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Metabolic strategies for the degradation of the neuromodulator agmatine in mammals

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Abstract

Agmatine (1-amino-4-guanidinobutane), a precursor for polyamine biosynthesis, has been identified as an important neuromodulator with anticonvulsant, antineurotoxic and antidepressant actions in the brain. In this context it has emerged as an important mediator of addiction/satiety pathways associated with alcohol misuse. Consequently, the regulation of the activity of key enzymes in agmatine metabolism is an attractive strategy to combat alcoholism and related addiction disorders.

Agmatine results from the decarboxylation of L-arginine in a reaction catalyzed by arginine decarboxylase (ADC), and can be converted to either guanidine butyraldehyde by diamine oxidase (DAO) or putrescine and urea by the enzyme agmatinase (AGM) or the more recently identified AGM-like protein (ALP). In rat brain, agmatine, AGM and ALP are predominantly localised in areas associated with roles in appetitive and craving (drug-reinstatement) behaviors. Thus, inhibitors of AGM or ALP are promising agents for the treatment of addictions. In this review, the properties of DAO, AGM and ALP are discussed with a view to their role in the agmatine metabolism in mammals.

Introduction

Agmatine (1-amino-4-guanidinobutane) is a primary amine that is generated from the decarboxylation of L-arginine by arginine decarboxylase (ADC; EC 4.1.1.19: **Fig. 1**). In mammals, agmatine has been directly associated with many important cellular functions, including the modulation of insulin release from pancreatic cells [1-3], renal sodium excretion [4,5] and neuroprotective effects [6-9]; furthermore, agmatine inhibits all known isoforms of nitric oxide synthase (NOS) in the brain [10] and increases the tolerance to morphine [11]. Agmatine also plays an essential role in the regulation of the expression of ornithine decarboxylase (ODC) [12] (**Fig.1**) and spermidine/spermine acetyl transferase [13], two enzymes that are involved in polyamine biosynthesis [14]. In the central nervous system (CNS), agmatine is considered to be a neurotransmitter/neuromodulator, because it is synthesized in the brain, stored in synaptic vesicles, accumulated by uptake and released by depolarization [15,16]. In addition, agmatine activates several membrane receptors, including nicotinic, imidazoline, α2-adrenergic, 5-HT2A, and 5HT3 [16], while it antagonizes *N*-methyl-D-aspartate (NMDA) receptors [16-19]. It also regulates the release of catecholamines and potentiates opioid analgesia [20]. However, a receptor specific for agmatine has not yet been described.

Agmatine administration to laboratory rodents modulates ethanol-induced anxiolysis and withdrawal anxiety [21], nicotine sensitization, and compulsive behaviors [21-23]. Injection of agmatine also produces anticonvulsant, antineurotoxic, and antidepressant-like actions in animals [24-27]. In recent years the number of studies with animal models has increased; these have demonstrated beneficial effects of agmatine administration for the following disorders: anxiety, hypoxic ischemia, nociception,

morphine tolerance, memory loss, Parkinson's disease, Alzheimer's disease, traumatic brain injury-related disorders, and epilepsy [17,28-32]. Furthermore, an increase in intracellular agmatine concentration has been associated with a decrease in the number of neoplastic cells. RNA interference targeting ADC resulted in a significant increase in proliferation of the human intestinal tumor cell line SW480, which was paralleled by a distinct decrease of the intracellular agmatine content [33]. On the other hand, addition of ADC to the culture medium of HeLa cells caused the arrest of cell growth [34]. These observations may be linked to the inhibitory effect of agmatine on ODC [35,36] and polyamine uptake [37-39], because ODC is the rate-limiting enzyme in polyamine synthesis and polyamines are required for a cell to enter into the cell cycle and to proliferate.

The cellular and regional distributions of agmatine were mapped in rat brain with the aid of a polyclonal anti-agmatine antibody [40]. The highest numbers of neurons displaying agmatine immunoreactivity were in hippocampus, hypothalamus, and the rostral midbrain, as well as in periventricular areas that included the dorsolateral nucleus, locus cœruleus, nucleus raphe dorsalis and the periaqueductal gray matter [40]. Notably, agmatine-containing neurons were concentrated in regions of the brain that regulate visceral and neuroendocrine control, the processing of emotions, pain perception, and cognition [15,16].

The capacity of rat brain to synthesize agmatine has been also investigated. The highest levels of ADC activity were detected in hypothalamus and cerebral cortex, while the lowest levels were found in the locus cœruleus and medulla [41,42]. ADC is also present in various other tissues and/or organs, with the highest levels recorded in the aorta and the lowest in testis [43]. ADC, extracted from rat brain mitochondrial preparations, is

thermally unstable but able to catalyze the decarboxylation of both arginine (K_m ~0.75 mM) and ornithine (K_m ~0.25 mM), in a reaction that is not inhibited by the specific ODC inhibitor difluoromethylornithine [43]. Agmatine is thus synthesized in proximity to mitochondria, and to a class of imidazoline receptors (I2) to which agmatine binds with high affinity [16,44]; indeed, while there is evidence for the localization of I2 on synaptic membranes, a higher proportion of I2 is found on mitochodrial membranes [45]. In rat liver, ADC is also associated with the mitochondrium; its *Km* value for arginine is 28-40 mM [46]. The importance of ADC in agmatine biosynthesis has been demonstrated with various cell models, in which agmatine could be detected upon transfection with the ADC-encoding gene [47-49]. However, since no data on the kinetic properties of isolated ADC are currently available, further studies are necessary to develop a better understanding of the role of this enzyme in the agmatine metabolism.

While only one enzyme appears to be involved in the formation of agmatine, several contribute to its degradation (**Fig. 1**). In one route agmatine is converted to guanidinobutyraldehyde by diamine oxidase (DAO), while in another path it is hydrolyzed to putrescine and urea by either agmatinase (AGM) or the more-recently described AGM-like protein (ALP). Guanidinobutyraldehyde is a precursor for the neurotransmitter 4-aminobutyrate (GABA) [50], whereas putrescine is a precursor for polyamines, which are essential for proliferation, differentiation and migration of mammalian cells and also act as positive modulators of glutamate-NMDA receptors. In addition, putrescine in the brain can also be converted via aminobutanal to GABA by the combined action of monoamine oxidases (MAOs) or DAOs and aldehyde dehydrogenase, respectively [51]. Aldehyde dehydrogenase 1A1 mediates a GABA synthesis pathway in midbrain dopaminergic

neurons; glial GABA, synthesized by MAO B, mediates tonic inhibition [52,53]. These pathways and those outlined in the next section may allow agmatine to participate indirectly in GABA-mediated inhibition in the brain, and thus augment its direct action as an inhibitor neurotransmitter. GABA is metabolized to succinate and thereby enters the citric acid cycle via succinate semialdehyde and its dehydrogenase, which is a target of the target of the antiepileptic valproate. Hence, augmentation of GABA concentration may contribute to agmatine's anticonvulsant activity (as mentioned above).

Diamine oxidase (DAO)

DAO (EC 1.4.3.6) catalyzes the conversion of agmatine to guanidinobutyraldehyde (**Fig. 1**), which is subsequently (i) oxidized to guanidinobutyrate by an aldehyde dehydrogenase, and (ii) then hydrolyzed to 4-amino butyrate (GABA) by a ureohydrolase; such an enzyme was initially identified in liver, kidney and intestinal mucosa samples from rabbit, and more recently in human brain samples [50,54,55]. DAO has been identified in various mammalian tissues, including the basal level of the skin epidermis, glomeruli, the decidua of the placenta, epithelial cells in the small intestine, blood and tissue eosinophils, macrophages, as well as smooth muscle cells of veins, arteries, bronchioles, stomach wall and skin [56]. Its distribution, detected by immunohistochemistry, is identical in human and mouse tissues [56]. Enzymatic activity assays and Northern blot analysis of guinea pig tissues indicate that the relative abundance of the enzyme is liver > small intestine > lung, kidney > stomach [57]. Only small but detectable amounts of DAO mRNA were observed in cerebellum, thalamus-hypothalamus and cerebral cortex [57]. As these distribution data suggest, the role of DAO is not specific for agmatine metabolism in the brain. Indeed, DAO purified from porcine kidney, not only acts on agmatine (K_m ~5 µM), but also on histamine

(K_m ~15 µM), putrescine (K_m ~0.2 mM) and spermidine (K_m ~1 mM) [50], and because it was originally identified for its ability to remove exogenous histamine from lung and liver samples it is also designated as histaminase [56]. Reduced levels of DAO are directly correlated with histamine intolerance [58], and inhibitors such as berenil and pentamidine are diamine derivatives used as drugs to treat Trypanosomiasis and *Pneumocystis pneumoniæ* [59,60]. The low prevalence of DAO in brain, together with its function as target for chemotherapeutics to treat infectious diseases, reduce the suitability of this enzyme as target for inhibitors that are geared towards treating addiction-related disorders.

Agmatinase (AGM)

AGM (EC 3.5.3.1.1) catalyzes the hydrolytic breakdown of agmatine to putrescine and urea (**Fig. 1**). In mammals, agmatine hydrolysis has been directly determined only in rat brain [61,62] and murine macrophages [63]. In subcellular fractions of rat brain, AGM activity has been associated principally to mitochondrial fractions. Distributed in the hypothalamus, medulla oblongata and hippocampus, and significantly lower levels in the striatum and the cerebral cortex of rat brain [61]. Generally, the reported values of AGM activity in the rat brain are extremely low (7.6–11.8 nmol urea/mg of protein/h), almost at the limit of sensitivity of the methods used [61,62] and the enzyme has not been purified.

The cloning and partial characterization of human AGM was reported almost simultaneously by two different groups [64, 65]. The protein shares 35% sequence identity with *Escherichia coli* AGM, requires Mn^{2+} for activity and, importantly, all the side chains essential for metal ion and substrate binding in members of the ureohydrolases superfamily, which include all known bacterial AGMs, arginases, ureases and some

organophosphate-degrading hydrolases [66-70], are conserved (**Fig. 2**). Interestingly, AGM has an N-terminal mitochondrial import sequence, and the enzyme's mitochondrial localization has indeed been confirmed in kidney cells [71]. Due to the extremely low catalytic activity of isolated, recombinant mammalian AGMs, little is currently known about their enzymatic properties [64,65]. The strongest demonstration of their AGM activity has come from a functional complementation test, not from a direct *in vitro* enzymatic assay. The test is based on the polyamine-requiring yeast strain yASG1–8 that contains a disruption in the *spe1* gene encoding ODC, which makes the cells fully dependent on exogenous polyamines for growth [65,72]. Transformants of yASG1–8, expressing either *E. coli* ADC (encoded by the gene *speA*) or *E. coli* AGM (*speB*) also require exogenous polyamines, but the simultaneous expression of both genes, *speA* and *speB*, allows the yeast strain to grow on minimal medium without exogenous polyamines. Yeast strain transfected with mouse AGM or human AGM grew in minimal medium, compared with the non-transfected strain (**Fig. 3**). Using the structure of rat arginase I as template, a homology model for human AGM was generated (the sequence identity between the two proteins is 20%) [64]. The modelled structure reveals significant structural homology, in particular in domains that are conserved in most ureahydrolases; these include an active site that can accommodate two closely spaced divalent metal ions (*i.e.* a di-managanese cluster) [64] . It is intriguing, however, that although preliminary crystallographic data of human AGM were reported, its structure has not yet been resolved [73]. Furthermore, isolated recombinant mammalian AGMs appear to lack metal ions [72]. These observations may indicate that mammalian AGMs adopt a fold different from that of their bacterial counterparts or the distantly related arginases. Indeed, while a sequence

comparison indicates that human AGM may have active site residues (*i.e.* manganese-coordinating residues) identical to those of other ureahydrolases, four out of these six residues are different in mouse AGM [72]. Indeed, if the predicted fold of mammalian AGMs were correct at least the mouse enzyme would not be expected to bind metal ions in its active site with high affinity since the four substitutions involve the replacement of side chains that form strong interactions with metal ions with residues that form either weak or no coordination bonds at all [72]. It has been speculated that mammalian AGMs require cofactors or chaperones similar to the Ni^{2+}-dependent urease to form an active, Mn^{2+}-containing form [72]. The identification of such cofactors will be essential to understand the regulation of mammalian AGMs and thus their role(s) in controlling the agmatine metabolism.

In human tissues, AGM mRNA is most abundant in liver and kidney, with much lower expression levels observed in muscle, small intestine, and brain [64]. Similarly, Western blot analysis has demonstrated that AGM is most abundant in the liver, with lower amounts present in the thalamus, cerebellum and cerebral cortex of rat brain [74]. The use of immunocytochemical methods showed that in human and rat brain AGM is predominantly present in distinct populations of neurons, especially cortical interneurons. Principal neurons in limbic regions such as the habenula, and in cerebellum, also express AGM [74]. In contrast, a proteomics approach showed that human AGM is diminished in the clear-cell type of renal carcinoma, an observation that is consistent with a reduced amount of AGM mRNA in such tumor cells [71]. AGM levels vary in mood disorders: human subjects affected by depression show reduced blood levels of agmatine, which is consistent with post-mortem findings of increased AGM levels in brain tissues from

depressed subjects [75,76]. The correlation between AGM/agmatine levels and mood was also illustrated in a mouse model of depression. An AGM upregulation in the prefrontal cortex and hippocampus was demonstrated by confocal immunofluorescence microscopy, notably in parvalbumin- and somatostatin-positive interneurons [77].

In summary, AGM is a poorly understood, Mn^{2+}-dependent ureohydrolase. Its inverse correlation with agmatine levels in brain tissue, together with its complex regulatory mechanism requiring as-yet unidentified cofactors or chaperones, make this enzyme an interesting target for the development of novel chemotherapeutics to treat neurological disorders.

Agmatinase-like protein (ALP)

Recently, a novel enzyme with AGM activity was cloned from a rat brain cDNA library, the AGM-Like Protein (ALP) [78-82]. However, its amino acid sequence bears no resemblance to known AGMs (from any source) nor to other ureohydrolases; no motif that may define the active site of this enzyme has yet been identified [69]. ALP is specific for the substrate agmatine, and is not active against arginine. Using a polyclonal antibody raised against ALP was shown to be localized mainly in astrocytes and neurons in rat hypothalamus and hippocampus [80]. Recombinant ALP expressed in *E. coli* and purified, is the first (and to date only) mammalian enzyme with *in vitro* AGM activity (k_{cat} ~1.0 s^{-1}; K_m ~3.0 mM for agmatine [78,79]. The ability of ALP to generate putrescine (**Fig. 1**) for polyamine synthesis, has also been demonstrated using the same complementation assay that was employed to demonstrate biological activity of mammalian AGMs in a yeast strain (see above and compare **Figs 3 and 4**) [72,79,81].

In addition to being active in isolated form ALP is distinct from mammalian AGMs in the regulation of its catalytic activity. The C-terminal section of the protein (residues 459–510) comprises the sequence motif C-X_{16}-H-X_2-C-X_2-C-X_2-C-X_{21}-C-X_2-C), characteristic of the LIM domain, a fold that plays important roles in protein-protein interactions [81]. Such domains are key components of the regulatory machinery of cells. Specifically, LIM domain-containing proteins have been implicated in cellular differentiation and the control of cell growth, and play crucial roles in cytoarchitecture, cell adhesion, cell motility and signal transduction, and as regulators of gene expression [83,84]. The LIM domain folds into two zinc fingers, each stabilized at its base by a Zn^{2+} ion [81]; a homology model for the LIM domain of rat brain ALP is shown in **Fig. 5** [82]. Notably, a deletion mutant of rat brain ALP that lacks the LIM domain is catalytically significantly more efficient than its wild-type counterpart; the truncated variant has a ten-fold higher k_{cat} and a three-fold lower K_m value for agmatine [81]. The altered kinetic parameters of the truncated mutant are accompanied by significant alterations in tryptophan fluorescence, which indicate that a lack of the LIM domain also affects overall ALP structure [81]. These structural changes are unlikely to be irreversible because addition of the isolated LIM domain to the truncated rat brain ALP variant restores the catalytic properties of the wild-type enzyme [82]. Mutation of one of the Zn^{2+} binding sites (replacement of Cys453 by Ala) leads to a similar degree of activation of catalytic efficiency as the complete removal of the LIM domain [82]. A similar activation mechanism was observed in a LIM domain-containing kinase; complete removal of the LIM domain or introduction of a specific Cys mutation also enhanced this enzyme's catalytic efficiency [85]. The LIM domain thus appears to act as an auto-inhibitory entity in

ALP; we speculate that the interaction of this domain with an as-yet unknown accessory protein may abolish this inhibition, thus providing an alternative avenue for the regulation of agmatine levels in the brain.

In the section above the relevance of Zn^{2+} for the regulation of ALP activity was discussed. However, the enzyme also requires Mn^{2+} for catalytic activity, as evidenced by its complete inactivation when incubated with the metal ion chelator EDTA. The enzymatic activity can, however, easily be recovered by the addition of Mn^{2+} to the metal-ion-free apoenzyme [82,86]. In this respect ALP behaves like all well-characterized Mn^{2+}-dependent members of the ureohydrolase family, which include human and rat arginases [87,88] and bacterial AGMs [65,66]. In their fully active states the active sites of arginases and bacterial AGMs contain a binuclear Mn^{2+} center. Crystal structures of *Deinococcus radiodurans* AGM [89] and the two putative AGMs from *Clostridium difficile* and *Burkholderia thailandensis* [90] have been reported (**Fig. 6**). These structures provide detailed insight into the active site of bacterial AGMs, which is virtually identical to those of arginases (as expected from the sequence homology shared by these enzymes). The lack of sequence homology between these enzymes and ALP limits, in part, our current knowledge of the structure of the active site of the latter. Metal-ion measurements indicate that ALP is likely to contain a bimetallic manganese center in its catalytically optimal form [82,86]. Mutagenesis studies identified up to two Histidine residues that play important roles in Mn^{2+} binding in ALP, but the precise structure of the bimetallic metal center awaits further structural studies [86].

The form of ALP initially obtained is encoded by a 1569 bp cDNA, but two additional transcripts with extended 5'-regions have since been detected. These are denoted

LIMCH1 isoforms I (3918 bp) and II (2871 bp); their 3'-regions are identical to that of their shorter counterpart [79]. Interestingly, in isoform I the N-terminal region is characteristic of a calponin homology (CH) domain. CH domains occur in three highly divergent groups: CH1 and CH2 bind actin, while CH3, present in LIMCH1 isoform I, binds to microtubules [91]. Despite their N-terminal extensions both LIMCH1 isoforms I and II catalyze the hydrolysis of agmatine and support polyamine biosynthesis *in vivo* (**Fig. 4**). In addition, removal of the C-terminal LIM-domain in these isoforms produces a moderate activation, albeit to an extent less significant than observed for their shorter counterpart [79]. Splicing mechanisms that may lead to different ALP variants in rat brain have not yet been investigated but it is likely that isoform I is the initial functional enzyme produced there, while isoform II and the shorter homolog represent proteolytically modified forms. Understanding the mechanism of protein processing of ALP will be essential to fully comprehend the role of this enzyme in brain metabolism, and to target it for the development of novel treatments for neurological disorders such as alcoholism. In the human genome there is a single sequence (accession code: Q9UPQO) that encodes a putative enzyme which is 85% identical to rat brain LIMCH1 isoform I; their C-terminal LIM domains share 98% identity. Preliminary data by our group indeed demonstrate that this human homolog of ALP indeed possesses AGM activity (unpublished data).

Conclusions

In 2004, Morris stated that in mammals the "*agmatine metabolism is still largely a mystery*" [92]. At present, the cloning and partial characterization of ADC and the enzymes involved in agmatine degradation (**Fig. 1**) have significantly enhanced our knowledge of the agmatine metabolism in particular in mammals. However, questions remain. For

instance, it is not known why mammalian AGMs are virtually inactive under *in vitro* conditions. It is speculated that the enzymes may adopt a fold different from that of their bacterial counterparts and other members of the ureohydrolase family, and that they are activated by protein-protein interactions in the cell as occurs with the Ni^{2+}-dependent urease [93]. This mechanism could provide the practical base for an efficient strategy to control AGM activity, and in turn agmatine concentrations. However, since AGM is not highly abundant in brain tissues it appears that this enzyme is unlikely to play a major role in this organ. In contrast, ALP is mostly found in the brain. Not only is this unusual enzyme an efficient AGM under *in vitro* and biological conditions, but its activity can be modulated *via* a C-terminal zinc finger (LIM) domain. Currently, only ALP from rat brain tissue has been partially characterized. While the enzyme requires manganese for its catalytic function its active-site geometry and metal-ion stoichiometry have not yet been determined. Also, possible accessory proteins that may regulate the activity of the enzyme *via* interaction with its LIM domain have not yet been identified. Addressing these current gaps in knowledge will be crucial for establishing ALP as a drug target, a role that is supported by the predominant co-localization of agmatine and ALP in rat brain areas associated with appetitive and craving (drug-reinstatement) behaviors [79,80,94]. Agmatine is considered relatively safe when taken orally but has a range of actions outside the brain [95,96]. An inhibitor of ALP (and possibly AGM) is anticipated to have an effect similar to that of exogenous agmatine injection (which reduces ethanol-withdrawal anxiety in rodents), but by targeting a specific pathway a lower risk of side effects is expected (**Fig. 7**).

Knowing where the agmatine receptor is localised in the brain is critical for understanding its function. This localisation is currently not known in either healthy

controls or individuals suffering from alcohol misuse. A PET ligand for the agmatine receptor IR2, [^{11}C]BU99008, has recently been developed [97]. It has high specificity and behaves well in *in vivo* imaging in primates [98].

In summary, agmatine offers a much-needed new avenue to address disorders such as alcohol misuse and related addictions [99]. The human and financial costs associated with alcoholism alone are enormous and novel treatment strategies are urgently needed. Annually, several million people die of alcohol misuse [100]. Treatment options are limited; currently approved drugs for treating alcoholism have questionable efficacy and limited uptake in the community [101]. AGM, and in particular ALP, are promising targets for new strategies to address this global problem.

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References

1. Chan, S.L. (1998). Clonidine-displacing substance and its putative role in control of insulin secretion: a minireview. *Gen. Pharmacol.* 31:525-9.

2. Su, C.H., Liu, I.M., Chung, H.H., Cheng, J.T. (2009). Activation of I2-imidazoline receptors by agmatine improved insulin sensitivity through two mechanisms in type-2 diabetic rats. *Neurosci. Lett*. 457:125-128.

3. Lee, J.P., Chen, W., Wu, H.T., Lin, K.C., Cheng, J.T. (2011). Metformin can activate imidazoline I-2 receptors to lower plasma glucose in type 1-like diabetic rats. *Horm. Metab. Res.* 43:26-30.

4. Penner, S.B., Smyth, D.D. (1996). Natriuresis following central and peripheral administration of agmatine in the rat. *Pharmacology*. 53:160-169.

5. Satriano, J., Cunard, R., Peterson, O.W., Dousa, T., Gabbai, F.B., Blantz, R.C. (2007). Effects on kidney filtration rate by agmatine requires activation of ryanodine channels for nitric oxide generation. *Am. J. Physiol. Renal. Physiol.* 294:795-800.

6. Cunha, A.S., Matheus, F.C., Moretti, M., Sampaio, T.B., Poli, A., Santos, D.B., Colle, D., Cunha, M.P., Blum-Silva, C.H., Sandjo, L.P., Reginatto, F.H., Rodrigues, A.L., Farina, M., Prediger, R.D. (2016). Agmatine attenuates reserpine-induced oral dyskinesia in mice: role of oxidative stress, nitric oxide and glutamate NMDA receptors. *Behav. Brain. Res.* 312: 64-76.

7. Moretti, M., Matheus, F.C., de Oliveira, P.A., et al. (2014). Role of agmatine in neurodegenerative diseases and epilepsy. *Front. Biosci.* 6: 341–59.

8. Lee, W.T., Hong, S., Yoon, S.H., Kim, J.H., Park, K.A., Seong, G.J., Lee, J.E. (2009). Neuroprotective effects of agmatine on oxygen-glucose deprived primary-cultured astrocytes and nuclear translocation of nuclear factor-kappa B. *Brain Res*. 1281:64-70.

9. Qiu, W.W., Zheng, R.Y. (2006). Neuroprotective effects of receptor imidazoline 2 and its endogenous ligand agmatine. *Neurosci. Bull.* 22:187-191.

10. Mun, CH., Lee, WT., Park, KA., Lee, J.E. (2010). Regulation of endothelial nitric oxide synthase by agmatine after transient global cerebral ischemia in rat brain. *Anat. Cell. Biol.* 43:230-40.

11. Li, F., Wu, N., Su, R., Chen, Y., Lu, X., Liu, Y. (2011). Imidazoline receptor antisera-selected/Nischarin regulates the effect of agmatine on the development of morphine dependence. *J. Addict. Biol.* 17:392-408.

12. Satriano, J., Matsufuji, S., Murakami, Y., Lortie, M.J., Schwartz, D., Kelly, C.J., Hayashi, S., Blantz, R.C. (1998). Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels, *J. Biol. Chem.* 273:15313– 15316.

13. Vargiu, C., Cabella, C., Belliardo, S., Cravanzola, C., Grillo, M.A., Colombatto, S. (1999). Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. *Eur. J. Biochem.* 259:933-938.

14. Ramani, D., De Bandt, JP., Cynober, L.(2014). Aliphatic polyamines in physiology and diseases. *Clin Nutr*. 33:14-22.

15. Reis, D., Regunathan, S. (2000). Is agmatine a novel neurotransmitter in brain?

Trends Pharmacol. Sci. 21:187– 193.

16. Piletz, J.E., Aricioglu, F., Cheng, J.T., Fairbanks, C.A., Gilad, V.H., Haenisch, B., Halaris, A., Hong, S., Lee, JE., Li, J., Liu, P., Molderings, GJ., Rodrigues, AL., Satriano, J., Seong, G.J., Wilcox, G., Wu, N., Gilad, G.M. (2013). Agmatine: clinical applications after 100 years in translation. *Drug Discov. Today* 18:880-893.

17. Neis, V.B., Rosa, P.B., Olescowicz, G., Rodrigues, A.L. (2017). Therapeutic potential of agmatine for CNS disorders. *Neurochem Int*. 108:318-331.

18. Piletz, J.E., Chikkala D.N., Ernsberger, P. (1995). Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and a-2 adrenergic receptors. *J. Pharmacol. Exp.Ther*. 272:581– 587.

19. Yeşilyurt, O., Uzbay, I.T. (2001). Agmatine potentiates the analgesic effect of morphine by an alpha-(2)-adrenoceptor-mediated mechanism in mice. *Neuropsychopharmacol.* 25:98-103.

20. Wu, N., Su, R.B., Li, J. (2008). Agmatine and imidazoline receptors: their role in opioid analgesia, tolerance and dependence. *Cell Mol Neurobiol*. 28:629-641.

21. Aglawe, M., Taksande, B.G., Kuldhariya, S., Chopde, C., Umekar, M.J., Kotagale, N. (2014). Participation of central imidazoline binding sites in antinociceptive effect of ethanol and nicotine in rats. *Fundam. Clin. Pharmacol*. 28:284-293.

22. Taksande, B.G., Kotagale, N.R., Gawande, D.Y., Bharne, A.P., Chopde, C.T., Kokare, D.M. (2010). Agmatine, an endogenous imidazoline receptor ligand, modulates ethanol anxiolysis and withdrawal anxiety in rats. *Eur. J. Pharmacol.* 637:89–101.

23. Dixit, M.P., Thakre, P.P., Pannase, A.S., Aglawe, M.M., Taksande, B.G., Kotagale, N.R. (2010). Imidazoline binding sites mediates anticompulsive-like effect of agmatine in marble-burying behaviour in mice. *Eur. J. Pharmacol.* 732:26–31.

24. Neis, V.B., Moretti, M., Manosso, L.M., Lopes, M.W., Leal, R.B., Rodrigues, A.L. (2015). Agmatine enhances antidepressant potency of MK-801 and conventional antidepressants in mice. *Pharmacol. Biochem. Behav*. 130:9-14.

25. Neis, V.B., Bettio, L.E., Moretti, M., Rosa, P.B., Ribeiro, C.M., Freitas, A.E.,Goncalves, F.M., Leal, R.B., Rodrigues, A.L. (2016). Acute agmatine administration, similar to ketamine, reverses depressive-like behavior induced by chronic unpredictable stress in mice. *Pharmacol. Biochem. Behav*. 150-151:108-114.

26. Neis, V.B., Moretti, M., Bettio, L.E., Ribeiro, C.M., Rosa, P.B., Goncalves, F.M., Lopes, M.W., Leal, R.B., Rodrigues, A.L. (2016). Agmatine produces antidepressant-like effects by activating AMPA receptors and mTOR signaling. *Eur. Neuropsychopharmacol*. 26:959-971.

27. Freitas, A.E., Neis, V.B., Rodrigues, A.L. (2016). Agmatine, a potential novel therapeutic strategy for depression. *Eur. Neuropsychopharmacol*. 26:1885-1899.

28. Ahn, S.K., Hong, S., Park, Y.M., Lee,W.T., Park, K.A., Lee, J.E. (2011). Effects of agmatine on hypoxic microglia and activity of nitric oxide synthase. *Brain. Res.* 1373:48-54.

29. Uranchimeg, D., Kim, J.H., Kim, J.Y., Lee, W.T., Park, K.A., Batbaatar, G., Tundevrentsen, S., Amgalanbaatar, D., Lee, J.E. (2010) Recovered changes in the

spleen by agmatine treatment after transient cerebral ischemia. *Anat. Cell. Biol.* 43:44-53.

30. Condello, S., Calabro, E., Caccamo, D., Curro, M., Ferlazzo, N., Satriano, J., Magazu, S., Ientile, R. (2012). Protective effects of agmatine in rotenone-induced damage of human SH-SY5Y neuroblastoma cells: fourier transform infrared spectroscopy analysis in a model of Parkinson's disease. *Amino acids* 42:775-781.

31. Song, J., Hur, B.E., Bokara, K.K., Yang, W., Cho, H.J., Park, K.A., Lee, W.T., Lee, K.M.,Lee, J.E. (2014). Agmatine improves cognitive dysfunction and prevents cell death in a streptozotocin-induced Alzheimer rat model. *Yonsei Med. J.* 55:689-699.

32. Kang, S., Kim, C.H., Jung, H., Kim, E., Song, H.T., Lee, J.E. (2017). Agmatine ameliorates type 2 diabetes induced-Alzheimer's disease-like alterations in high-fat diet-fed mice via reactivation of blunted insulin signalling. *Neuropharmacol.* 113:467-479.

33. Molderings, G.J. (2006). The many faces of agmatine. *Pharmacol. Reports.* 58:266-267.

34. Philip, R., Campbell, E., Wheatley, D.N. (2003). Arginine deprivation, growth inhibition and tumour cell death:Enzymatic degradation of arginine in normal and malignant cell cultures. *Br. J. Cancer.* 88:613-623.

35. Matés, J.M., Sánchez-Jiménez, F., López-Herrera, J., de Castro, I.N. (1991). Regulation by 1,4-diamines of the ornithine decarboxylase activity induced by ornithine in perifused tumor cells. *Biochem. Pharmacol.* 42:1045-1052.

36. Satriano, J., Matsufuji, S., Murakami, Y., Lortie, M.J., Schwartz, D., Kelly, C.J.,

Hayashi, S., Blantz, R.C. (1998). Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels. *J. Biol. Chem.* 273:15313– 15316.

37. Molderings, G.J., Bönisch, H., Göthert, M., Brüss, M. (2001). Agmatine and putrescine uptake in the human glioma cell line SK-MG-1. *Naunyn-Schmiedeberg´s Arch. Pharmacol.* 363: 671-679.

38. Satriano, J., Isome, M., Casero, R.A. Jr., Thomson, S.C., Blantz, R.C. (2001). Polyamine transport system mediates agmatine transport in mammalian cells. *Am. J. Physiol. Cell Physiol.* 281:C329-C334.

39. Babál, P., Ruchko, M., Olson, J.W., Gillespie, M.N. (2000). Interactions between agmatine and polyamine uptake pathways in rat pulmonary artery endothelial cells. *Gen. Pharmacol*. 34:255-261.

40. Otake, K., Ruggiero, D.A., Regunathan, S., Wang, H., Milner, T.A., Reis, D.J. (1998). Regional localization of agmatine in the rat brain: an immunocytochemical study. *Brain Res.* 787:1-14.

41. Iyo, A., Zhu, M., Ordway, G., Regunathan, S. (2006). Expression of arginine decarboxylase in brain regions and neuronal cells. *J. Neurochem.* 96:1042-1050.

42. Peters. D., Berger, J., Langnaese, K., Derst, C., Madai, V.I., Krauss, M., Fischer, K.D., Veh, R.W., Laube, G (2013). Arginase and Arginine Decarboxylase - Where Do the Putative Gate Keepers of Polyamine Synthesis Reside in Rat Brain? *PLoS One* 8:e66735.

43. Regunathan, S., Reis, D.J. (2000). Characterization of arginine decarboxylase in rat

brain and liver: distinction from ornithine decarboxylase. *J. Neurochem.* 74:2201-2208.

44. Dixit, M.P., Thakre, P.P., Pannase, A.S., Aglawe, M.M., Taksande, B.G., Kotagale, N.R. (2014). Imidazoline binding sites mediates anticompulsive-like effect of agmatine in marble-burying behavior in mice. *Eur. J. Pharmacol*. 732:26-31.

45. Chung, S., Ahn, DS., Kim, YH., Kim, YS., Joeng, JH., Nam, TS. (2010) Modulation of N-type calcium currents by presynaptic imidazoline receptor activation in rat superior cervical ganglion neurons. *Exp Physiol.* 95:982-93.

46. Horyn, O., Luhovyy, B., Lazarow, A., Daikhin, Y., Nissim, I., Yudkoff, M., Nissim, I. (2005). Biosynthesis of agmatine in isolated mitochondria and perfused rat liver: studies with 15N-labelled arginine. Biochem. J. 388:419-425.

47. Zhu, M.Y., Iyo, A., Piletz, J.E., Regunathan, S. (2004). Expression of human arginine decarboxylase, the biosynthetic enzyme for agmatine. *Biochim. Biophys. Acta* 1670:156-64.

48. Moon, S.U., Kwon, K.H., Kim, J.H., Bokara, K.K., Park, K.A., Lee, W.T., Lee, J.E. (2010). Recombinant hexahistidine arginine decarboxylase (hisADC) induced endogenous agmatine synthesis during stress. *Mol. Cell Biochem*. 345:53-60.

49. Hong, S., Son, M.R., Yun, K., Lee, W.T., Park, K.A., Lee, J.E. (2014). Retroviral expression of human arginine decarboxylase reduces oxidative stress injury in mouse cortical astrocytes. *BMC Neurosci*. 15:99.

50. Cabella, C., Gardini, G., Corpillo, D., Testore, G., Bedino, S., Solinas, S.P., Cravanzola, C., Vargiu, C., Grillo, M.A., Colombatto, S. (2001). Transport and

metabolism of agmatine in rat hepatocyte cultures. *Eur. J. Biochem.* 268:940–947.

51. Kim, JI., Ganesan, S., Luo, SX., Wu, YW., Park, E., Huang, EJ., Chen, L., Ding, JB. (2015) Aldehyde dehydrogenase 1a1 mediates a GABA synthesis pathway in midbrain dopaminergic neurons. *Science.* 350:102-106.

52. Yoon, BE., Woo, J., Chun, YE., Chun, H., Jo, S., Bae, JY., An, H., Min, JO., Oh, SJ., Han, KS., Kim, HY., Kim, T., Kim, YS., Bae, YC., Lee, CJ. (2014) Glial GABA, synthesized by monoamine oxidase B, mediates tonic inhibition. *J Physiol.* 592:4951-68.

53. Caron, PC., Kremzner, LT., Cote, LJ. (1987) GABA and its relationship to putrescine metabolism in the rat brain and pancreas. *Neurochem Int.*10:219-29.

54. Pisano, J., Abraham, D., Udenfriend, S (1963) Biosynthesis and disposition of γ-guanidinobutyric acid in mammalian tissues. *Arch. Biochem. Biophys.* 100:323–9.

55. Shugalei, VS., Chupich, ZH., Iapundzhich, I., Rakich, L. (1977) Subcellular distribution of arginase and gamma-guanidino-butyrate-ureohydrolase in brain sections, neurons and glia. *Biokhimiia.* 42:67-73.

56. Lortie, M., Novotny, W., Peterson, O., Vallon, V., Malvey, K., Mendonca, M., Satriano, J., Insel, P., Thomson, S., Blantz, R. (1996). Agmatine, a bioactive metabolite of arginine. Production, degradation, and functional effects in the kidney of the rat. *J. Clin. Invest.* 97:413-420.

57. Kitanaka, J., Kitanaka, N., Tsujimura, T., Terada, N., Takemura, M. (2002). Expression of diamine oxidase (histaminase) in guinea-pig tissues. *Eur. J. Pharmacol*. 437:179-185.

58. Maintz. L., Novak, N. (2007) Histamine and histamine intolerance. *Am. J. Clin. Nutr*. 85:1185–1196.

59. Brown, D.G., Sanderson, M.R., Garman, E., Neidle, S. (1992) Crystal-Structure of a Berenil-D(Cgcaaatttgcg) Complex - an Example of Drug DNA Recognition Based on Sequence-Dependent Structural Features. *J. Mol. Biol*. 226:481–490.

60. Edwards, K.J., Jenkins, T.C., Neidle, S. (1992) Crystal structure of a pentamidine-oligonucleotide complex: implications for DNA-binding properties. *Biochemistry* 31:7104–7109.

61. Sastre, M., Regunathan, S., Galea, E., Reis, D. (1996). Agmatinase activity in rat brain: a metabolic pathway for the degradation of agmatine. *J. Neurochem.* 67:1761-1765.

62. Gilad, G., Wollam, Y., Iaina, A., Rabey, J., Chernihovsky, T., Gilad, V. (1996). Metabolism of agmatine into urea but not into nitric oxide in rat brain. *Neuroreport* 7:1730-1732.

63. Sastre, M., Galea, E., Feinstein, D., Reis, D., Regunathan, S. (1998). Metabolism of agmatine in macrophages: modulation by lipopolysaccharide and inhibitory cytokines. *Biochem. J.* 330:1405-1409.

64. Iyer, R.K., Kim, H.K., Tsoa, R.W., Grody, W.W., Cederbaum, S.D. (2002). Cloning and characterization of human agmatinase. *Mol. Genet. Metab.* 75:209-18.

65. Mistry, S.K., Burwell, T.J., Chambers, R.M., Rudolph-Owen, L., Spaltmann, F., Cook, W.J., Morris, S.M. Jr. (2002). Cloning of human agmatinase. An alternate path for polyamine synthesis induced in liver by hepatitis B virus. *Am. J. Physiol.*

Gastrointest. Liver Physiol. 282:375-381.

66. Carvajal, N., López, V., Salas, M., Uribe, E., Herrera, P., Cerpa, J. (1999). Manganese is essential for catalytic activity of *Escherichia coli* agmatinase. *Biochem. Biophys. Res. Commun.* 258:808-11.

67. Salas, M., Lopez, V., Uribe, E., Carvajal, N. (2004). Studies on the interaction of *Escherichia coli* agmatinase with manganese ions: structural and kinetic studies of the H126N and H151N variants. *J. Inorg. Biochem.* 98:1032-1036.

68. Schenk, G., Mateen, I., Ng, T., Pedroso, M.M., Mitić, N., Jafelicci Jr., M., Marques, R., Gahan, L.R., Ollis, D.L. (2016). Organophosphate-degrading metallohydrolases: Structure and function of potent catalysts for applications in bioremediation. *Coord. Chem. Rev.* 317:122–131.

69. Selleck, C., Guddat, L.W., Ollis D.L., Schenk G., Pedroso M.M. (2017). High resolution crystal structure of a fluoride-inhibited organophosphate-degrading metallohydrolase. *J. Inorg. Biochem.* [Epub ahead of print; DOI: 10.1016/j.jinorgbio.2017.06.013]

70. Mitić, N., Miraula, M., Selleck, C., Hadler, K.S., Uribe, E., Pedroso, M.M., Schenk G. (2014). Catalytic mechanisms of metallohydrolases containing two metal ions. *Adv. Protein Chem. Struct. Biol*. 97:49-81.

71. Dallmann, K., Junker, H., Balabanov, S., Zimmermann, U., Giebel, J., Walther, R. (2004). Human agmatinase is diminished in the clear cell type of renal cell carcinoma. *Int. J. Cancer.* 108:342-347.

72. Romero, N., Benítez, J., Garcia, D., González, A., Bennun, L., García-Robles,

M.A., López, V., Wilson, L.A., Schenk, G., Carvajal, N., Uribe, E. (2017). Mammalian agmatinases constitute unusual members in the family of Mn^{2+}-dependent ureahydrolases. *J. Inorg. Biochem*. 166:122-125.

73. Kim, K., Ahn, H., Kim, D., Lee, H., Ha, J., Kim, H., Yoon, H., Suh, S. (2005). Expression, crystallization and preliminary X-ray crystallographic analysis of human agmatinase. *Acta Crystallogr.* 61:889-891.

74. Bernstein, H.G., Derst, C., Stich, C., Prüss, H., Peters, D., Krauss, M., Bogerts, B., Veh, R.W., Laube, G. (2011). The agmatine-degrading enzyme agmatinase: a key to agmatine signaling in rat and human brain? *Amino Acids* 40:453-465.

75. Halaris, A., Zhu, H., Feng, Y., Piletz, J.E (1999). Plasma agmatine and platelet imidazoline receptors in depression. *Ann. NY Acad. Sci.* 881:445–451.

76. Bernstein, H.G., Stich, C., Jager, K., Dobrowolny, H., Wick, M., Steiner, J.,Veh, R., Bogerts, B., Laube, G. (2012). Agmatinase, an inactivator of the putative endogenous antidepressant agmatine, is strongly upregulated in hippocampal interneurons of subjects with mood disorders. *Neuropharmacol.* 62:237–246.

77. Meylan, E.M., Breuillaud, L., Seredenina, T., Magistretti, P.J., Halfon, O., Luthi-Carter, R., Cardinaux, J.R. (2016). Involvement of the agmatinergic system in the depressive-like phenotype of the Crtc1 knockout mouse model of depression. *Transl Psychiatry* 6:1-11.

78. Uribe E., Salas, M, Enriquez, S., Orellana, MS, Carvajal, N. (2007). Cloning and functional expression of a rodent brain cDNA encoding a novel protein with agmatinase activity, but not belonging to the arginase family. *Arch. Biochem.*

Biophys. 461: 146-150.

79. García, D., Ordenes, P., Benítez, J., González, A., García-Robles, MA., López, V., Carvajal, N., Uribe, E. (2016). Cloning of two LIMCH1 isoforms: characterization of their distribution in rat brain and their agmatinase activity. *Histochem. Cell Biol.* 145:305-313.

80. Mella, C., Martínez, F., García, M., Nualart, F.,Castro, V., Bustos, P., Carvajal, N. and Uribe, E. (2010). Expression and localization of agmatinase-like protein in rat brain. *Histochem. Cell Biol.* 134:137-144.

81. Castro, V., Fuentealba, P., Henríquez, A., Vallejos, A., Benítez, J., Lobos, M., Díaz, B., Carvajal, N., Uribe E. (2011). Evidence for an inhibitory LIM domain in a rat brain agmatinase-like protein. *Arch. Biochem. Biophys.* 512:107-10.

82. Cofré, J., Montes, P., Vallejos, A., Benítez, J., García, D., Martínez-Oyanedel, J., Carvajal, N., Uribe, E. (2014). Further insight into the inhibitory action of a LIM/double zinc-finger motif of an agmatinase-like protein. *J. Inorg. Biochem.* 132:92-5.

83. Feuerstein, R., Wang, X., Song, D., Cooke, N. E. & Liebhaber, S. A. (1994). The LIM/double zinc-finger motif functions as a protein dimerization domain. *Proc. Natl. Acad. Sci.* 91:10655–10659.

84. Kadrmas, J., Beckerle, M. (2004). The LIM domain: from the cytoskeleton to the nucleus. *Nat. Rev. Mol. Cell Bio.* 5:920-931.

85. Nagata K., Ohashi K., Yang N., Mizuno K. (1999). The N-terminal LIM domain negatively regulates the kinase activity of LIM-kinase 1. *Biochem. J.* 343:99-105.

86. Quiñones, M., Cofre, J., Benítez, J., García, D., Romero, N., González, A., Carvajal, N., García, M., López, V., Schenk, G., Uribe, E. (2015). Insight on the interaction of an agmatinase-like protein with Mn^{2+} activator ions. *J. Inorg. Biochem.* 145:65-69.

87. Alarcón, R., Orellana, M.S., Neira, B., Uribe, E., Garcia, J.R., Carvajal, N. (2006). Mutational analysis of substrate recognition by human arginase type I—agmatinase activity of the N130D variant. *FEBS J.* 7324:5625-5631.

88. Cama, E., Pethe, S., Boucher, J.L., Han, S., Eming, F.A., Ash, D.E., Viola, R.E., Mansuy, D., Chistianson, D.W. (2004). Inhibitor coordination interactions in the binuclear manganese cluster of arginase. *Biochemistry* 43:8987-8999.

89. Ahn, H., Kim, K., Lee, J., Ha, J., Kim, D., Yoon, H., Kwon, A., Suh, S. (2004). Crystal structure of agmatinasa reveals structural conservation and inhibition mechanism of the ureohydrolase superfamily. *J. Biol. Chem.* 279:50505-50513.

90. Baugh, L., Gallagher, LA., Patrapuvich, R., Clifton, MC., Gardberg, AS., Edwards, TE., Armour, B., Begley, DW., Dieterich, SH., Dranow, DM., Abendroth, J., Fairman, JW., Fox, D 3rd., Staker, BL., Phan, I., Gillespie, A., Choi, R., Nakazawa-Hewitt, S., Nguyen, MT., Napuli, A., Barrett, L., Buchko, GW., Stacy, R., Myler, PJ., Stewart, LJ., Manoil, C. and Van Voorhis, WC. (2013). Combining functional and structural genomics to sample the essential Burkholderia structome. *PLoS One* 8:53851.

91. Sjöblom, B., Ylänne, J., Djinoviae-Carugo, K. (2008). Novel structural insights into F-actin-binding and novel functions of calponin homology domains. *Curr. Opin. Struct. Biol.* 18:702-708.

92. Morris S.M. Jr. (2004). Recent advances in arginine metabolism. *Curr. Opin. Clin. Nutr. Metab. Care*. 7:45-51.

93. Zambelli, B., Musiani, F., Benini, S., Ciurli, S. (2011). Chemistry of Ni^{2+} in Urease: Sensing, Trafficking, and Catalysis. *Acc. Chem. Res.* 44:520–530.

94. Uzbay, T.I. (2012). The pharmacological importance of agmatine in the brain. *Neurosci. Biobehav. Rev.* 36:502–519.

95. Maltsev, A.V., Kokoz, Y.M., Evdokimovskii, E.V., Pimenov, O.Y., Reyes, S., Alekseev, A.E. (2014). α-2 adrenoceptors and imidazoline receptors in cardiomyocytes mediate counterbalancing effect of agmatine on NO synthesis and intracellular calcium handling. *J. Mol. Cell Cardiol.* 68:66–74.

96. Huynh N.N., Chin-Dusting J. (2006). Amino acids, arginase and nitric oxide in vascular health. *Clin. Exp. Pharmacol. Physiol.* 33:1–8.

97. Tyacke, R.J., Fisher, A., Robinson, E.S., Grundt, P., Turner, E.M., Husbands, S.M., Hudson, A.L., Parker, C.A., Nutt, D.J. (2012). Evaluation and initial *in vitro* and *ex vivo* characterization of the potential PET ligand, BU99008 (2-(4,5-dihydro-1H-imidazol-2-yl)-1-methyl-1H-indole), for the $imidazoline_2$ binding site. *Synapse* 66:542–51.

98. Parker, C.A., Nabulsi, N., Holden, D., Lin, S.F., Cass, T., Labaree, D., Kealey, S., Gee, A.D., Husbands, S.M., Quelch, D., Carson, R.E., Nutt, D.J., Huang, Y., Tyacke, R.J. (2014). Evaluation of ^{11}C-BU99008, a PET ligand for the $imidazoline_2$ binding sites in rhesus brain. *J. Nucl. Med.* 55:838–44.

99. Laube, G., Bernstein, H.G. (2017). Agmatine: multifunctional arginine metabolite

and magic bullet in clinical neuroscience? *Biochem. J.* 474:2619-2640.

100. Gowing, L.R., Ali, R.L., Allsop, S., Marsden, J., Turf, E.E., West, R., Witton, J. (2015). Global statistics on addictive behaviours: 2014 status report. *Addiction* 110:904–919.

101. Müller, CA., Geisel, O., Banas, R., Heinz, A. (2014). Current pharmacological treatment approaches for alcohol dependence. *Expert Opin. Pharmacother*. 15:471–81.

Figure legends

Figure 1. Metabolic pathways for agmatine synthesis and conversion to polyamines or GABA. Agmatine, produced from L-arginine by ADC, may be hydrolyzed to putrescine, either by AGM or ALP in the polyamine biosynthetic pathway, or converted to guanidinobutyraldehyde by DAO, for the synthesis of GABA. ODC decarboxylates arginine and is also capable to produce putrescine.

Figure 2. Multiple sequence alignment of human, *E. coli*, *D. radiodurans* agmatinases and human arginase. Sequences obtained from Uniprot were aligned by Clustal Omega and displayed using the GeneDoc. Locations of the three histidines and four aspartate residues, required for metal ligand binding and enzymatic activity, which are conserved among members of the ureohydrolase superfamily, are indicated by stars below the alignment.

Figure 3. The cell growth of yeast strains unable to polyamine biosynthesis transfected with AGM mouse (*i.e.* clone 1 (□) and 2 (■)) and human AGM (▲), compared with the non-transfected strain (●). The yeast strain lacks the *ODC* gene, making it deficient in polyamine biosynthesis and necessitating the presence of exogenous polyamines for growth. The cultures were depleted of endogenous polyamines and then grown in minimal medium. All media contained 10 μg/mL agmatine and growth was estimated by nephelometry at 600 nm. The data were previously published [72] and reproduced with permission from the *Journal of Inorganic Biochemistry.*

Figure 4. Yeast strains unable to polyamine biosynthesis, transfected with isoform I of the *Limch1* gene (o); Yeast transformed with isoform II of the *Limch1* gene (o); yeast transformed with the *ALP* gene (□); untransformed control cells (●). After 30 h, spermidine was added to the control culture to verify the viability and dependence of polyamines for growth of this yeast strain. All other details are identical to those described for Fig. 3 (see for more details [79]).

Figure 5. Homology model of the LIM domain of ALP. Left, schematic representation of the LIM-domain of ALP. (A), structural model of the LIM-domain of ALP, constructed using MODELLER 9v10. (B) and (C), geometric environments of the Zn^{2+} ions in the two fingers of the LIM domain (see Cofré *et al.* for more details [82]).

Figure 6. Active site structure of AGM from *D. radiodurans*. A and B represent the two Mn^{2+} binding sites (adapted from Ahn *et al*.[89]).

Figure 7. ALP and AGM as promising targets for novel addiction treatments. AGM and ALP hydrolyse agmatine to urea and putrescine. The latter is further metabolized to spermine. An inhibition of these two enzymes thus has the same effect as direct agmatine injection.

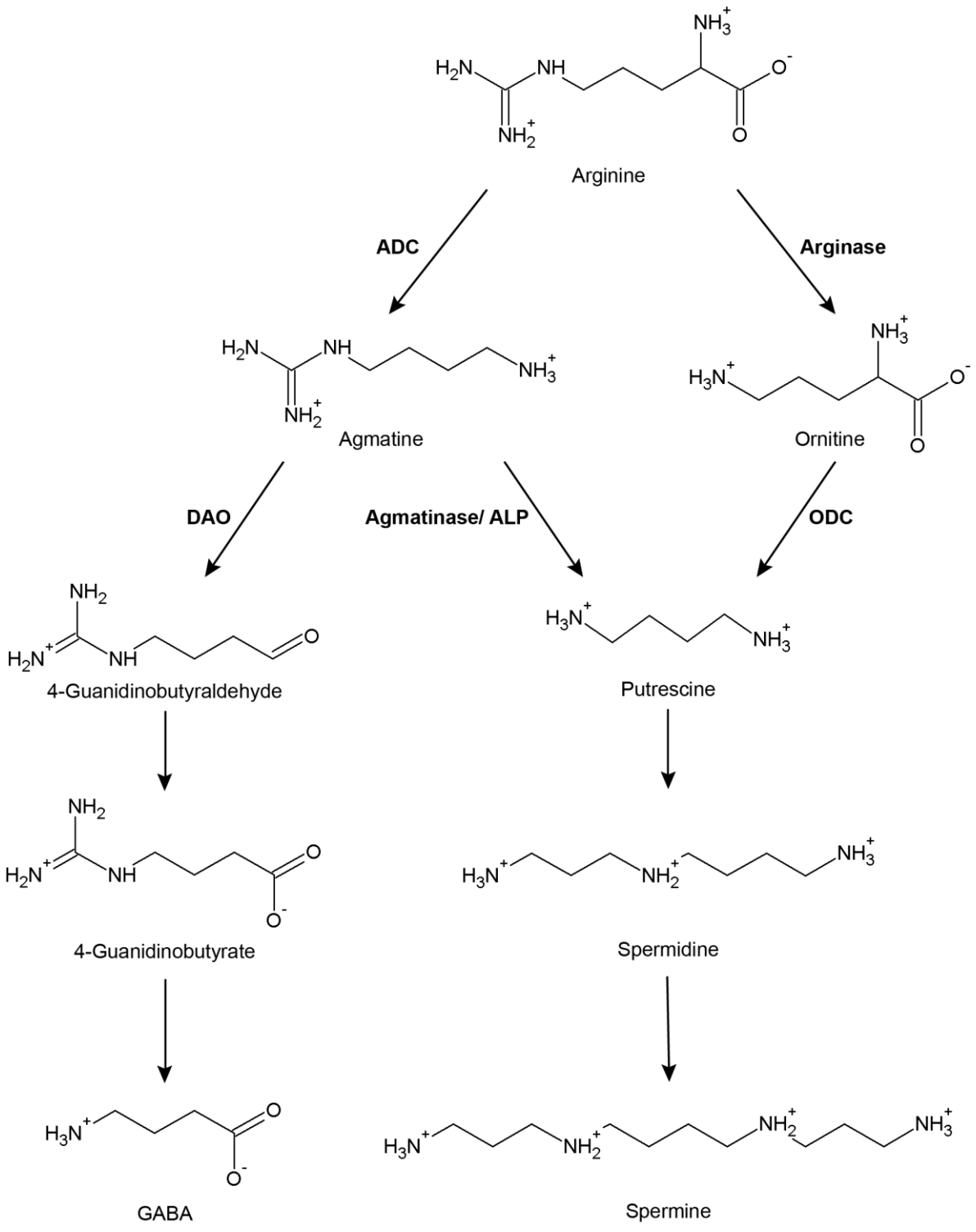

Figure 1

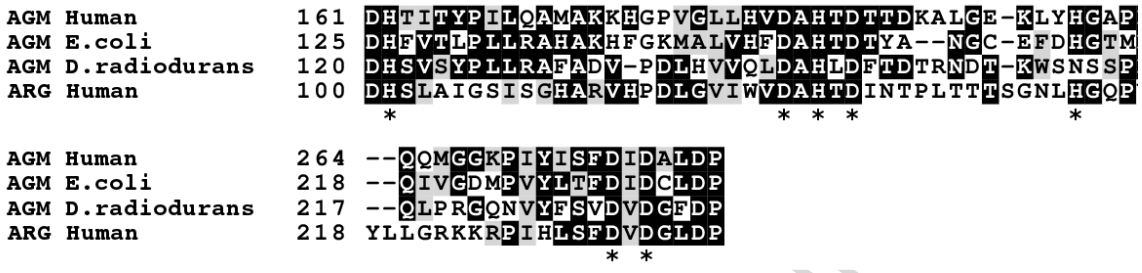

Figure 2

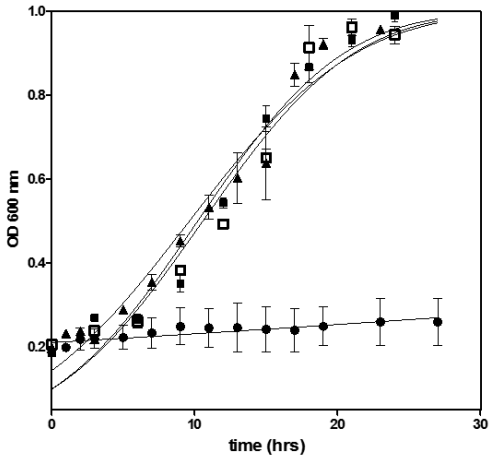

Figure 3

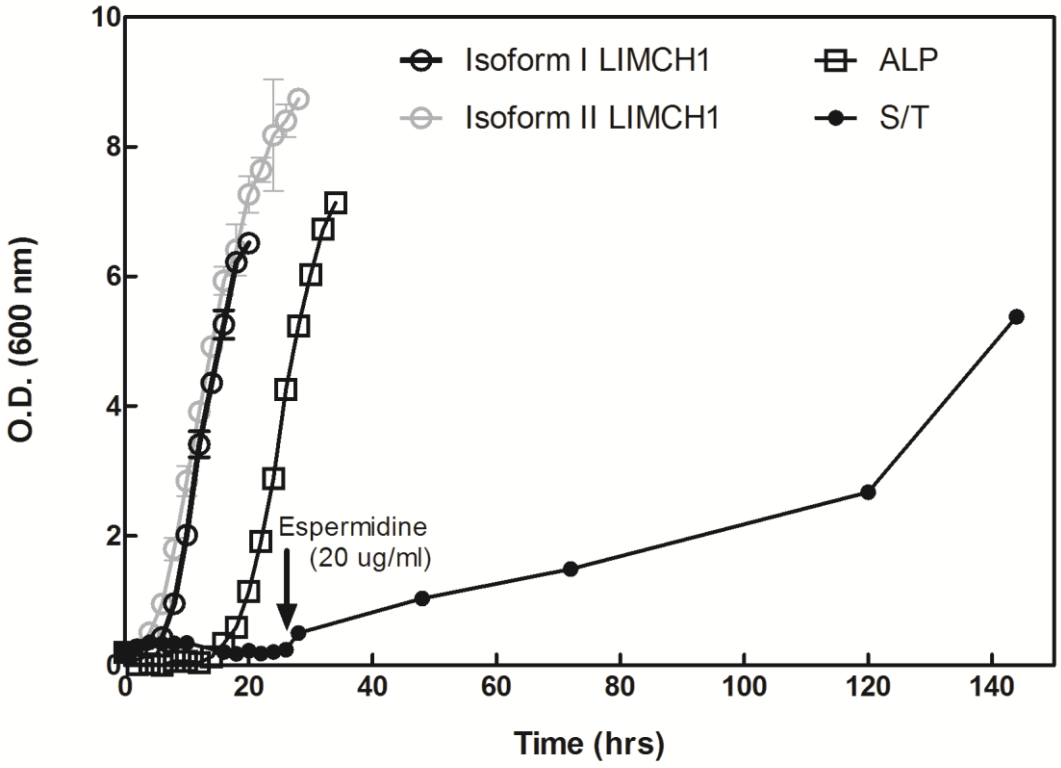

Figure 4

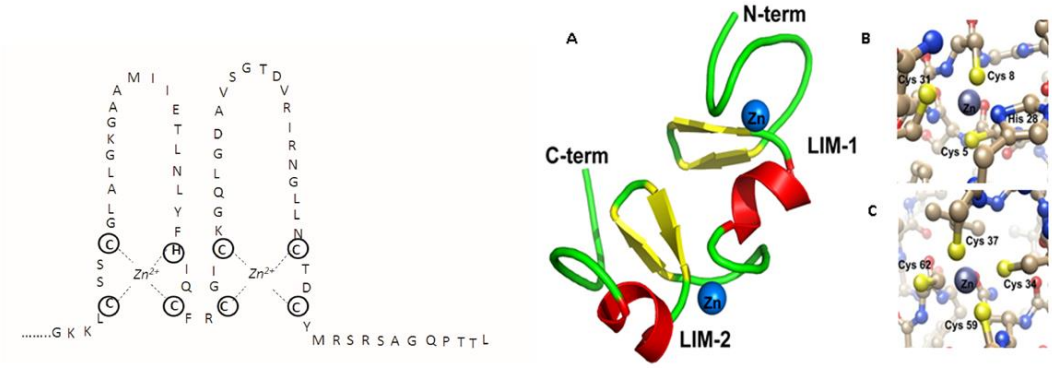

Figure 5

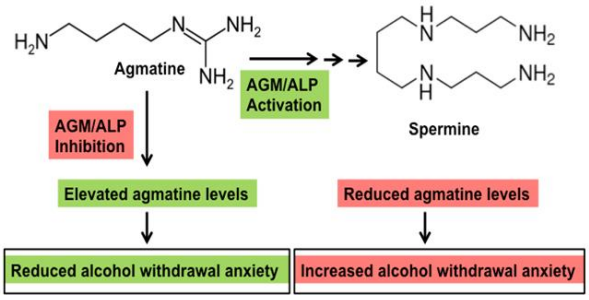

Figure 6

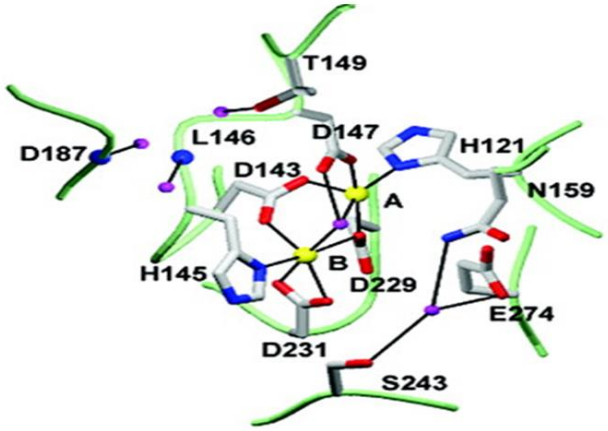

Figure 7