 15 different families

for molecular targets that interact with homocysteine or its derivatives. In second messenger generation assays, we tested the regulatory activity of the acidic homocysteine derivatives, including L-HCSA, L-HCA, L-CSA and L-CA, on metabotropic glutamate receptors. We found that metabotropic glutamate receptors are the receptor candidates for several acidic oxidized derivatives of homocysteine.

Materials and Methods

Chemical Compounds. D,L-homocysteine (D,L-HCY), L-cysteine (L-CYS), D,L-homocysteic acid (D,L-HCA), D-homocysteic acid (D-HCA), L-homocysteic acid (L-HCA), L-cysteic acid (L-CA), D-homocysteine sulfinic acid (D-HCSA), L-homocysteine sulfinic acid (L-HCSA), L-cysteine sulfinic acid (L-CSA), D-homocysteine thiolactone (D-HCYT), L-homocysteine thiolactone (L-HCYT), D-cystine (D-CTN), L-cystine (L-CTN), D-methionine (D-MET), L-methionine (L-MET), 4-aminophosphonobutyrate (AP4), and L-glutamate (L-GLU) were purchased from Sigma Chemical Co. (St. Louis, MO). Figure 1 shows the chemical structure of the tested compounds. Radioligands were purchased from Perkin Elmer, except for GR-125,743 that was purchased from the Amersham Pharmacia Biotech. Myo-[^{3}H]-inositol was purchased from NEN.

Receptors. Cell lines stably transfected with recombinant cDNA encoding receptors or cell lines that express endogenous receptors were used for the drug screen. The recombinant cDNA included (1) human adrenergic receptors, α1A, α1B, α2A, α2B, α2C, and rat adrenergic receptors, β1 and β2; (2) rat cannabanoid CB1 receptor; (3) dopaminergic receptors, hD1, hD2,

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hD3, rD4, hD5; (4) human histamine receptors, H1, H2, and H4; (5) rat imidazoline receptor; (6) human muscarinic acetylcholine receptors, M1, M2, M3, M4, M5; (7) human nicotinic acetylcholine receptors, α2/β2, α2/β4, α3/β2, α3/β4, α4/β2, α4/β4; (8) human opiate receptors, μ, δ and κ; (9) human peptide receptors, V1, V2, V3, OT; (10) serotonergic receptors, h5-HT1A, h5-HT1Da, h5-HT1Db, r5-HT2A, r5-HT2C, h5-HT3, h5-HT5A, h5-HT6, h5-HT7; (11) human transporters of serotonin (SERT), norepinephrine (NET), and dopamine (DAT) as previously described (Roth et al., 1998;Roth et al., 2001;Rothman et al., 2000;Shapiro et al., 2002; Roth et al, 2002); (12) rat metabotropic glutamate receptors, mGluR1a, mGluR2, mGluR4, mGluR5a, mGluR6, and mGluR8 as previously described (Gomeza et al., 1996;Kozikowski et al., 1998;Wroblewska et al., 1997). The endogenous receptors included (1) GABA receptors, $GABA_A$, $GABA_B$, $GABA_{BZP}$ from rat forebrain; (2) histamine receptors H1 from rat forebrain; (3) rat nicotinic acetylcholine receptor, α4/β2; (4) ionotropic glutamate receptor NMDA from rat forebrain; and (5) voltage-sensitive Ca^{2+} channel from rat heart.

Radioligand Competitive Binding Assays. Receptor-binding affinity of homocysteine and its derivatives was determined by radioligand binding competitive experiments. The assays were performed with cell lysates extracted from the cells stably transfected with the recombinant-receptor-expression vectors (Roth et al., 1998;Roth et al., 2001;Rothman et al., 2000;Shapiro et al., 2002), or with forebrain membrane preparation containing the endogenous receptors of interest (Roth et al., 1991). Competitive binding assays were carried under standard conditions as previously detailed (Roth et al., 2001, 2002; Rothman et al., 2000). The conditions for the different binding assays and *K*i values for reference ligands are summarized in Table 1. On-line protocols for binding assays are available at http://pdsp.cwru.edu/nimh/binding.htm.

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Homocysteine and its derivatives were dissolved in 0.05 % L-ascorbic acid and incubated with radioligands as indicated Table 1. Binding data were determined in duplicate.

Phosphoinositide Hydrolysis Assays Using Recombinant Receptors. mGluR1a and mGluR5a (Group I) were stably expressed in Chinese hamster ovary (CHO) cells (Wroblewska et al., 1997). mGluR8 (Group III) was stably expressed in a CHO cell line that expressed a chimeric G protein, $G_{\alpha qi9}$, and experimentally allowed a positive coupling of mGluR8 to phospholipase C (Gomeza et al., 1996). Cultured in 96-well plates, the receptor-expressing cells were incubated overnight in glutamine-free culture medium supplemented with 0.75 μCi myo-[^{3}H]-inositol to label the cell membrane phosphoinositides. Incubations with homocysteine derivatives were carried out for 45 min at 37°C in Locke's buffer (156 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, 5.6 mM glucose, and 20 mM Hepes, pH 7.4) containing 20 mM LiCl that blocks the degradation of inositol phosphates (IPs). The reaction was terminated by aspiration and addition of 0.1 M HCl. IPs were extracted with 0.1 M HCl. [^{3}H]-IPs were separated by anion exchange chromatography and determined in duplicate by liquid scintillation counter (LKB) as described previously (Raulli et al., 1991).

Cyclic AMP Formation Assay. Receptor-mediated inhibition of the forskolin-induced elevation of cyclic AMP formation was described previously (Wroblewska et al., 1997). In brief, mGluR2 (Group II) and mGluR6 (Group III) were stably expressed in CHO cells whereas mGluR4 (Group III) was stably expressed in baby hamster kidney (BHK) cells (Wroblewska et al., 1997). Cultured in 96-well culture plates, the receptor-expressing cells were preincubated for 10 min at 37°C in Locke's medium containing 300 μM isobutylmethylxanthine that inhibits the activity of

phosphodiesterases to prevent the degradation of cAMP. Then, 5 μM forskolin was added without or with the test compounds, and the incubation was continued for 10 min. After incubation, the medium was rapidly aspirated. cAMP was extracted with 0.1 M HCl and measured by radioimmunoassay using a magnetic Amerlex RIA kit (Amersham).

Data Analysis. For binding assays, data analysis and curve generation were carried out with GraphPad Prizm 3.02 (GraphPAD, San Diego, CA). *K*i values were calculated with LIGAND software (Munson and Rodbard, 1981) as previously detailed (Roth et al, 2002; Rothman et al., 2000). For second messenger assays, EC_{50} values were determined by fitting the normalized data to the logistic equation by non-linear regression using SigmaPlot software (SPSS Science, Chicago, IL) whereas curve generation was carried out with GraphPad Prizm 3.02.

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Results

Receptor Binding Affinity of Homocysteine and Its Derivatives. Radioligand competitive binding assays were performed to test the specific binding affinity of homocysteine and its derivatives against a panel of receptors, ion channels, and transporters via the resources of the NIMH Psychoactive Drug Screening Program (Table 2). The survey was conducted in two steps, a preliminary screen followed by *K*i determination. The preliminary screen was performed to determine percent inhibition of the specific radioligand binding by homocysteine and its derivatives at 10 μM. Determination of *K*i values were conducted on the compounds whose inhibition of specific radioligand binding to the corresponding receptors was greater than 50 percent. As determined in the preliminary screen, significant inhibition (i.e., >50%) by compounds was seen for α1B-adrenergic receptor (by L-HCA, D-HCA, and D,L-HCY), imidazoline receptor (by D,L-homocysteine), and serotonin receptors of 5-HT1A (by L-CSA, D,L-HCA, D- and L-HCYT, and L-CTN), 5-HT1Da (by L-CTN), 5-HT2A (by L-HCA and D,L-HCY), 5-HT2C (by L-HCA and D,L-HCY), 5-HT3 (by L-CSA and D-HCSA) and 5-HT6 (by D,L-HCA) (data not shown). No significant inhibition (i.e., ≤50%) was found for adrenergic receptors (α1A, α2A, α2B, α2C, β1, and β2), dopaminergic receptors (D1~D5), cannabanoid CB1 receptor, GABA receptors ($GABA_A$, $GABA_B$, and $GABA_{BZP}$), ionotropic NMDA glutamate receptor (except for L-cystine), histamine receptors, muscarinic acetylcholine receptors (M1~M5), nicotinic receptors, opiate receptors (μ, δ, κ), peptide receptors (V1~V3, OT), serotonin receptors (5-HT1Db, 5-HT5A, and 5-HT6), voltage-sensitive calcium channel, and transporters of serotonin, norepinephrine and dopamine.

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Homocysteine and its derivatives had *K*i values greater than 10,000 nM for the adrenergic receptor and serotonin receptors that initially passed the preliminary screen, suggesting that homocysteine and its derivatives have minimal affinities for these functional membrane proteins at least under the assay conditions that were used. It is likely that high concentrations of homocysteine and its metabolites non-specifically inhibit binding to 5-HT and adrenergic receptors.

Stimulation of Phosphoinositide Hydrolysis through Activation of mGluR1, mGluR5 and mGluR8. Metabotropic glutamate receptors (mGluRs) are well-characterized G-protein coupled receptors composing a large family of eight different mGluR subtypes in three different groups based on their molecular structure and pharmacological behavior (De Blasi et al., 2001). Group I mGluRs includes mGluR1 and 5, Group II mGluRs includes mGluR2 and 3, and Group III mGluRs includes mGluR4, 6, 7 and 8. Stimulation of mGluRs regulates intracellular signaling through changing the levels of intracellular second messengers. In general, Group I mGluRs are involved in activation of phospholipase C (PLC) via $G_{\alpha q}$ or $G_{\alpha o}$ whereas Group II and III mGluRs participate in inhibition of adenylyl cyclase via $G_{\alpha i}$ (De Blasi et al., 2001). In the present study, recombinant cDNAs for mGluR1, mGluR5 and mGluR8 were stably transfected and expressed in cells (see Materials and Methods). It should be noted that the *in vitro* expressed mGluR8 in the present study is artificially coupled to PLC by co-expression of a recombinant chimeric $G_{\alpha qi9}$ because the endogenous mGluR8 is not naturally coupled to PLC (Gomeza et al., 1996). L-homocysteine sulfinic acid (L-HCSA), L-homocysteic acid (L-HCA), L-cysteine sulfinic acid (L-CSA), and L-cysteic acid (L-CA) were used to investigate whether they were able to stimulate mGluR1, mGluR5 or mGluR8 expressed in the cells. As positive controls, we

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used L-glutamate, a known endogenous ligand for the mGluRs, and L(+)-2-amino-4-phosphonobutyric acid (AP4), a known agonist for Group III mGluRs (Wroblewska et al., 1997). The stimulatory activity of these homocysteine derivatives at the mGluRs was determined by measurements of their ability to increase intracellular inositol phosphates (IPs), products from the hydrolysis of membrane phosphoinositides (PI).

The dose response curves of the relative stimulatory activity are plotted in Figure 2. Based on the EC_{50} values of the curves (Table 3), the relative potencies are ranked as follows for mGluR1: L-glutamate ≈ L-HCSA > L-CA ≈ L-CSA >> L-HCA; mGluR5: L-HCSA ≈ L-HCA ≈ L-glutamate > L-CA ≈ L-CSA; mGluR8: AP4 >> L-HCSA ≈ L-HCA ≈ L-CA > L-CSA. In the ranking, "≈" indicates the fold difference of EC_{50} is less than 2; ">" indicates the fold difference is equal to or greater than 2, but less than 10; ">>" indicates the fold difference is equal to or greater than 10. The results demonstrate that the acidic homocysteine derivatives stimulated mGluR1, mGluR5 and mGluR8 expressed *in vitro* with varying potency and efficacy. In particular, L-HCSA was as potent and efficacious as glutamate at mGluR1; L-HCSA and L-HCA were as potent and efficacious as glutamate at mGluR5; L-CSA and L-CA were less potent than glutamate but similarly efficacious as glutamate at mGluR5 (Figure 2).

Inhibition of Cyclic AMP Formation through Activation of mGluR2, mGluR4, and mGluR6. mGluR2, mGluR4 and mGluR6 are negatively coupled to adenylyl cyclase. To investigate whether the homocysteine derivatives stimulate mGluR2, mGluR4 or mGluR6, we treated the cells that stably expressed each of the three receptors with L-HCSA, L-HCA, L-CSA and L-CA. The stimulatory activity of these compounds at the mGluRs was determined by

measurements of their ability to reduce forskolin-stimulated cAMP. Dose response curves of the relative stimulatory activity are shown in Figure 3 and EC50 values are in Table III. The relative potencies are ranked as the following, for mGluR2: L-glutamate >> L-CA ≈ L-HCSA ≈ L-HCA > L-CSA; mGluR4: AP4 >> L-HCA ≈ L-HCSA > L-CA > L-CSA; mGluR6: AP4 > L-HCSA > L-HCA >> L-CA ≈ L-CSA. The results demonstrate that the homocysteine derivatives stimulated mGluR2, mGluR4 and mGluR6 with different potencies and efficacies, but they are less potent than L-glutamate and AP4. Of the homocysteine derivatives, L-HCSA and L-HCA were more potent than L-CSA and L-CA at mGluR4 and mGluR6, whereas their potency was similar to that of L-CA at mGluR2 (Table 3 and Figure 3).

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Discussion

The major finding of the present study is that various acidic homocysteine derivatives, especially L-HCSA and L-HCA, potently and specifically activate cloned rat mGluRs expressed *in vitro*. These findings imply that Groups I, II, and III mGluRs represent specific receptors for acidic homocysteine derivatives. One prior radioligand binding study demonstrated that L-HCSA, L-CSA, and L-CA had high binding affinities for mGluR1$_\alpha$ with *K*i values of 440, 3,510 and 8,050 nM, respectively (Kingston et al, 1998). Herein, we further demonstrate that, with respect to the binding-site specificity of radioligands, homocysteine or its derivatives do not have detectible binding affinity for 54 other GPCRs, ion channels or transporters. Taken together, these results suggest that acidic homocysteine derivatives, if present at high enough concentrations, are likely to preferentially modulate the activity of mGluRs and thereby regulate intracellular signaling events and neural transmission.

GPCRs have a single ligand-binding pocket although some receptors, channels and transporters may contain more than one ligand-binding site (Lu et al., 2002;Ma et al., 2002). In the present survey, we mainly used radioligand competitive binding assays to test whether the test ligands have potential capacity to regulate the tested receptors, channels or transporters. We also tested the PCP and MK-801 sites for the NMDA receptor in our competitive binding assay. We found (Table 2) that L-cystine had medium affinity (*K*i = 3,240 nM) for the MK801 site on the NMDA receptor whereas L-HCA or the other compounds had low binding-affinity for the MK801 site. Moreover, their binding-affinity for the PCP site was also quite low. A previous study showed that L-HCA bound to the NMDA receptor through the glutamate-binding site with extremely low affinity (*K*i = 9,500 nM) in comparison to that L-glutamate (*K*i = 500 nM) at the same receptor (Olney et al., 1987). In the present paper, we were unable to estimate the binding

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affinity of homocysteine and its derivatives for the glutamate-binding site on the NMDA receptor because we did not measure significant inhibition (i.e. >50% at 10,000 nM).

Our analysis of the dose-response curves revealed that the agonist properties, i.e., potencies and efficacies, of the acidic homocysteine derivatives (L-HCSA, L-HCA, L-CSA and L-CA) were similar to each other at mGluR5, but displayed profound differences at the other five mGluRs tested (i.e., mGluR1, 2, 4, 6, and 8). A close examination of the chemical structures reveal (1) L-HCSA and L-HCA are sulfur-containing 4-carbon acidic amino acids, whereas L-CSA and L-CA are sulfur-containing 3-carbon acidic amino acids, and (2) L-HCSA and L-CSA are sulfinic acids, whereas L-HCA and L-CA are sulfonic acids. In comparison to the endogenous neurotransmitter L-glutamate, the chain length of L-HCSA and L-HCA molecules is similar to that of L-glutamate. By contrast, the chain length of L-CSA and L-CA are one carbon shorter than that of L-glutamate. Furthermore, the distal sulfonate groups of L-HCA and L-CA contain one more oxygen atom than the distal carboxylate group of L-glutamate and the distal sulfinate groups of L-HCSA and L-CSA (Figure 1). The endogenous full agonist glutamate probably fits to a binding pocket on the mGluRs in a “perfect” manner. The closer to glutamate’s chemical structure the chemical structure of a glutamate analogue is the better fitting to the binding pocket and the stronger agonist property the glutamate analogue will be. The structure of L-HCSA is the most similar to L-glutamate among the four tested glutamate analogues; this may explain why L-HCSA is generally the most potent and efficacious metabolite tested. Because all of the present studies were performed with cloned receptors expressed in heterologous expression systems, the issue of receptor reserve and relative agonist potency must be considered. Since there are no adequate commercially available radioligands available for most of these receptors, the absolute receptor number is impossible to quantify. Nonetheless, we

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have In this regard, we have obtained preliminary data with rat cortical neurons in culture that L-HCSA is equipotent with L-glutamate for activation of PI hydrolysis (Shi et al, manuscript in preparation) as is predicted from our current studies with cloned receptors.

Kingston et al reported that L-HCSA, L-CSA and L-CA stimulated PI hydrolysis through activation of human mGluR1α and mGluR5a expressed in a transformed Syrian hamster cell line (Kingston et al., 1998). In their study, L-HCSA was less potent than L-glutamate at human mGluR5. In our study, L-HCSA was more potent than L-glutamate and L-HCA was as potent as L-glutamate at rat mGluR5 expressed in Chinese hamster ovary cells. The different results could be due to differences in the host cell lines, the assay conditions, or receptor species used in the two independent studies. Our functional studies demonstrated that the acidic homocysteine derivatives also stimulated Group II and III mGluRs. Therefore, all three classes of mGluRs can be regulated by acidic homocysteine derivatives. In the brain, Group I mGluRs are located mainly postsynaptically whereas Group II and III mGluRs are located presynaptically (De Blasi et al., 2001); others have suggested that mGluR5 receptors may also be presynaptic (Romano et al, 2002). In terms of spatial distribution, mGluR1 and mGluR5 are located in a variety of brain regions (Daggett et al., 1995;Shigemoto et al., 1992) whereas the distribution of Group II and III mGluRs in the brain is more restricted than that of Group I mGluRs (Bennett and Balcar, 1999;Kinzie et al., 1995;Nakajima et al., 1993;Ohishi et al., 1993;Tanabe et al., 1993). Knockout mouse studies reveal that mGluRs are involved in a variety of physiological functions including motor coordination and spatial learning (Conquet et al., 1994), associative learning (Aiba et al., 1994), hippocampal long-term depression (Yokoi et al., 1996), complex motor learning (Pekhletski et al., 1996), and visual transmission (Masu et al., 1995). If the acidic homocysteine

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derivatives are present in high enough local concentrations, they could functionally modulate mGluR activity.

L-HCSA, L-HCA, L-CSA, and L-CA have been long speculated to be endogenous neurotransmitters (Curtis and Watkins, 1960) although there has been no strong support for this hypothesis. High-performance liquid chromatographic studies showed that L-HCSA and L-HCA were detectable in astrocytes (72 and 49 pmol/mg protein respectively) (Grieve and Griffiths, 1992), and acute exposure to β-adrenergic receptor agonists induced a 3.7-fold increase in the concentration of L-HCA (Do et al., 1997). One prior clinical study showed that patients suffering methotrexate-induced neurotoxicity had high concentrations of homocysteic acid (119.1 μM) and cysteine sulfinic acid (28.4 μM) in their cerebrospinal fluid (Quinn et al., 1997), implying that acidic homocysteine derivatives can be produced in the central nervous system under certain circumstances.

The identification of metabotropic glutamate receptors as the molecular targets for acidic homocysteine derivatives could be important for clarifying the molecular mechanisms responsible for the pathogenesis of various neuropsychiatric diseases associated with moderate hyperhomocysteinemia (Spence et al., 1999). Moderate hyperhomocysteinemia is also a risk factor for stroke and cardiovascular disease in general (Beckett and Marsden, 1997;Spence et al., 1999) although it has been unclear how elevated levels of homocysteine increase the risk for stroke and other cardiovascular diseases. Quite recent studies (Collard et al, 2002) in which human brain endothelial cells were demonstrated to have functional mGluR's illuminates a potential mechanisms by which elevations of homocysteine and acidic homocysteine derivatives

may increase the risk for cardiovascular disease. In these studies Collard et al (2002) demonstrated that activation of endothelial mGluR's modulates vascular permeability. Other studies have demonstrated the presence of cardiac mGluR (Gill et al, 1999) whose expression may be altered by nicotine administration (Hu et al, 2002).

Animal studies have established that the glutamate-mediated excitotoxicity is a cause of neuronal apoptosis in cerebral ischemia (Collingridge and Lester, 1989). Recent studies with cultured neurons showed that activation of Group I mGluR can exacerbate NMDA-induced excitotoxic injury (Mukhin et al., 1997) although this exacerbation is not abrogated in mGluR1 knockout mice (Ferraguti et al., 1997). An additional study suggests that non-competitive mGluR5 antagonists inhibit NMDA-induced apoptosis in primary cortical neuron cultures, indicating the involvement of mGluR5 (Bruno et al., 2000). It is conceivable, therefore, that elevations of acidic homocysteine derivatives may predispose individuals to the neurotoxic consequences of stroke via alterations in mGluR functioning.

Another disease for which moderate hyperhomocysteinemia is a risk factor is dementia, including Alzheimer's disease (Miller, 2000) and schizophrenia (Levine et al, 2002). Recent epidemiological studies reported that serum homocysteinemia concentrations >14.0 μM increase the risk for AD (Miller, 2000;Seshadri et al., 2002) and schizophrenia (Levine et al, 2002). A previous *in vivo* study showed that activation of mGluRs induced the intraneuronal production of amyloid beta fragments (Aβ) together with degeneration of pyramidal neurons in CA1 region of hippocampus (Stephenson and Clemens, 1998). Additional findings showed that the aggregated-Aβ-mediated activation of mGluRs and the aggregated-Aβ-elicited intracellular inositol phosphate formation are two consecutive events preceding the aggregated Aβ-induced neuronal

apoptosis *in vitro* (Allen et al., 1999;Kanfer et al., 1998;Singh et al., 1998). These prior studies suggest that activation of mGluRs may contribute to the pathogenesis of Alzheimer's disease although these ideas are still quite speculative. The mechanism by which an elevation of acidic homocysteine derivatives may exacerbate schizophrenia is unknown, although mGluR's are known to affect a number of signaling processes thought to be altered in schizophrena and mGluR's have been suggested to be a target for antipsychotic drug development (see for instance Kalkman, 2002).

In conclusion, we have demonstrated that acidic homocysteine derivatives are selective mGluR-family agonists. It is conceivable that if certain acidic homocysteine derivatives are elevated sufficiently under normal and pathological conditions they could modulate the activity of a variety of mGluRs. Future experiments will be needed to determine whether the local concentrations of acidic homocysteine derivatives are elevated in moderate hyperhomocysteinemia and if, via mGluRs, they influence other risk factors to contribute to the pathogenesis of the diseases associated with moderate hyperhomocysteinemia.

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Legends for Figures.

Figure 1. Chemical Structure of Homocysteine and its derivatives.

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Figure 2. Stimulatory effect of homocysteine derivatives on phosphoinositide hydrolysis in the CHO cells stably expressing metabotropic glutamate receptors, mGluR1 (A), mGluR5 (B), and mGluR8 (C). After preincubation with myo-[^{3}H]-inositol overnight, cells were treated for 45 minutes at 37°C with L-HCSA, L-homocysteine sulfinic acid (▲); L-HCA, L-homocysteic acid (△); L-CSA, L-cysteine sulfinic acid (●); L-CA, L-cysteic acid (○); L-Glu, L-glutamic acid (□); and AP4, L(+)-2-amino-4-phosphonobutyric acid (■). Determination of the produced [^{3}H]-IPs is described in Materials and Methods. Data were normalized to the maximal response induced by 1 mM glutamate for mGluR1 and mGluR5, or by 0.1 mM AP4 for mGluR8, and are presented as percentage of the maximal response (mean ± SEM). Dose-response curves of the induced inositol phosphates (IP) formation were generated with the IP data from cells expressing (A) mGluR1 in 5~9 independent experiments, (B) mGluR5 in 4~6 independent experiments, and (C) mGluR8 and $G_{\alpha qi9}$ in 4~15 independent experiments.

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Figure 3. Inhibitory affect of homocysteine derivatives on the forskolin-induced cyclic AMP formation in the CHO cells stably expressing mGluR2 (A) and mGluR6 (C), and in the baby hamster kidney (BHK) cells stably expressing mGluR4 (B). After blockade of phosphodiesterases for 10 minutes, cells were treated for 10 minutes at 37°C with 5 μM forskolin without or with L-HCSA (▲); L-HCA (△); L-CSA (●); L-CA (○); L-Glu (□); and AP4 (■). Cyclic-AMP concentration was determined by radioimmunoassay. Data were normalized to the maximal response induced by 0.1 mM glutamate for mGluR2, or by 0.1 mM AP4 for mGluR4 and mGluR6, and are presented as percentage of the maximal response (mean ± SEM). Dose-response curves of the inhibited cyclic AMP formation were generated with the cyclic AMP data from cells expressing (A) mGluR2 in 4~12 independent experiments, (B) mGluR4 in 4~12 independent experiments, and (C) mGluR6 in 4~12 independent experiments.

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Table 1. Conditions for Radioligand Competitive Binding Assays

Membrane-Bound Proteins	Species	Radioligand	Reference Ligand (*K*i in nM)	Binding Buffer
Receptors				
Adrenergic α1A	human, cloned	[^{125}I]-Heat	Prazosin (U/A)	20mM Tris pH 7.4 + 145 mM NaCl
Adrenergic α1B	human, cloned	[^{125}I]-Heat	Prazosin (U/A) Cyclazosin (U/A)	20mM Tris pH 7.4 + 145 mM NaCl
Adrenergic α2A	human, cloned	Clonidine	Epinephrine (U/A) Oxymetazoline (U/A)	Alpha 2 Binding Buffer
Adrenergic α2B	human, cloned	Clonidine	Epinephrine (U/A) Prazosin (U/A)	Alpha 2 Binding Buffer
Adrenergic α2C	human, cloned	Clonidine	Epinephrine (U/A) Prazosin (U/A)	Alpha 2 Binding Buffer
Adrenergic β1	rat, cloned	[^{125}I]-Pindolol	Atenolol (U/A)	50mM Tris pH 7.7 + 3 mM $MnCl_2$
Adrenergic β2	rat, cloned	[^{125}I]-Pindolol	ICI-118,551 HCl (U/A)	50mM Tris pH 7.7 + 3 mM $MnCl_2$
Cannabinoid CB1	rat brain	CP-55,940	WIN 55212-2 (8.2)	Cannabinoid Binding Buffer
Dopaminergic D1	human, cloned	SCH23390	SKF38393 (978.0000) Fluphenasine (21.0000)	D1 Dopamine Binding Buffer
Dopaminergic D2	human, cloned	N-Methylspiperone	Haloperidol (2.5000 Avg)	Dopamine Binding Buffer
Dopaminergic D3	human, cloned	N-Methylspiperone	Clozapine (155.0000)	Dopamine Binding Buffer
Dopaminergic D4	rat, cloned	N-Methylspiperone	Clozapine (29.0000)	Dopamine Binding Buffer
Dopaminergic D5	human, cloned	SCH23390	Olanzapine (74.0000)	D1 Dopamine Binding Buffer
$GABA_A$	rat forebrain	Muscimol	GABA (38.0000)	50mM Tris-Acetate pH 7.4
$GABA_B$	rat forebrain	Baclofen	GABA (U/A)	50mM Tris-HCl pH 7.4 + 2.5mM $CaCl_2$
$GABA_{BZP}$	rat forebrain	RO-15-1788	Diazapam (20.0000)	50mM Tris-HCl pH 7.4 + 2.5mM $CaCl_2$
Histamine 1	human, cloned	Pyrilamine		50mM Tris-HCl pH 7.4 + 0.5mM EDTA
Histamine 1	rat forebrain	Pyrilamine	Chlorpheniramine (U/A)	50mM Tris-HCl pH 7.4 + 0.5mM EDTA
Histamine 2	human, cloned	Pyrilamine		50mM Tris-HCl pH 7.4 + 0.5mM EDTA
Histamine 4	human, cloned	Pyrilamine		50mM Tris-HCl pH 7.4 + 0.5mM EDTA
Imidazoline I_1	rat PC12 cells	[^{125}I]Clonidine	Naphazoline (860.0)	Imidazoline I_1 Binding Buffer
Muscarinic M1	human, cloned	QNB	Pirenzepine (13.0000)	50mM Tris-HCl pH 7.7
Muscarinic M2	human, cloned	QNB	Methoctramine (42.0000)	50mM Tris-HCl pH 7.7
Muscarinic M3	human, cloned	QNB	4-DAMP (U/A)	50mM Tris-HCl pH 7.7
Muscarinic M4	human, cloned	QNB	Tropicamide (U/A)	50mM Tris-HCl pH 7.7
Muscarinic M5	human, cloned	QNB	Pirenzepine (U/A)	50mM Tris-HCl pH 7.7
Nicotinic α2/β2	human, cloned	Epibatidine	Nicotine (U/A)	50mM Tris-HCl pH 7.4
Nicotinic α2/β4	human, cloned	Epibatidine	Nicotine (9,900.0000)	50mM Tris-HCl pH 7.4
Nicotinic α3/β2	human, cloned	Epibatidine	Nicotine (U/A)	50mM Tris-HCl pH 7.4
Nicotinic α3/β4	human, cloned	Epibatidine	Nicotine (40,300.0000)	50mM Tris-HCl pH 7.4
Nicotinic α4/β2	human, cloned	Epibatidine	Nicotine (U/A)	50mM Tris-HCl pH 7.4
Nicotinic α4/β2	rat forebrain	Epibatidine	Nicotine (0.8400)	50mM Tris-HCl pH 7.4
Nicotinic α4/β4	human, cloned	Epibatidine	Nicotine (6,690.0000)	50mM Tris-HCl pH 7.4
Opiate μ	human, cloned	Diprenorphine	Naloxone (1.0000)	Standard Binding Buffer
Opiate δ	guinea pig forebrain	DPDPE	Naltrindole (0.0900)	Standard Binding Buffer
Opiate κ	guinea pig forebrain	Bremazocine	Naloxone (3.8000)	Standard Binding Buffer
Peptide V1	human, cloned	Vasopressin	Vasopressin (1.0000)	Vasopressin Binding Buffer
Peptide V2	human, cloned	Vasopressin	Vasopressin (0.7800)	Vasopressin Binding Buffer
Peptide V3	human, cloned	Vasopressin	Vasopressin (U/A)	Vasopressin Binding Buffer
Peptide OT	human, cloned	Oxytocin	Oxytocin (U/A)	Oxytocin Binding Buffer
Serotonergic 1A	human, cloned	8-OH-DPAT	WAY 100,635 (0.1000)	Standard Binding Buffer
Serotonergic 1Da	human, cloned	GR-125,743	Ergotamine (2.0000)	Standard Binding Buffer
Serotonergic 1Db	human, cloned	GR-125,743	Ergotamine (3.0000)	Standard Binding Buffer
Serotonergic 2A	rat, cloned	Ketanserin	Clozapine (10.9647)	Standard Binding Buffer
Serotonergic 2C	rat, cloned	Mesulergine	Clozapine (28.1890 Avg)	Standard Binding Buffer
Serotonergic 3	human, cloned	Zacopride	LY-278,584 (U/A)	50 mM Tris-HCl pH 7.4
Serotonergic 5A	human, cloned	LSD	Ergotamine (37.0000)	Standard Binding Buffer
Serotonergic 6	human, cloned	LSD	Clozapine (9.5000)	Standard Binding Buffer
Serotonergic 7	human, cloned	LSD	Clozapine (20.0000)	Standard Binding Buffer
Ion channels				
NMDA (MK801)	rat forebrain	MK801	MK801 (U/A)	5mM Tris-HCl pH 7.4
NMDA (PCP)	rat forebrain	TCP	PCP (66.0000)	5mM Tris-HCl pH 8.0
Ca^{2+} channel	rat heart	Nitrendepine	Nifedipine (0.67)	Ca^{2+} channel Binding Buffer
Transporters				
SERT	human, cloned	Citalopram	Fluoxetine (0.9000)	Transport Binding Buffer
NET	human, cloned	Nisoxetine	Nortriptyline (1.8000)	Transport Binding Buffer
DAT	human, cloned	GBR12935	4',4"-Diflouro-3a-(diphenylmethoxy) tropane hydrochloride (32.0)	Transport Binding Buffer

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Note: All radioligands except for [^{125}I]-Heat are labeled with ^{3}H. Alpha 2 Binding Buffer, 5mM Hepes, 5mM Tris-HCl, 0.5mM EGTA, 0.5mM EDTA, 0.5 mM $MgCl_2$, pH 8.0; Ca^{2+} channel Binding Buffer, 50mM Tris pH 7.4, 50mM NaCl, 1 mM $CaCl_2$; Cannabinoid Binding Buffer, 50mM Tris pH 7.4, 1 mM EDTA, 3mM $MgCl_2$, 5 mg/ml BSA; Dopamine Binding Buffer, 50mM Tris-HCl, pH 7.4, 50mM NaCl, 5mM $MgCl_2$, 0.5mM EDTA; D1 Dopamine Binding Buffer, 50mM Tris-HCl, pH 7.4, 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$; Imidazoline I_1 Binding Buffer, 5mM Tris pH 7.7, 1 mM EDTA, 0.5 mM $MgCl_2$; Oxytocin Binding Buffer, 50mM HEPES and 10 mM $MnCl_2$ pH 7.4; Standard Binding Buffer, 50 mM Tris-HCl, pH 7.4, 10mM $MgCl_2$, 0.1mM EDTA; Transport Binding Buffer, 50mM Tris-HCl, pH 7.4, 150mM NaCl, 5mM KCl; Vasopressin Binding Buffer, 20mM Tris-HCl, pH 7.4, 100mM NaCl, 10mM $MgCl_2$, 0.1mg/ml Bacitracin, 1mg/ml BSA. Avg, an average of several *K*i from different papers. U/A, unavailable in the on-line PDSP Drug Database in CWRU.

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Table 2. *K*i Values of Homocysteine and Derivatives in Competitive Binding Assays (mean ± SEM, nM)

Membrane-Bound Proteins	Compounds														
	D,L-HCY	L-CYS	D-HCYT	L-HCYT	D-HCSA	L-HCSA	L-CSA	D,L-HCA	D-HCA	L-HCA	L-CA	D-CTN	L-CTN	D-MET	L-MET
Receptors															
Adrenergic α1A	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adrenergic α1B	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adrenergic α2A	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adrenergic α2B	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	NT	>10,000	>10,000
Adrenergic α2C	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adrenergic β1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Adrenergic β2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Cannabanoid CB1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopaminergic D1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopaminergic D2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopaminergic D3	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopaminergic D4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopaminergic D5	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
$GABA_A$	NT	NT	NT	NT	NT	NT	NT	>10,000	NT	NT	NT	NT	NT	NT	NT
$GABA_B$	NT	NT	NT	NT	NT	NT	NT	>10,000	NT	NT	NT	NT	NT	NT	NT
$GABA_{BZP}$	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Histamine 1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Histamine 1*	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Histamine 2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Histamine 4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Imidazoline	164% Inh	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Muscarinic M1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Muscarinic M2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Muscarinic M3	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Muscarinic M4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Muscarinic M5	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α2/β2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α2/β4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α3/β2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α3/β4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α4/β2	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α4/β2*	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Nicotinic α4/β4	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Opiate μ	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Opiate δ	NT	NT	>10,000	>10,000	NT	NT	NT	>10,000	NT	NT	NT	NT	NT	NT	NT
Opiate κ	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Peptide V1	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Peptide V2	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Peptide V3	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Peptide OT	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT
Serotonergic 1A	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 1Da	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 1Db	>10,000	>10,000	NT	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 2A	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 2C	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 3	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 5A	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 6	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Serotonergic 7	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Ion channels															
NMDA (MK801)	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	**3,240 ±1,046**	>10,000	>10,000
NMDA (PCP)	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Ca^{2+}	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Transporters															
Serotonin	>10,000	>10,000	>10,000	>10,000	>10,000	NT	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Norepinephrine	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
Dopamine	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000

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Abbreviations: D,L-HCY, D,L-homocysteine; L-CYS, L-cysteine; D-HCYT, D-homocysteine thiolactone; L-HCYT, L-homocysteine thiolactone; D-HCSA, D-homocysteine sulfinic acid; L-HCSA, L-homocysteine sulfinic acid; L-CSA, L-cysteine sulfinic acid; D,L-HCA, D,L-homocysteic acid; D-HCA, D-homocysteic acid; L-HCA, L-homocysteic acid; L-CA, L-cysteic acid; D-CTN, D-cystine; L-CTN, L-cystine; D-MET, D-methionine; L-MET, L-methionine. NT, not tested. *, rat. Inh, inhibition.

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Table 3. Stimulatory effects of acidic homocysteine derivatives on mGluR's.

	mGluR1 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM	mGluR5 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM	mGluR2 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM	mGluR4 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM	mGluR6 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM	mGluR8 pEC_{50}±SEM (EC_{50} in μM) E_{max}±SEM
L-GLU	4.89±0.02 (13) 96±2	5.22±0.05 (6.1) 102±6	5.9±0.1 (1) 116±7	NT	NT	NT
L-AP4	NT	NT	NT	6.4±0.2 (0.4) 98±8	6.4±0.2 (0.4) 95±6	5.9±0.1 (1) 111±6
L-CA	4.07±0.08 (85) 65±4	2.66±0.04 (22) 108±3	4.87±0.06 (14) 55±1	3.3±0.1 (500) 120±20	4.5±0.2 (30) 22±2	5±1 (6) 63±3
L-HCA	3.8±0.6 (200) 30±10	5.2±0.3 (6) 130±40	4.9±0.3 (10) 85±7	4.52±0.08 (30) 91±2	5.1±0.1 (8) 98±3	4.5±0.2 (30) 54±2
L-CSA	3.92±0.03 (120) 82±3	4.6±0.2 (30) 100±10	3.9±0.2 (100) 100±10	2.7±0.2 (2000) 160±50	4.0±0.2 (100) 39±3	3.94±0.08 (110) 74±2
L-HCSA	4.72±0.05 (190) 94±4	5.24±0.04 (5.8) 106±3	4.7±0.2 (20) 96±7	4.1±0.1 (80) 112±6	5.21±0.03 (6.1) 106±1	4.4±0.1 (40) 105±6

Data represent mean +/- sem of pEC_{50} and E_{max} values for N=3-6 separate dose-response experiments. Emax values are normalized to maximal stimulation with 100 uM L-glutamate or L-AP4. NT=not tested.

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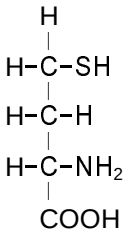
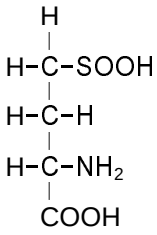
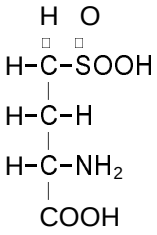
Figure 1.



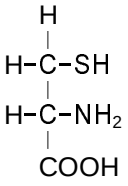
L-homocysteine (L-HCY)



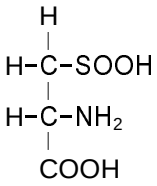
L-cysteine sulfinic acid (L-HCSA)



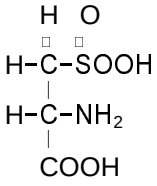
L-cysteic acid (L-HCA)



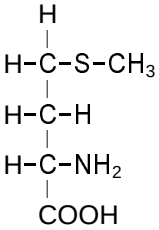
L-cysteine (L-CYS)



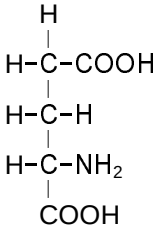
L-cysteine sulfinic acid (L-CSA)



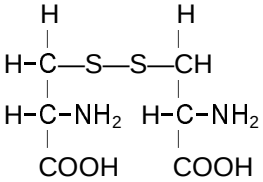
L-cysteic acid (L-CA)



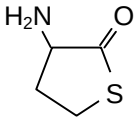
L-methionine (L-MET)



L-glutamate (L-GLU)



L-cystine (L-CTN)



L-homocysteine thiolactone (L-HCYT)

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Figure 2.

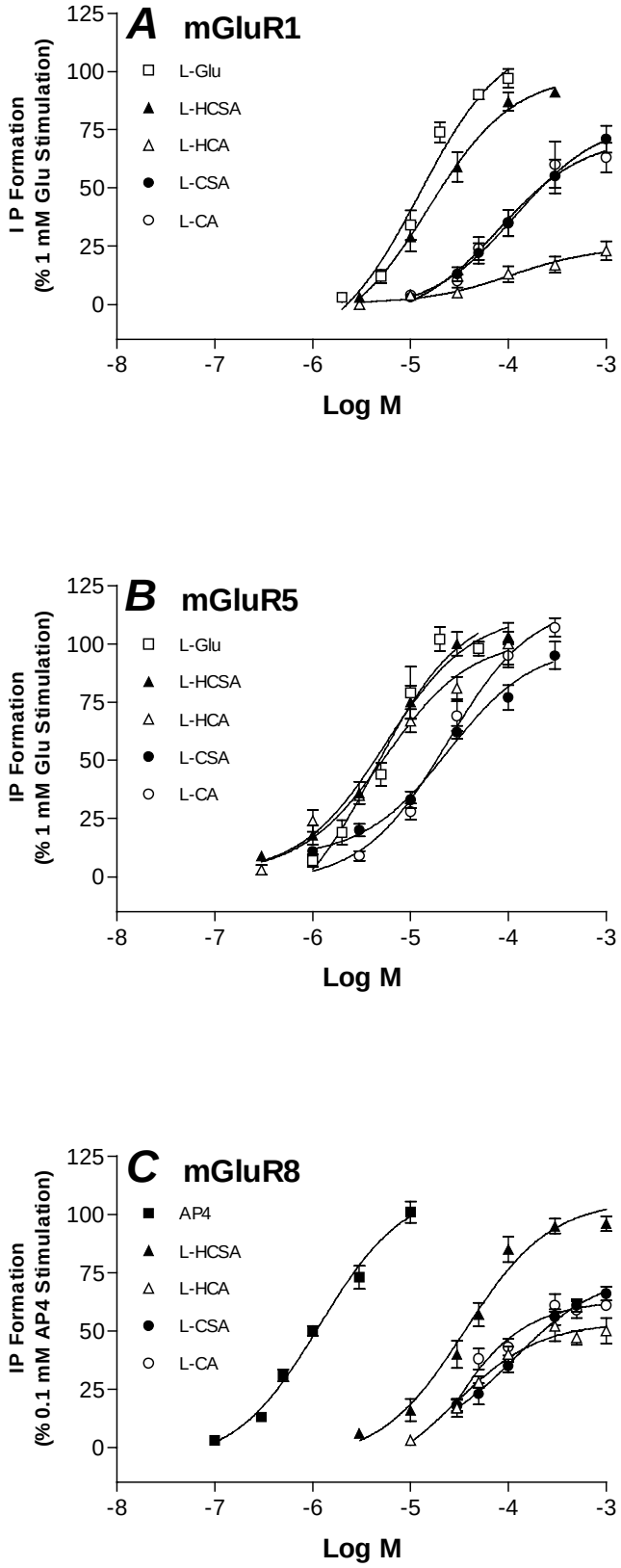

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Figure 3.