Excitatory Amino Acid Transporter 5 is widely expressed in peripheral tissues

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Abstract

It is routinely stated in the literature that Excitatory Amino Acid Transporter 5 (EAAT5) is a retina-specific glutamate transporter. EAAT5 is expressed by retinal photoreceptors and bipolar cells, where it serves as a slow transporter and as an inhibitory glutamate receptor, the latter role is due to the gating of a large chloride conductance. The dogma of an exclusively retinal distribution has arisen because Northern blot analyses have previously shown only modest hybridisation in non-retinal tissues. Others have re-interpreted this as indicating that EAAT5 was only present in retinal tissues. However, this view appears to be erroneous; recent evidence demonstrating abundant expression of EAAT5 in rat testis prompted us to re-examine this dogma. A new antibody was developed to an intracellular loop region of rat EAAT5. This new tool, in concert with RT-PCR and sequencing, demonstrated that EAAT5 was abundantly expressed in the canine cerebellum and lens tissue,6 whilst EAAT5 expression has been demonstrated in several other tissues, including liver, kidney, intestine, heart, lung, and skeletal muscle. We conclude that EAAT5 is a widely distributed protein. Whether it functions in all locations as a glutamate transporter, or mainly as a glutamate-gated chloride conductance, remains to be determined.

I

ntroduction

High affinity sodium-dependent glutamate transporters play a central role in the homeostasis of extracellular glutamate concentrations in tissues such as the central nervous system, where glutamate is used as a neurotransmitter and must therefore be tightly regulated.1 In the nervous system a variety of glutamate transporters are utilised. The five key types are Excitatory Amino Acid Transporter 1 (EAAT1; also called GLAST), EAAT 2 (also called GLT-1), EAAT3 (also called EAAC1), EAAT4 and EAAT5. Each of these types in turn probably exists as multiple splice variants.2 EAAT5, which was cloned by Arizza et al.,1,2 is the least studied of all the EAATs, possibly because of the widely stated view that it is a retina-specific glutamate transporter, and thus not implicated in glutamate homeostasis in other tissues. This view has been promulgated by our team1 as well as many others but seems, in retrospect, to reflect (at least from the perspective of our own team), from a misinterpretation of the findings of Arizza et al.1 In their study, strong hybridisation signals were observed in Northern blots of human retina, but less intense signals were evident in other tissues, with some bands at different sizes. Whilst the significance of these bands was briefly discussed as possibly representing splice variants, the overall focus of the discussion on the retina appears to have lead to the assumption that this meant it was a retina-specific glutamate transporter. Aside from the misinterpretations of the original findings of Arizza et al.1 by ourselves and others, technical issues may have also influenced the results of the study. These technical issues may have included parameters such as the quality of the mRNA used to generate the original human cDNA library from which EAAT5 was cloned. Retinal cDNA is relatively easy to source with minimal degradation (as donor human eyes are harvested rapidly for corneal donation), when compared to other tissues such as brain, where post-mortem delays tend to be greater. Perhaps more significantly, Northern hybridisation is very sensitive to alternate-splicing of RNA; changes in overall sequence significantly reduce the hybridisation efficiency. This may influence subsequent interpretations as to the presence or absence of mRNA encoding a specific gene. Our team have now identified multiple splice variants of EAAT5 (Genbank accession JF422064, JF422065, JF422066, JF422067, JF422068). Accordingly, the Northern hybridisation studies may have under-represented the overall abundance and tissue distribution of EAAT5 variants. Indeed, Ochiai et al. recently showed expression of EAAT5 message and protein in the canine cerebellum and lens tissue,6 whilst EAAT5 expression has been demonstrated in the vestibular system.7 The final catalyst for our re-evaluation was our recent finding that EAAT5 was abundantly expressed in the testis where it may have a role in directing sperm motility.6 EAAT5 is particularly interesting since this protein exhibits a relatively large chloride conductance, associated with modest transport activity, suggesting a function more closely related to ligand gated chloride channels than classic transporters. This has lead to the finding that wild type EAAT5 functions as an inhibitory presynaptic glutamate receptor in retinal bipolar cells.1,3,10 Thus, the chloride conductance properties of EAAT5 may be more important than the transport function. In this study, we have re-evaluated the dogma that EAAT5 is a retina-specific glutamate transporter, using PCR, immunocytochemistry and Western blotting. One key issue, which has become increasingly apparent in the glutamate transporter literature, is that the carboxyl and amino terminals of transporters may become inaccessible to antibodies either because of modification of the protein or the cleavage off of such terminal regions.1,2,11 This possibility has been raised with respect to EAAT5 to explain the abrupt loss of immunoreactivity for amino and carboxyl terminal regions of EAAT5 as it was transported out of the cell bodies of the retinal bipolar neurons.4 To counter this possibility, a new antibody was generated in this study against an intracellular epitope of EAAT5 corresponding to a region encoded by exon 6 of the EAAT5 gene. This was chosen because all of the EAAT5 splice variants we have cloned retained exon 6. Accordingly, it was deemed that an antibody against this region would potentially detect all known forms of EAAT5. Moreover, it was considered probable that this intracellular epitope in the middle of the protein was unlikely to be eliminated by cleavage events under normal physiological circumstances.
Cloning of EAAT5

To confirm the veracity of our PCR data, EAAT5 was cloned from several representative tissues (retina, kidney, lung and small intestine). PCR was performed using KOD Hot Start DNA Polymerase (Toyobo, Osaka, Japan) and primers [F-rEAAT5 (1200) and R-rEAAT5 (1643)]. The PCR mixture (50 µL) contained: 2 µL cDNA, 200 µM dNTPs, 0.2 µM each of sense and antisense primers, 1 mM MgSO4, and 2U of KOD Hot Start DNA polymerase in 1X PCR buffer. Polymerase activation was at 95°C for 2 min followed by 30 cycles of amplification (95°C for 20 s, 60 s for 15 s, 70°C for 40 s). The reaction products were separated on a 1.5% agarose gel and visualized by staining with 0.5 µg/mL ethidium bromide (Sigma-Aldrich, Castle Hill NSW, Australia). The PCR products were excised from the gel, purified using a Gel extraction kit (Qiagen Pty Ltd., Doncaster VIC, Australia) and subcloned into the pCR Blunt II-Topo vector (Invitrogen) according to the manufacturer’s instructions and sequenced in both directions by the Australian Genome Research Facility (Brisbane, Qld, Australia).

Antibodies

A rabbit was immunized according to standard protocols14,15 using a peptide with the unique amino acid sequence TLPSVFQ-CNLEVS, corresponding to EAAT5 amino acid residues 254-267 (Genbank accession NM_031144.3 corresponding to rat β-actin; densitometry units of 1:500-1:50,000. Detection was revealed by enhanced chemiluminescence using the SuperSignal West Dura Extended Duration Substrate Kit (Pierce, Rockford, IL, USA). Preabsorption of antisera (50 µg of antigen peptide per millilitre of diluted antiserum) was used to confirm the specificity of the EAAT5 antisera. The peptide was added to the diluted antibody and incubated in a refrigerator at 4°C for 6 h, prior to use.

Cell culture and transfections

The HEK293 cell line was maintained in high glucose Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and incubated at 37°C in 5% CO2. Cells were plated into T-25 cm2 flasks and at ~80% confluency were transfected with pcDNA3-EAAT5, pBK-CMV:GLT-1a or pBK-CMV:GLAST using X-tremeGENE HP (Roche) following the manufacturer’s instructions. Three to four days after transfection, cells were rinsed with ice-cold PBS and harvested in lysis buffer containing 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40, and protease inhibitor mixture (Roche). After lysis for 60 min at 4°C, lysates were clarified by centrifugation for 20 min at 17,000× g at 4°C. Lysates (20-50 µg) were used in Western blotting as described above.

Immunocytochemistry

Immunoperoxidase labeling for EAAT5 was performed as previously described using standard methods14 on paraffin-wax embedded sections of rat tissues. Briefly, rat tissues were fixed by perfusion with 4% paraformaldehyde in 0.1 M sodium phosphate buffer, then dehydrated through a graded series of water/aceton solutions, cleared in xylene and embedded in paraffin wax. Serial sections (8 µm in thickness) were cut on a Leica rotary microtome and mounted onto silanated micro-
scope slides. Sections were de-waxed with xylene and rehydrated through a graded series of ethanol/water solutions. Sections were pre-treated with 3% hydrogen peroxide in methanol for 10 min (during the rehydration process) to inhibit any endogenous peroxidase activity. All sections were blocked in 0.5% bovine serum albumin/0.05% saponin/0.05% sodium azide in 0.1 M sodium phosphate buffer for 30 min before primary antibodies were applied. Biotinylated secondary antibodies (GE Healthcare) and streptavidin-biotin-horseradish peroxidase conjugates (GE Healthcare) were subsequently applied at a dilution of 1:300. Labelling of sections was revealed using 3,3'-diaminobenzidine as a chromogen, and sections were mounted using DePex. Preabsorption of antisera was always used to confirm the specificity of such.

Microscopy

Bright field imaging was performed using a Nikon 80i equipped with an Olympus DP70 camera. All images were imported into Adobe Photoshop for minor brightness and contrast adjustments prior to composition of plates using Adobe Freehand.

Results

EAAT5 mRNA expression in multiple tissues

Expression of EAAT5 mRNA in various rat tissues was determined by RT-PCR (Figure 1A). A dominant EAAT5 amplification product of 443-bp (corresponding to exons 7-10) was obtained in mRNA samples from retina, small intestine, large intestine, heart and lung. Lower levels of EAAT5 mRNA expression were observed in liver, kidney, pancreas and muscle. By normalizing the EAAT5 mRNA signal to that of β-actin, the relative abundance of EAAT5 in small and large intestine, heart and lung were determined to be approximately 70-80% as that of retina (Figure 1B). By comparison, levels of EAAT5 mRNA in liver, kidney, pancreas and muscle were approximately 20% of that in retina (Figure 1B). The identities of the 443-bp amplicons in several representative tissues [retina (control), lung and kidney] were subsequently confirmed by direct cloning and sequencing. Other primer combinations (e.g., F1139 and R1594 corresponding to exons 7 to 9) were also used to validate expression of EAAT5 mRNA in retina and all tissues examined (data not shown).

Specificity of the exon-6-directed EAAT5 Antibody

The polyclonal antibody used in the present study was raised against a peptide corresponding to an intracellular loop region of EAAT5 (encoded by exon 6) (Figure 2A) with very little homology to other EAAT members (Figure 2B). Specificity of the new EAAT5 antibody for its tar-

Figure 1. Tissue distribution of EAAT5 mRNA. A) RNA from various rat tissues (as indicated) were reverse transcribed and used in PCR with EAAT5 (lower bands) and β-actin (upper bands) primers. An aliquot of each PCR was electrophoresed on a 2% agarose gel and visualised with ethidium bromide staining. A water control (blank) is shown. B) Densitometric analysis of EAAT5 amplification products from three separate experiments; data are shown as mean ± SD.

Figure 2. The EAAT5 loop epitope. A) Schematic representation of wild-type EAAT5 secondary structure with exon boundaries overlaid onto the predicted topology; an antibody was generated against an intracellular epitope of EAAT5 (as indicated) corresponding to a region encoded by exon 6 of the EAAT5 gene. B) Alignment of the EAAT5 epitope sequence against that of other EAAT members (GLAST, GLT-1a, EAAC1 and EAAT4); identical residues are highlighted in grey.
get was initially verified using dot blots of the immunizing peptide. The antisera specifically detected the target peptide but not irrelevant peptides (Figure 3A). To further assess antibody specificity, HEK293 cells were transfected with a vector encoding wild-type EAAT5 as well as vectors encoding GLAST (EAAT1) and GLT-1a (EAAT2) as controls. Western blotting was performed on cell extracts from the transfected cells to detect specificity of antibody-antigen interaction. The EAAT5 loop antibody detected a single band migrating at approximately 61–62 kDa in lysates of cells transfected with EAAT5 but not in lysates of cells transfected with GLAST or GLT-1a (Figure 3B). The relative mass of the EAAT5 immunoreactive band corresponded to the predicted molecular weight of EAAT5 (61,340.23 Da; predicted by ExPASY Bioinformatic Resource Portal). Pre-absorption of the antisera resulted in no detectable labelling (data not shown). An antibody directed against the C-terminal region of EAAT5 also yielded similar results, demonstrating that the EAAT5 loop antibody was in fact detecting the correct target protein in transfected cells (Figure 3B).

Tissue distribution of EAAT5

Western blot analysis showed that EAAT5 was identified in protein extracts of all tissues examined (Figure 4). In retina (control), a band at approximately 62 kDa was evident (corresponding to wild-type EAAT5) along with smaller sized bands of ~57 to ~43 kDa that corresponded to the sizes of the splice variants of EAAT5 that have been recently identified. Each of the other tissues examined showed expression of a band at around 62 kDa, presumably corresponding to wild-type EAAT5. Additional smaller sized bands were detected in liver, kidney and heart, which may be indicative of differential splicing of EAAT5 in such tissues.

Immunolocalization of EAAT5

Each of the tissues chosen for immunocytochemical investigation displayed immunoreactivity for EAAT5. In the retina (Figure 5A), EAAT5 immunoreactivity was associated with the previously described locations for EAAT5, labelling being notable in photoreceptors including their terminals in the outer plexiform layer (Figure 5B), in bipolar cells and in ganglion cells. The kidney exhibited strong labelling in the proximal tubules, labelling accordingly being evident in the outer stripe of the cortex, with weaker labelling in the inner stripe and no labelling in the medullary region (Figure 5C). In the gut, strong labelling was evident in structures such as the goblet cells of the small intestine (Figure 5D). Similarly, consistent labelling was also evident in the lung; punctate labelling appeared to be associated with the membranes of epithelial cells that form the alveoli (Figure 5E). In skeletal and heart muscle labelling was also evident, such labelling being localised around the individual muscle fibres (Figure 5F).

Discussion

In the present study, EAAT5 mRNA and protein was demonstrated in multiple peripheral tissues in the mammalian body, in addition to the known localisation in retina. Subsequent sequencing data, using lung, kidney and small intestine as representative peripheral tissues, showed that the larger amplicons detected in such tissues corresponded to EAAT5. Our new antibody detected multiple bands in several tissues including the retina. This observation is compatible with our prior observations that EAAT5 can exist as multiple differentially spliced isoforms (see Genbank accession #JF422064, JF422065, JF422066, JF422067, JF422068), with a range of predicted molecular weights between 43 kDa and 57 kDa. The consistency between the molecular data and the protein data in this study strongly supports the view that the tissues investigated express EAAT5. These observations are, however, in apparent contradiction to the commonly articulated dogma that EAAT5 is a retina-specific glutamate transporter. It is plausible however that the dogma has arisen primarily as a response to a lack of evidence; accordingly in the light of the current evidence, the dogma should be revised to indicate that EAAT5 is widely expressed. This view is supported by the recent findings of EAAT5 in the anterior segment of the eye, cerebellum, testis and vestibular system.

Our findings now evoke questions as to the relevance of EAAT5 in the tissues that express such. The two properties that are central to the known function of EAAT5 in the retina (namely the slow transport of glutamate and the gating of a chloride conductance which may cause hyperpolarisation of the cells expressing EAAT5) are presumably also evident in other cells in other locations. Skeletal and heart muscle both contain high concentrations of glutamate, and at least in the hypoxic heart, it
is known to be released in response to stimuli such as hypoxia, and that one mechanism by which this might be mediated is by the reversal of sodium-dependent glutamate transport. There appears to be an association between glutamate transport and exercise in skeletal muscles, with fluxes of glutamate, both out of, and into the muscles. If EAAT5 mediates glutamate uptake under such exercise conditions then it is plausible that release of glutamate from cells that are actively contracting could evoke a hyperpolarising response that feeds back to modify the contractile event. In the gut, it is known that multiple glutamate transporters may be present though their localisation is not well understood and their roles have not been established beyond the possibility of removal of glutamate from the lumen of the gut as part of ongoing digestive processes. Whether high luminal concentrations of glutamate generated by the digestion of protein cause hyperpolarisation of gut cells, such as the goblet cells and thus influence their secretory activity, is unclear.

The general roles of glutamate transporters in the kidney are unclear. EAAT3 has previously been identified in the kidney using in situ hybridisation studies, whilst Northern analyses have suggested the possible additional expression of GLT-1. However, their roles have not been clearly understood, beyond the view that these transporters provide substrates for a variety of transamination reactions. A close analysis of the literature reveals that EAAT5 has been detected in gene array studies of kidney but never commented upon, but again supports the proposed view that EAAT5 is widely expressed by mammalian tissues. The presence of EAAT5 in what appears to be the proximal tubules (based upon morphological criteria) suggests that EAAT5 may be implicated in reabsorption events. Whether fluxes of glutamate are the intended consequences of EAAT5 expression or chloride fluxes, or a combination of both is unclear. In the lung, the widespread expression of EAAT5 is associated with the transport epithelia. The lung utilises glutamate in a variety of ways, including uptake of such to synthesise glutamine as well as glutathione. Whilst much of the uptake of glutamate appears to be mediated via the cystine-glutamate antiporter, around 10% is via a sodium-dependent transporter; our data would suggest that this sodium-dependent transporter could be EAAT5. Many tissues also express glutamate receptors including heart, kidney, lungs and testis, and such receptors may mediate cell-to-cell communication. It is plausible that EAAT5 may be used as a glutamate transporter in such tissues to reduce extracellular levels of glutamate to levels where these receptors can function appropriately. It has previously been suggested that EAAT5 might serve to regulate sperm motility in the testis, possibly also generating directional cues for the sperm by virtue of the polarised expression of EAAT5 on the heads of sperm and the gradient of glutamate that exists in the reproductive tract. Other comparable hypothetical scenarios can be invoked to explain the functional significance of the glutamate transporters expression and distribution.

![Figure 4](image-url)  
**Figure 4.** Western blot demonstrating expression of EAAT5 in various rat tissues (as indicated). A ~62 kDa band (corresponding to wild-type EAAT5) is labelled in retina (control) and all other tissues examined. Additional smaller bands (presumed to represent tissue-specific alternate splicing of EAAT5) are also evident in retina, liver, kidney and heart. All lanes were loaded with ~20 µg of total lysate with exception of kidney (~60 µg input).

![Figure 5](image-url)  
**Figure 5.** Immunolabelling for EAAT5 in retina (A and B), kidney (C), small intestine (D), lung (E), skeletal muscle (F) and heart (G). In retina (A) labelling is associated with somata of photoreceptors (p) as well as their outer segments (os) and inner segments (is). Strong labelling is associated with the synaptic terminals of the photoreceptors in the outer plexiform layer (OPL). Labelling is similarly evident in somata of bipolar cells (b) and processes in the inner plexiform layer (IPL). Ganglion cell somata in the ganglion cell layer (gcl) are also labelled. (B) arrow indicates labelling of photoreceptor synaptic terminals. In kidney (C), heaviest labelling is in the outer stripe (os) of the cortex, with lesser labelling in the inner stripe (is); labelling was absent from the medulla (m). In the intestine (D), labelling was associated with goblet cells (g) whilst in lung (E), labelling was associated with epithelia of the alveoli. In skeletal muscle (F) and heart (G), labelling was around the periphery of muscle fibres. Scale bars: A, C, F, G, 25 µm; B, 500 µm; D, 50 µm; E, 10 µm.
expression of EAAT5 in these and other tissues such as liver. In all instances these hypotheti-
cal scenarios require experimental validation.
Many of the roles of EAAT5 will presumably be
clarified in due course as a validated EAAT5
knockout mouse becomes available, but such
animals are not yet currently available.

In this study the dominant PCR amplicon for
mRNA from kidney, lung and small intestine
was cloned and sequenced. In each case the
amplicon was demonstrated to represent
EAAT5. Similar analyses have not yet been
performed on the less abundant and smaller
mRNA bands that are present in some of the
tissues, but we presume such to represent
some of the smaller splice variants we have
previously cloned from the retina. There is cur-
tently no available data as to whether the
splice variants are functional transporters,
whether they represent functionally inactive
proteins or whether they serve more complex
roles. Glutamate transporters that have been
studied to date appear to form trimeric com-
plexes with other glutamate transporter mole-
cules. The possibility exists that the formation
of heterotrimers containing wild type (full
length) EAAT5 and alternately spliced forms
could result in modification of the trafficking
and function of the resultant heterotrimer.1,2

A more extensive analysis of the range of
EAAT5 splice variants and their expression at
the mRNA and protein levels using splice-spe-
cific antibodies is the subject of another forth-
coming manuscript.

Conclusions

EAAT5 is present in multiple tissues. The
abundance of this protein as well as an abun-
dance of mRNA strongly argues against the
expression of such being an epiphenomenon.
Instead, we suggest that EAAT5 may have an as
yet range of roles in the body, which embrace
both the glutamate transport properties of the
protein and the co-associated chloride conduc-
tance. This latter property, which sets EAAT5
aside from other transporters such as GLAST
and GLT-1, may hyperpolarise cells that express
such during events that require auto-regula-
tion via modulation of membrane potential,
such as regulation of secretion from the goblet
cells. These possibilities await further studies.

References

1. Danbolt NC. Glutamate uptake. Prog

2. Lee A, Pow DV. Astrocytes: glutamate trans-
  port and alternate splicing of transporters. Int J

3. Arriza JL, Eliasof S, Kavanaugh MP, Amara
  SG. Excitatory amino acid transporter 5, a
  retinal glutamate transporter coupled to a
  chloride conductance. Proc Natl Acad Sci
  USA 1997;94:4155-60.

4. Pow DV, Barnett NL. Developmental expres-
  sion of excitatory amino acid transporter 5:
  a photoreceptor and bipolar cell glutamate

5. Lee A, Anderson AR, Barnett NL, Stevens
  MG, Pow DV. Alternate splicing and expres-
  sion of the glutamate transporter EAAT5 in

6. Ochiia H, Saito M, Maruo T, Kanemaki N.
  Molecular cloning of canine excitatory
  amino acid transporter 5 and its detection
  in primary lens epithelial cells. Exp Anim

7. Lim R, Kindig AE, Lee A, Pow DV, Callister
  RJ, Brichta AM. EAAT5 mediates glutamate
  transport in mouse vestibular epithelium.
  Proceedings of the Australian Physiological
  Society, No. 408.3P. Australian Neuro-
  science, Sydney, NSW, 2010.

8. Dalet A, Bonsacquet J, Gaboyard-Niay S,
  Calin-Jageman I, Chidavaenzi RL, Venteo S,
  et al. Glutamate transporters EAAT4 and
  EAAT5 are expressed in vestibular hair cells

9. Lee A, Anderson AR, Barnett AC, Chan A,
  Pow DV. Expression of multiple glutamate
  transporter splice variants in the rodent

10. Wersinger E, Schwab Y, Sahel JA, Rendon A,
    Pow DV, Picaud S, et al. The glutamate
    transporter EAAT5 works as a presynaptic
    receptor in mouse rod bipolar cells. J
    Physiol 2006;577(Pt 1):221-34.

11. Veruki ML, Monkve SH, Hartveit E. Acti-
    vation of a presynaptic glutamate trans-
    porter regulates synaptic transmission
    through electrical signaling. Nat Neurosci
    2006;9:1388-96.

12. Susarla BT, Seal RP, Zeleniaio A, Watson DJ,
    Wolfe JH, Amara SG, et al. Differential reg-
    ulation of GLAST immunoreactivity and
    activity by protein kinase C. evidence for
    modification of amino and carboxyl termi-

13. Williams SM, Sullivan RK, Scott HI, Finke-
    lstein DJ, Colditz PB, Lingwood BE, et al.
    Glial glutamate transporter expression
    patterns in brains from multiple mam-

14. Pow DV, Sullivan R, Scott H. Antibody pro-
    duction and immunocytochemical localiza-
    tion of amino acid transporters. Methods

15. Rauen T, Wiessner M, Sullivan R, Lee A,
    Pow DV. A new GLT1 splice variant: cloning
    and immunolocalization of GLT1c in the
    mammalian retina and brain. Neurochem
    Int 2004;45:1095-106.

16. Pow DV. Immunocytochemistry of amino-
    acids in the rodent pituitary using extreme-
    ly specific, very high-titer antisera. J

17. Fuk-Kolodziej B, Qin F, Dzhagaryan A,
    Pourcho RG. Differential cellular and sub-
    cellular distribution of glutamate trans-
    porters in the cat retina. Vis Neurosci 2004;

18. Song D, O’Regan MH, Phillips JW. Mechani-
    sms of amino acid release from the
    isolated anoxic/regperfused rat heart. Eur J

19. Rennie MJ, Low SY, Taylor PM, Khogali SE,
    Yao PC, Ahmed A. Amino acid transport dur-
    ing muscle contraction and its relevance to
    305.

20. Mourtzakis M, Graham TE. Glutamate
    ingestion and its effects at rest and during
    exercise in humans. J Appl Physiol 2002;93:
    1251-9.

21. Howell JA, Matthews AD, Swanson KC,
    Harmon DL, Matthews JC. Molecular iden-
    tification of high-affinity glutamate trans-
    porters in sheep and cattle forestomach,
    intestine, liver, kidney, and pancreas. J

22. Shayanuk C, Kanai Y, Lee WS, Brown D,
    Rothstein JD, Hediger MA. Localization of
    the high-affinity glutamate transporter
    EAAC1 in rat kidney. Am J Physiol 1997;

23. Welbourne TC, Matthews JC, Glutamate
    transport and renal function. Am J Physiol
    1999;277:F501-5.

24. Valerius MT, McMahon AP. Transcriptional
    profiling of Wnt4 mutant mouse kidneys
    identifies genes expressed during nephron
    formation. Gene Expr Patterns 2008;28:297-
    306.

25. Labow BI, Abcouwer SF, Lin CM, Souha
    WW. Glutamine synthetase expression in
    rat lung is regulated by protein stability. Am

    WW. Characterization of glutamine and glu-
    tamate transport in rat lung plasma mem-

27. Gill SS, Pulido OM. Glutamate receptors in
    peripheral tissues: current knowledge,
    future research, and implications for toxi-

28. Vallejo-Illarramendi A, Domercq M, Matute
    C. A novel alternative splicing form of exci-
    tatory amino acid transporter 1 is a nega-
    tive regulator of glutamate uptake. J