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Agmatine: clinical applications after 100 years in translation

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Agmatine (decarboxylated arginine) has been known as a natural product for over 100 years, but its biosynthesis in humans was left unexplored owing to long-standing controversy. Only recently has the demonstration of agmatine biosynthesis in mammals revived research, indicating its exceptional modulatory action at multiple molecular targets, including neurotransmitter systems, nitric oxide (NO) synthesis and polyamine metabolism, thus providing bases for broad therapeutic applications. This timely review, a concerted effort by 16 independent research groups, draws attention to the substantial preclinical and initial clinical evidence, and highlights challenges and opportunities, for the use of agmatine in treating a spectrum of complex diseases with unmet therapeutic needs, including diabetes mellitus, neurotrauma and neurodegenerative diseases, opioid addiction, mood disorders, cognitive disorders and cancer.

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Introduction and historical perspective

Discovered in 1910 by the Nobel Laureate Albrecht Kossel [1], agmatine is a ubiquitous compound biosynthesized from arginine by the enzyme arginine decarboxylase (ADC), hence also known as decarboxylated arginine. Its discovery generated immediate interest and, in the same year, Engeland and Kutscher reported [2] that agmatine could induce contractions of the isolated cat uterus and increase blood flow in rabbits. A year later, Dale and Laidlaw confirmed these findings, but questioned their physiological relevance given the high concentrations (high μM range) required [3], an issue resolved only a century later (Box 1). Marking the centenary of agmatine discovery, we address this and other historical controversies, the essence of scientific progress, and discuss dogmas that stifled research.

The scarcity of agmatine research during most of the 20th century is clear (Box 1). The most often cited reason behind this neglect was the difficulty in demonstrating ADC activity in mammals, which led many to assume erroneously that mammals, compared with bacteria, plants and fish, did not synthesize agmatine [4]. It was not until the breakthrough discovery in 1994 by Reis and colleagues of agmatine and ADC activity in the mammalian brain, that this false dogma finally began to erode [5]. Today, there is overwhelming evidence that mammalian agmatine biosynthesis occurs via this enzyme and the human gene encoding ADC has been cloned (Gene ID: 113451) [6]. However, there also remains controversy regarding the very identity of mammalian ADC. For one, the enzyme protein has yet to be purified. Furthermore, mammalian ADC activity is, curiously, not inhibited by known ADC inhibitors of other species, but rather by a classic inhibitor of ornithine decarboxylase (ODC, the enzyme catalyzing the first step in polyamine biosynthesis) [7–9]. This has suggested that ODC is the enzyme that catalyzes arginine decarboxylation in mammals [7–10] (Box 2, Fig. 2a).

The 'rediscovery of agmatine' in 1994 [5] occurred serendipitously during a search for the elusive endogenous ligand of the type-1 imidazoline receptor (I_1R), identifying agmatine as a candidate for clonidine displacing substance (CDC; reviewed in [11]). However, this CDS candidacy soon generated controversy because agmatine was found to lack binding specificity and functional selectivity and to exert mixed physiological effects, probably via either I_1R or I_2R, as well as via the related α_2-adrenoceptors [12,13]. This preoccupation with imidazoline receptors eventually subsided when further research showed that agmatine acts as a neuromodulator at several other types of neurotransmitter receptor (Box 3) and substantial evidence emerged implicating agmatine as a novel co-transmitter ([14]; reviewed in [15,16]).

By 1995, the first neuroprotective effects of agmatine had been documented by Gilad and colleagues [7]. Ensuing research variegated rapidly on several fronts, implicating the cytoprotective properties of agmatine in neuroprotection, nephroprotection and cardioprotection. Additionally, its neuromodulatory characteristics have been implicated in opiate actions, psychiatric disorders and cognitive functions, and its modulation of cell proliferation in arresting cancer [15,16]. Importantly, the first human clinical trials, published in 2010, indicate that oral agmatine is safe and effective in alleviating neuropathic pain [17]. In addition, a recent case study suggests that agmatine is efficacious in treating depressive disorders [18].

Burgeoning research has unraveled the regulation of mammalian agmatine synthesis and metabolism and implicated multiple vital molecular targets in its pharmacological actions. These include key neurotransmitter receptors, membrane transporters, NO synthesis and polyamine metabolism (Boxes 2 and 3). Agmatine is now considered to be capable of exerting modulatory actions simultaneously at multiple target sites, thus fitting the therapeutic profile of a 'magic shotgun' for complex disorders (Box 3) [19].

In this article, we advocate that agmatine and its newly discovered derivatives are poised for expanded drug development efforts and clinical trials. The article represents the consensus concern of scientists from 16 independent laboratories and draws attention to the new understandings of agmatine mechanisms of action, the substantial preclinical evidence for its therapeutic effects, and early clinical trials indicating effectiveness in treating complex diseases. We begin with a focus on agmatine metabolism and proposed key regulatory roles at the intersection of biochemical pathways critical to arginine metabolism. The concept of agmatine modulatory function is developed and the feasibility of using agmatine, or related analogs, for treating various disorders, is discussed.

Endogenous agmatine: regulation of metabolic pathways

Agmatine biosynthesis by arginine decarboxylation is well positioned to compete with the principal arginine-dependent pathways, namely: nitrogen metabolism (urea cycle), polyamine and NO synthesis, as well as protein synthesis (Box 2, Fig. 2a) [20,21].

BOX 1

Centenary milestones in agmatine biomedical research

As depicted in Fig. 1, most of the 20th century since Kossel's 1910 discovery [1] and well into the 1990s, was characterized by sparse research activity into the effects of agmatine. The initial excitement generated by Kossel's discovery soon subsided and research resumed only during the 1920s, after a 10-year period that coincided with World War I, when agmatine was investigated as a potential nontoxic antidiabetic aminoguanidine analog in the clinic of Oskar Minkowski [59–61]. However, chemical derivatives of agmatine then proved more potent and the course of drug discovery diverged to modern-day biguanidine antidiabetics [61], leading eventually to the development of metformin, the first-line therapeutic for type 2 diabetes mellitus. Thereafter, aside from a few isolated studies, biomedical research on agmatine remained scarce well into the 1990s.

During the 1960s, it was found that agmatine can stimulate the proliferation of thymocytes and lymphocytes [127]. During the 1970s and 1980s, a few studies uncovered mechanisms of the glucoprotective effects of agmatine [63]. In addition, during the late 1980s, research using [^{3}H]-agmatine as a radiotracer led Loring to discover that agmatine acts as an antagonist of neuronal nicotinic receptors [130]. Unfortunately, this latter finding generated little attention. It was only in 1994 that the breakthrough discovery of endogenous agmatine in mammals by Reis and colleagues reignited the field and led to the recent avalanche in new discoveries and sharp increase in publications [5,131].

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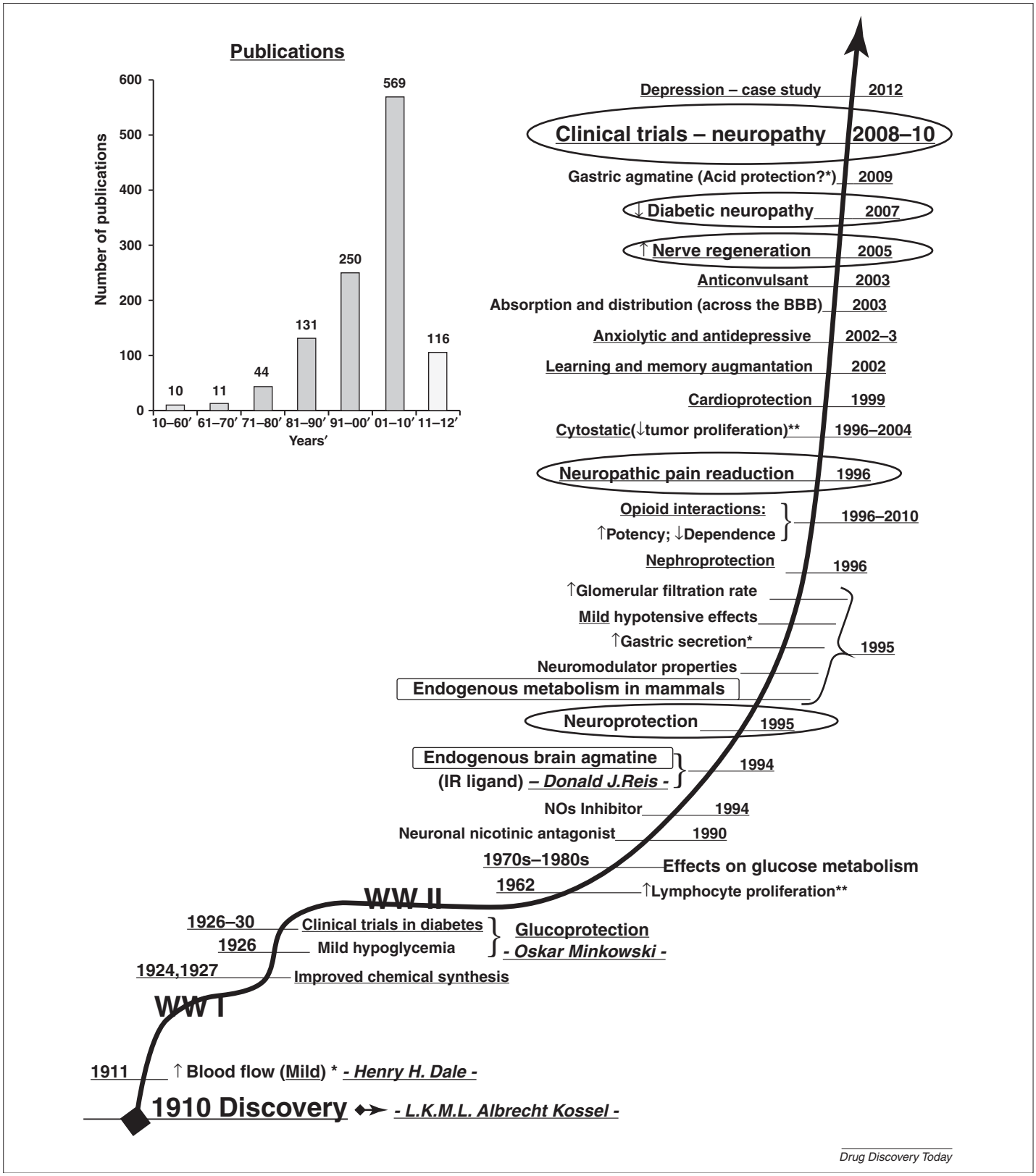

FIG. 1

Milestones in agmatine biomedical research. Key preclinical findings are emphasized by larger or underlined type set, landmark discoveries concerning metabolism in mammals are framed, experimental studies with clinical implications are underlined, and discoveries leading to clinical trials in neuropathy are encircled. The years covering World Wars I (WWI) and II (WWII) coincided with extended periods of few scientific publications, which are signified as plateaus. The inserted bar graph indicates the number of publications: the first bar represents 1910 to 1960, the following bars each decade thereafter and the final bar shows years 2011 and 2012 (searched in PubMed: http://www.ncbi.nlm.nih.gov and reference lists of historical publications). *Key*: *, controversy; **, differential effects on proliferation of various cell types. ↑, increase; ↓, decrease. *Abbreviations*: BBB, blood–brain barrier; IR, imidazoline receptors.

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BOX 2

Agmatine modulation of NO production and the polyamine stress response

A temporally synchronized increase in cellular agmatine biosynthesis is proposed to have important role in down regulating NO synthesis (Fig 2a), specifically iNOS and polyamine biosynthesis (Fig 2b) [20]. Agmatine might reduce NO overproduction by three proposed mechanisms: (i) by exerting direct inhibition of iNOS [25,42,132]; (ii) by DAO-catalyzed agmatine oxidation into agmatine aldehyde, a metabolite that is an even more potent and selective inhibitor of iNOS than agmatine itself [Fig 2b, step (2)] [25]; and (iii) by inhibition of aldehyde dehydrogenase (AldDH) activity, leading to agmatine aldehyde accumulation and further inhibition of iNOS [Fig 2b, step (3)] [25]. The latter two mechanisms however, appear to be unique to adult peripheral tissues rather than to the CNS [24]. Importantly, in addition to direct iNOS inhibition, agmatine might lead to induction of endothelial NOS (eNOS) [133]. This indicates that agmatine might best be considered as a modulator of cellular NO concentrations.

A concomitant transient stress-induced increase in ornithine and agmatine biosynthesis [9,26,31] is proposed as a part of the adaptive PSR, whereby agmatine serves as a direct precursor for polyamine synthesis [4]. Increased cellular agmatine could also have a three-part role in the transient characteristic of the PSR by providing a feed-forward inhibition [20]: first, by reducing polyamine synthesis via induction of antizyme, the endogenous inhibitor of ODC [Fig 2b, step (1)] [20]; second, by increasing polyamine metabolism via upregulation of the degradative enzyme, polyamine-*N*-acetyltransferase [20]; and third, by competing at the polyamine transport system, thus reducing cellular polyamine content [20] (Fig 2b). Thus, agmatine is also considered a modulator of cellular polyamine concentrations. Note: when stress becomes chronic, it can lead within weeks to a persistent downregulation of agmatine biosynthesis [30], which might in turn remove those agmatine 'brakes' and allow sustained polyamine and NO accumulation with potential pathological consequences [27].

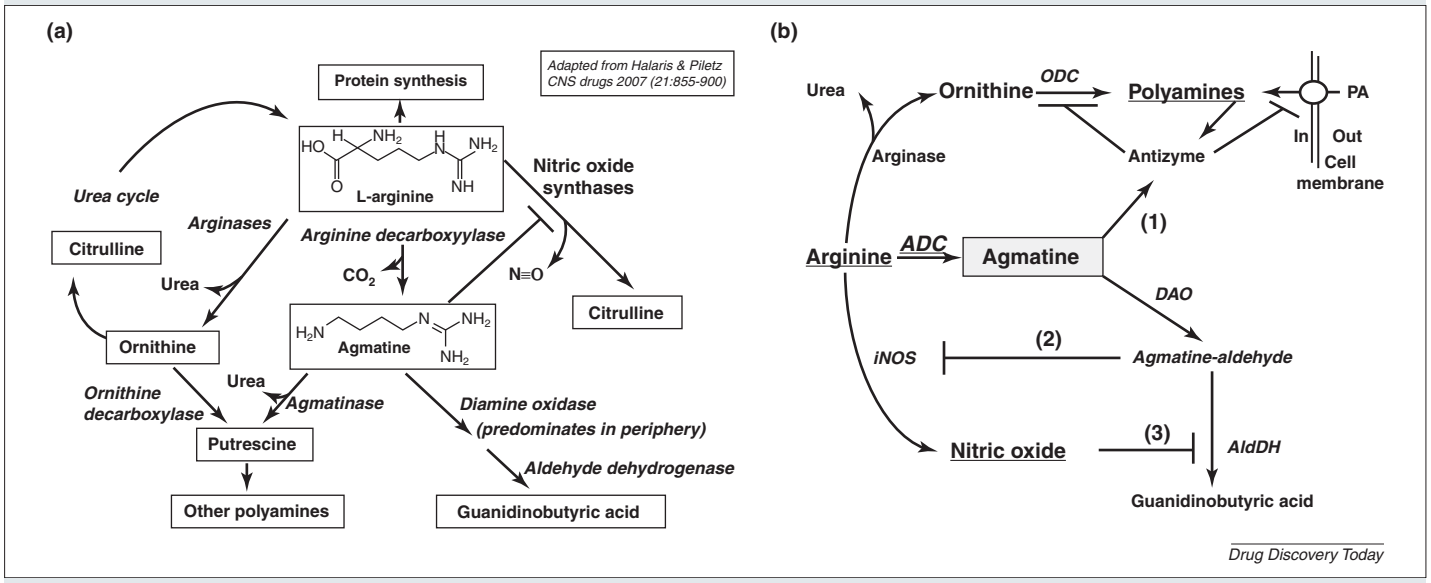

FIG. 2

(a) Illustration of agmatine biosynthesis and degradation, highlighting some of the interrelated pathways of arginine metabolism that regulate nitrogen (urea cycle), polyamines, N≡O (nitric oxide, NO), and protein metabolism. Because the recycling of arginine from protein degradation is not direct, but involves methylation of arginine residues, this metabolic loop is not shown. Line ending with a vertical line indicates inhibition of NO synthases (NOS) by agmatine. **(b)** Modulation by agmatine of key control mechanisms of arginine metabolism, illustrating the 'Pendulum Hypothesis' [20]. Illustrated are: (1) agmatine induction of antizyme protein leading to ornithine decarboxylase (ODC) inactivation, with additional suppression of polyamine transport; (2) suppression of inducible (i)NOS via agmatine aldehyde; and (3) NO inhibition of aldehyde dehydrogenase (AldDH) activity. The oval in the cell membrane represents the polyamine transporter. Lines ending with a vertical line indicate inhibition. Other abbreviations: ADC, arginine decarboxylase; DAO, diamine oxidase; PA, polyamines. Adapted from [32] (a).

Agmatine degradation occurs mainly by hydrolysis, catalyzed by agmatinase into urea and putrescine, the diamine precursor of polyamine biosynthesis (Box 2, Fig. 2a) [22,23] and a known minor precursor of the neurotransmitter GABA (γ-aminobutyric acid) [15]. An alternative pathway, mainly in peripheral tissues, is by diamine oxidase (DAO)-catalyzed oxidation into agmatine aldehyde, which is in turn converted by aldehyde dehydrogenase into guanidinobutyrate and secreted by the kidneys (Box 2, Fig. 2a,b) [24,25].

Role in arginine metabolism

The principal arginine-dependent pathways (Box 2) are important in the cellular response to stressful stimuli [20,21]. Depending on spatiotemporal and cellular compartmentalization, these pathways often coexist and compete for the same substrate pool of arginine. Being a conditional amino acid, arginine supply can also be limiting upon demanding physiopathological conditions, such as growth, inflammation, traumatic stress or injury [21]. These conditions tend to perturb the balance of arginine metabolism, with a consequential selective shortage of arginine availability among the various pathways [20].

An immediate early response of NO synthesis (seconds to minutes) followed by an early transient increase in polyamine metabolism (minutes to hours), coined the 'Polyamine-Stress-Response' (PSR) by Gilad and colleagues [26,27], are crucial for proper adaptive

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BOX 3

Multiple putative molecular targets of agmatine

Agmatine has been found to exert modulatory actions directly and/or indirectly at multiple key molecular targets underlying cellular control mechanisms of cardinal importance in health and disease (see below). The following list indicates the categories of control mechanisms and identifies their molecular targets:

Neurotransmitter receptors and receptor ionophores
Nicotinic [130], imidazoline I_1 and I_2 [5,56,105], α_2-adrenergic [12,131], glutamate NMDAR [75], and serotonin 5-HT2A and 5HT-3 [110] receptors.

Ion channels
ATP-sensitive K^+ channels [64], voltage-gated Ca^{2+} channels [81], and acid-sensing ion channels (ASICs) [91].

Membrane transporters
Agmatine specific-selective uptake sites [15], organic cation transporters (mostly OCT2 subtype) [69], extraneuronal monoamine transporters (ENT) [69], and polyamine transporters [20]. Mitochondria also have an agmatine specific-selective transport system [50].

NO synthesis modulation
Differential inhibition of all isoforms of NOS [20,42,132]. Induction of eNOS [133].

Polyamine metabolism
Precursor for polyamine synthesis [4], inhibition of polyamine transport [20], induction of spermidine/spermine acetyltransferase (SSAT) [20], and induction of antizyme [20].

Protein ADP-ribosylation
Inhibition of protein arginine ADP-ribosylation [134].

Matrix metalloproteases
Downregulation of *Matrix metalloproteases* (MMP) 2 and 9 [86].

AGE formation
Inhibition of AGE formation [45,67].

Comment on pharmacological mechanisms of actions
Although classic rules of pharmacodynamics (e.g., Bmax, Km and Ki) would apply for agmatine at the appropriate molecular targets, they individually do not appear to account for the observed therapeutic effects of agmatine (as reviewed in the main text). Indeed, some debate hinges on which one of these targets selectively underlies a given disorder phenotype. The argument for this singularity has roots in Paul Ehrlich's 'magic bullet' concept of more than 100 years ago, or the 'lock and key' concept that still prevails in drug industry today. Yet, as previously suggested for the beneficial effects of agmatine in neuroprotection [16,97], a single drug capable of modulating, potentially synergistically, the multiple targets implicated in complex disease etiology, would be advantageous and also presents a preferred therapeutic solution to combination therapy with unavoidable broad side effects. Therefore, agmatine is proposed to act as a 'magic shotgun' (a metaphor previously used to describe newer antipsychotics [19]), capable of synergistically modulating, directly or indirectly, multiple dysregulated molecular targets to ameliorate various complex clinical disorders.

responses of quiescent cells to stressful conditions [20,26,27]. Yet unbridled overproduction of NO and/or polyamines can lead to improper arginine utilization, nitrogenous stress and, in turn, to pathological consequences and even cell death [20,26,27]. Thus, it stands to reason that tight regulatory mechanisms must control the overproduction of NO and polyamines. As illustrated in Box 2, the 'Pendulum Hypothesis' proposed by Satriano and colleagues [28] posits that agmatine is center stage as a key modulator of NO and polyamine overproduction.

Tissue concentrations

As summarized in Table 1, there are concentration differences between tissues, with the highest agmatine concentrations occurring in the stomach [29]. Interestingly, increased agmatine concentrations are associated with stressful stimuli in rat brain [30,31] and with major depression in human blood [32]. Additionally, low plasma concentrations have been observed in human metabolic syndrome [33]. The significance of these findings awaits further investigation.

The observed high stomach concentration (Table 1) is of interest because agmatine, which has been implicated in aggravating stomach ulcers [15], was recently found instead to exert rather protective effects against gastric ischemia [34]. Although these findings remain to be reconciled, agmatine is now implicated as a cytoprotective agent in gastric acid functions [34].

Concern has also been raised about the approximately tenfold discrepancy between pharmacodynamic concentrations required at the proposed targets (Box 3) and the tissue concentrations of agmatine measured mostly below 1 μM (Table 1). Localized agmatine biosynthesis coupled to intracellular sequestration and release into confined intercellular spaces, such as the synaptic cleft (as discussed below), could conceivably create the required high concentrations and, thus, could explain this discrepancy.

Implications for neurotransmission

Substantial evidence now supports agmatine as a co-neurotransmitter. First, agmatine has been shown to be taken up by presynaptic axon terminals, localized in synaptic vesicles, and released upon membrane depolarization involving calcium and potassium fluxes [14,35]. Second, agmatine and its biosynthetic enzyme ADC show an overlapping cellular localization in neurons [36,37]. ADC can also be localized in astrocytes, which might serve as an agmatine reservoir [38]. Third, agmatine and its central nervous system (CNS) degradative and/or inactivating enzyme, agmatinase, have been colocalized in various brain regions both pre- and postsynaptically [39]. Fourth, agmatine has been colocalized with other neurotransmitters, notably with glutamate in nerve cell bodies and synaptic terminals [40,41]. Fifth, ADC activity and extracellular (i.e., released) agmatine are increased in response to stressful and traumatic stimuli [9,31,42,43]. Sixth, agmatine binds to and modulates several postsynaptic membrane receptors (Box 3). In sum, short only of identifying specific ('own') agmatine postsynaptic receptors, agmatine in fact fulfills Henry Dale's criteria for a neurotransmitter and, hence, is considered a neuromodulator and co-transmitter. The identification of agmatinergic neuronal systems, if they exist, awaits future research.

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TABLE 1

Tissue concentrations of agmatine in different species and strains, and changes after stress in rats and in depressed humans[a]

Species, strain, gender and treatment	Tissue	*N*	Mean concentration	Detection method	Refs
Peripheral tissues (ng/g)					
Sprague Dawley rats, 2-month-old naive males	Stomach	5	71.0 (wet wt)	HPLC-FI	[29]
	Small intestine	5	55.4 (wet wt)	HPLC-FI	[29]
	Spleen	5	17.4 (wet wt)	HPLC-FI	[29]
	Lung	5	10.2 (wet wt)	HPLC-FI	[29]
	Kidney	5	6.5 (wet wt)	HPLC-FI	[29]
Brain (ng/g) or cerebrospinal fluid (ng/ml)					
Bovine	Brain cortex	4	200–400 (dry wt)	HPLC–MS	[5]
Sprague Dawley rats, 2-month-old naive males	Whole brain	5	2.4 (wet wt)	HPLC-FI	[30]
Long Evans rats, 3-month-old naive males	Brain cortex	6	12.6 (wet wt)	HPLC-FI	[30]
Sprague Dawley rat cultures	Cortical astrocytes	–	9,400[b] (wet wt)	HPLC–MS	[38]
Healthy human spinal taps	Cerebrospinal fluid	10	40.4	HPLC-FI	[30]
Effects of stress					
Sprague Dawley rats, adults; untreated controls	Hippocampus	8	90 (wet wt)	HPLC-FI	[30]
Sprague Dawley rats, adults; after repeated stress	Hippocampus	5	180[c] (wet wt)	HPLC-FI	[30]
Sprague Dawley rats, adults; untreated controls	Prefrontal cortex	8	110 (wet wt)	HPLC-FI	[30]
Sprague Dawley rats, adults after repeated stress	Prefrontal cortex	5	198[c] (wet wt)	HPLC-FI	[30]
Blood (ng/ml)					
Wistar rats, naïve adults	Aortic blood plasma	5	91.1	HPLC-FI	[24]
Human adults, untreated, non-psychiatric healthy controls	Venous blood plasma	17	25.8	HPLC-FI	[113]
Human adults (untreated); depressed	Venous blood plasma	13	31.9[c]	HPLC-FI	[113]

[a] *Abbreviations*: HPLC-FI, high performance liquid chromatography with fluorescence detection; HPLC-MS, HPLC with mass spectroscopy detection; wt, weight.
[b] Might indicate high intracellular concentrations.
[c] Significant differences compared with controls.

Therapeutic strategies

Strategies to modify endogenous agmatine levels

As already discussed, fluctuations in endogenous agmatine concentrations are implicated in adaptive cellular responses to stressful and traumatic stimuli. Accordingly, several experimental approaches have been devised to modify endogenous agmatine to achieve therapeutic benefits, as listed below.

- Specific agmatine antibodies for immunoneutralization of agmatine have been used to sensitize mice to subsequent induction of tolerance to opioid analgesia [44].
- Recombinant retrovirus containing cloned human ADC has been used to transfect target cells, resulting in elevated cellular agmatine concentrations and increased resistance to oxidative stress [45]. Human agmatinase has also been cloned and transfected cells show increased utilization of agmatine as precursor for polyamine synthesis [46,47].
- Development of small interference RNA (siRNA) to down-regulate agmatinase is proposed as an alternative option to increasing agmatine levels.
- Specific enzyme inhibitors of mammalian ADC are not yet available. As indicated above, mammalian ADC is not inhibited by classic nonmammalian enzyme inhibitors, such as DL-alpha-difluoromethylarginine, but ADC is inhibited by the classic ODC inhibitor, alpha-difluoromethylornithine (DFMO) [8,9]. Inhibition of both ODC and ADC activities by DFMO (inhibition of both polyamines and agmatine, respectively) carries important implications to understanding the extensively characterized pharmacological effects of this inhibitor.
- Agmatinase inhibitors are another target for drug development. Based on the observation that agmatinase predominates over DAO in the brain (Box 2) [24], Piletz and colleagues found two guanidino group-containing structures to be effective inhibitors [48]. Treatment of rats with one of the compounds, piperazine-1-carboxamidine, led to a selective increase in brain agmatine concentration and to reduced hippocampal damage in a newborn rat model of brain hypoxic ischemia, without obvious adverse effects [49].
- Developing blockers of agmatine cell membrane transporters (Box 3) is herein proposed as another possible therapeutic target for manipulating endogenous agmatine.
- Mitochondrial transport inhibitors have been developed that are capable of reducing agmatine transport in liver mitochondria, a mechanism involved in the protective effects of agmatine against mitochondrial damage [50] (see 'Mitochondrial protection' section). This might have implications for designing blockers of uptake sites in the plasma membrane.
- Agmatine analogs that are able to cross the blood–brain barrier (BBB) effectively have been developed [51]. *N*,*N*′-disubstituted-2-nitroethene-1,1-diamine and *N*,*N*′-disubstituted-*N*′-cyanoguanidines derivatives with the best predicted BBB permeation profile were found to have analgesic activity similar to, but not better than, agmatine [51].

Application of exogenous agmatine

Administration of exogenous agmatine is the most straightforward strategy for achieving therapeutic effects. Of the various routes available for drug administration, the oral one is considered most convenient. Being a ubiquitous naturally occurring compound, agmatine is present in small amounts in plant-, animal-, and fish-derived foodstuff [52]. Significant gut microbial production is considered a main source of agmatine [53]. Animal studies have demonstrated that oral agmatine is absorbed from the gastrointestinal tract and readily distributed throughout the body [53], with a limited amount crossing the BBB [54]. Rapid elimination of

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ingested (unmetabolized) agmatine by the kidneys has indicated a blood half life of approximately 2 h [55].

Evidently, diet alone seems incapable of delivering the quantity of agmatine needed to modulate its molecular targets [52]. However, the sulfate salt of agmatine, which has been marketed for several years as a dietary ingredient for bodybuilding, albeit with completely unsubstantiated claims, is now available as a nutraceutical [17]. The low incidence of adverse effects observed with gram quantities indicates a major advantage for using oral agmatine as a therapeutic [17].

Clinical indications

The remainder of this article provides a concise overview of the therapeutic potential of agmatine, indicating strengths and weaknesses where applicable and offers a practical perspective for investment in drug development (summarized in Table 2). The topical organization of this section does not indicate priorities for drug development.

Effects with general clinical implications

Cardiovascular effects

A full century since the initial controversy regarding its concentration-dependent cardiovascular effects [2,3], it has been ascertained that agmatine does produce reductions in heart rate (HR) and blood pressure (BP), although both are mild [56]. These effects of agmatine are apparently a vectorial outcome of activating both central and peripheral control systems by modulating several of its molecular targets, including imidazoline

TABLE 2

Summary of progress made in pursuing the therapeutic potential of agmatine in the various clinical indications and implications for future research

Clinical Indication	Support by Scientific Evidence[a]							Strength of support[a]	Comments[b]
	Pre-Clinical			Clinical					
	In-Vitro	*Ex-Vivo*	*In-Vivo*	*Phase*	*1*	*2*	*3*		
Cardiovascular Effects:	----------▶							**	Mild hypotensive effects. Significant implications for other clinical indications.
Cardioprotection	----------▶							*	Only hemodynamic recovery demonstrated.
Glucoprotection	----------.....▶							**	Mild hypoglycemic effects. Historical evidence for clinical trials in diabetes. Significant implications for other clinical indications.
Nephroprotection	----------▶							**	May cause enhanced GFR.
Gastroprotection	----------▶							*	May modulate gastric acid damage. But may enhance stress-induced ulceration.
Neuroprotective Effects:									
Stroke	----------▶							**	May ameliorate BBB disruption.
Traumatic CNS Injuries	----------▶							**	May reduce astrocytic scar formation.
Neuropathic Pain	----------▶							**	Effective orally and is available as a nutraceutical.
Epilepsy	----------▶							**	Several molecular targets related to neuroprotection.

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TABLE 2 (*Continued*)

Glaucoma	----------▶	*	Effective by topical application.
Neurodegenerative Disorders	----------▶	*	*In-vivo* studies only in a Parkinson's disease model.
Opioids Modulation	----------▶	**	Potentiation of opioid analgesia. Prevention of tolerance and inhibition of opioid dependence.
Psychiatric Disorders:			
Antidepressant Properties	----------▶	**	Human biomarker studies and one, 3-patient, pilot clinical study.
Anxiolytic Properties	----------▶	**	Involvement of increased endogenous agmatine metabolism.
Schizophrenia	----------▶	*	Lack of extra pyramidal side effects.
Cognitive Enhancement	----------▶	**	Involvement of increased endogenous agmatine metabolism.*In-vivo* studies on dementia models are lacking.
Anti-Cancer potential	----▶	*	Cytostatic effects without cytotoxicity.

[a]One asterisk indicates evidence supported by only a few studies; two asterisks indicate support from several independent studies.
[b]Comments refer to systemic applications of agmatine sulfate except where indicated otherwise.

receptors subtypes, norepinephrine release and NO production [56].

Furthermore, agmatine appears to be cardioprotective. Agmatine treatment given either pre- or postischemia can enhance hemodynamic recovery in the isolated perfused rat heart model [57]. Additionally, in a rat hemorrhagic shock model, agmatine was shown to increase survival by restoring BP [58]. Together, these cardiovascular effects might be of added therapeutic value for the salutary effects of agmatine in other clinical indications.

Effects on glucose regulation: implication for diabetes

Despite earlier interest during the 1920s [59–61], it was not until the 1980s and more so during the 1990s that various agmatine hypoglycemic mechanisms of action were elucidated, including: (i) direct insulin-like effects on end organs [62]; (ii) interactions with pancreatic islet β cells to increase insulin release [63,64]; and (iii) enhanced adrenal endorphin secretion via imidazoline receptors activation, leading to increased cellular glucose uptake, as demonstrated by Cheng and colleagues [65,66]. The mild hypoglycemic effects of agmatine might be important not only for diabetes therapy, but also for complex disorders where glucose dysregulation has been implicated (e.g., neuropathy, Alzheimer's disease, depression and seizures).

Agmatine might be important in mitigating diabetes-associated systemic malfunctions by: (i) inhibiting advanced glycation end products (AGE) formation [67]; (ii) enhancing nerve regeneration and neuropathic pain reduction in diabetic neuropathy [68]; (iii) increasing kidney glomerular filtration rate (GFR) [24]; and (iv) enhancing metformin action, the first-line therapeutic for type 2 diabetes mellitus (Box 1), both by competing at skeletal muscle I_{2B}-receptors [66] and inhibiting metformin transport by cation transporters (Box 3) [69]. Thus, substantial evidence supports agmatine as an adjunctive therapy for diabetes, with implications for other complex disorders.

Mitochondrial protection

Agmatine has been shown to exert direct protective effects on mitochondria at nanomolar concentrations. It has also been shown to alleviate oxidative stress-induced mitochondrial swelling, possibly by acting as a free radical scavenger, and prevent Ca^{2+}-dependent induction of mitochondrial permeability transition (MPT) by modulating mitochondrial membrane potential and NF-kappaB activation ([70–72] and references therein). Importantly, these effects are implicated in apoptotic cell death. Therefore, mitochondrial protection is considered essential in contributing to the general cytoprotective effects of agmatine in various bodily systems and, thus, to its beneficial effects in a spectrum of disease models. Of special

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interest is a potential for agmatine utility in neurodegenerative diseases where mitochondrial malfunctions have been implicated (e.g., Parkinson's disease [71]).

Implication for kidney functions

As mentioned above, agmatine has been shown to enhance GFR [24]. Possible mechanisms include activation of ryanodine receptor-mediated Ca^{2+} release, leading to induction of endothelial NO synthase (eNOS) and consequent increased NO levels, resulting in vasodilation ([73] and references therein). These mechanisms, together with the previously discussed cytoprotective mechanisms involved in the stress response (Box 2), suggest that agmatine should be considered for nephroprotection [20].

Neuroprotective effects

Potential in stroke

The neuroprotective and/or neurorescue effects of agmatine first discovered in 1995 [7] have been confirmed by numerous studies using various stroke and CNS injury models [16,32,74–78], even when treatment is initiated several hours after ischemia [79]. Several mechanisms have been implicated in these neuroprotective effects: (i) modulation of neurotransmitter receptors (notably: NMDA, α2-adrenoceptors and imidazoline receptors) [80]; (ii) modulation of ion channels (including ATP-sensitive K^+ [64], and voltage-gated Ca^{2+} [81] channels); and (iii) inhibition of NO synthesis [42,77]. Therefore, agmatine is promising both as a preventive (neuroprotective) agent and as a neurorescue treatment after cerebrovascular events.

Implications for traumatic CNS injuries

Agmatine treatment also attenuates motor function deficits and tissue damage after traumatic brain or spinal cord injuries in animal models [78,82–84]. These neuroprotective effects might be mediated by inducible (i)NOS inhibition or NMDA receptor (NMDAR) blockade [78]. Furtheremore, after complete spinal cord transection, agmatine can reduce collagen scar formation and enhance functional recovery associated with decreased tumor growth factor beta 2 (TGFβ2)and increased bone morphogenetic protein (BMP)-7 expression [83].

It is well known that the BBB is compromised early after ischemic and traumatic CNS injuries and remains thus for extended periods, making drug access into the brain easier. At the same time, however, this also contributes to hazardous post-injury intracranial pressure increase. Lee and colleagues found that agmatine treatment can attenuate postischemic brain edema and increase in intracranial pressure [85], and this was associated with decreased endothelial cell expression of aquaporin-1 (AQP-1) and matrix metalloproteinases (MMPs) 2 and 9, known biomarkers of BBB disruption and vasogenic edema [86]. Therefore, agmatine is implicated in accelerating functional recovery and reducing tissue damage after traumatic CNS injuries.

Treatment for neuropathic pain

Molecular mechanisms underlying neuroprotection are known to be common to those involved in neuropathic pain reduction (Box 3). Therefore, it is not surprising that agmatine also shows capacity for reducing pain-associated behaviors in rodent models of neuropathic sensory hypersensitivity [68,87,88]. But, the analgesic mechanisms of agmatine appear different from morphine-based analgesics [68,88–90]. Thus, specific modulation of NMDAR and NO signaling are implicated in the analgesic effects of intrathecal and systemic agmatine applications rather than μ-opioid and/or α2-adrenoceptors [89,90]. Indeed, the route of agmatine administration also appears important, as evidenced by intraplantar agmatine application, which appears, rather exceptionally, to evoke nociceptive behavior through a peripheral acid-sensing ion channel (ASIC-3) [91]. Interactions of agmatine with other functionally important ASICs, especially CNS ASICs, remain to be determined.

Cumulative evidence clearly indicates the therapeutic potential of agmatine in neuropathy and, indeed, recent human clinical trials strongly support it [17]. These trials began with a phase-1, open-label, dose-escalation study, which demonstrated the safety of oral agmatine sulfate [17]. This was followed by a Phase-2 randomized, placebo-controlled trial to assess the effectiveness of oral agmatine as add-on treatment for lumbar disc-associated radiculopathy (sciatica). Patients taking agmatine experienced significantly more pain relief and improved health-related quality of life compared with those taking a placebo [17]. These studies suggest that oral agmatine can be considered safe and effective for sciatica and provide a landmark 'proof-of-concept' for using agmatine in other neuropathies. Based on these studies, agmatine has already been introduced to market as a nutraceutical.

Implications for epilepsy

Considering its multiple molecular targets (Box 3) and neuroprotective effects, it is also of no surprise that agmatine has shown to exert antiseizure effects in preclinical models [92,93]. Additionally, it has been shown to enhance the anticonvulsant effect of lithium on pentylenetetrazole-induced seizures in mice, probably involving the L-arginine–NO pathway [94]. Therefore, it appears that agmatine should prove effective as an antiepileptic agent.

Implications for glaucoma

Retinal ganglion cell (RGC) death is the hallmark of glaucoma. Studies by Hong and colleagues showed that agmatine protected RGCs *in vitro* from apoptosis caused by exposure to hypoxic conditions or tumor necrosis factor alpha (TNFα) ([95] and references therein). The authors further reported [96] that 6 weeks of daily topical applications of agmatine to hypertensive rat eyes could significantly lower intraocular pressure and reduce RGC loss. These studies indicate potential for agmatine treatment in glaucoma.

Implications for neurodegenerative disorders

Less than a handful of studies have investigated agmatine in neurodegenerative disorders. Agmatine treatment has produced neuroprotective and/or neurorescue effects in two Parkinson's disease models. One, in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model [97], a finding recently confirmed [98], and the other, in neuron-like cell cultures using the neurotoxin rotenone, where protection of mitochondria was implicated as an important mechanism [71] (see 'Mitochondrial protection' above). There is also one study implicating agmatine in averting memory deficits in an animal counterpart of Alzheimer's disease (see 'Cognitive enhancement: age-related changes and relevance to dementia'), but neuroprotection was not explored [99]. In view of the robust neuroprotective and/or neurorescue effects of agmatine, the lack of neuroprotective therapeutics for

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neurodegenerative disorders is a glaring unmet need. It is expected that more research in these areas will be forthcoming.

Attenuation of opioid liability

As first reported in 1996 by Kolesnikov and coworkers [89], systemic agmatine can potentiate opioid analgesia and prevent tolerance to chronic morphine in mice. Since then, cumulative evidence amply shows that agmatine inhibits opioid dependence and relapse in several animal species by different routes of administration [100–104]. Notably, Li and coworkers found that agmatine pretreatment can inhibit acquisition of morphine self-administration, and that extended agmatine administration during the withdrawal period can selectively diminish the reacquisition of morphine self-administration in rats [104]. Most importantly, considering its general therapeutic effects, agmatine by itself neither produces self-administration nor drug discrimination [100,104]. Several mechanisms have been implicated in these agmatine-opioid interactions, including modulation of neurotransmitter receptors (notably glutamate, imidazoline I_1R, α2-adrenoceptors and dopamine receptors) and of NO synthesis [105–107]. Thus, there is a large body of evidence indicating the potential utility of agmatine in opioid analgesia and in treating opiate dependence and withdrawal syndrome.

Implications for psychiatric disorders

Antidepressant properties

Zomkowski *et al.* [108] were first to show that agmatine administrations dose-responsively produced antidepressant-like behaviors in animals. Various molecular targets have since been implicated in these effects, including NO synthesis modulation, inhibition of K^+ channels, and modulation of several key neurotransmitter receptors (notably: NMDAR, α_2-adrenoceptors, 5-$HT_{1A/1B}$ and 5-HT_2, δ- and μ-opioid, and I_1R and I_2R receptors) [109–111]. Surprisingly, there has been little evidence supporting the involvement of serotonin in the antidepressant-like effects of agmatine, with most evidence indicating imidazoline receptors involvement [109–112].

A recent case study in humans reported for the first time antidepressant effects of oral agmatine after 3–4 weeks of treatment in three patients [18]. None of the patients relapsed when subsequently given the serotonin-depleting drug parachlorophenylalanine (PCPA) together with agmatine [18]. This initial observation points again to a novel, non-serotonergic mechanism of the antidepressant action of agmatine. Furthermore, Halaris and Piletz reported in humans [113,114] that 8–12 weeks of standard antidepressant treatments with either bupropion or venlafaxine, led to reduced endogenous agmatine plasma concentrations, most prominently in treatment-responsive patients with depression. Recently, Bernstein *et al.* reported a robust increase of agmatinase immunoreactivity in a subset of hippocampal interneurons observed in brain autopsies from patients who were chronically depressed [115]; however, the historic usage of antidepressants in these cases made it impossible to determine whether *in situ* processes or the medications were the primary culprits [115]. In sum, there is growing evidence for the utility of agmatine in antidepressant therapy.

Anxiolytic properties

As previously discussed, stressful stimuli tend to alter brain structural plasticity in parallel with increased endogenous agmatine synthesis and metabolism, implicating agmatine as a natural modulator of stress responses [31,32] (Box 2). In animal studies using behavioral stress paradigms, exogenous agmatine treatments exert significant anxiolytic-like effects [43,111,116]. Additionally, agmatine can potentiate alcohol-induced anxiolytic effects as well as block the anxiogenic behaviors of withdrawal from chronic alcohol in rats, and these effects are probably mediated by imidazoline receptors [16,117]. These studies point to the feasibility of agmatine as an anxiolytic in humans.

Implications for schizophrenia

Using the pre-pulse inhibition (PPI) animal model of schizophrenia, agmatine treatment had no effect by itself. However, pretreatment with agmatine attenuated the disruptive effects of the noncompetitive NMDA antagonist, phencyclidine (PCP) on PPI [118]. These effects were dose dependent, suggesting involvement of dopamine neurotransmission [119]. In a subsequent study using drug-induced models of schizophrenia, agmatine attenuated characteristic behavioral patterns and potentiated the inhibitory effect of known antipsychotics (haloperidol and olanzepine) in the conditioned avoidance response test [120]. These studies suggest that agmatine could be a novel therapeutic for schizophrenia, unlikely to produce extra pyramidal adverse effects [120].

Cognitive enhancement: age-related changes and relevance to dementia

Studies by Liu and coworkers amply indicate that endogenous agmatine concentrations, mainly in the hippocampus and prefrontal cortex, correlate positively with the degree of learning and memory in rats ([121,122] and references therein). These observations complement previous observations of lower endogenous agmatine concentrations in the cortex of aged, 24-month-old, male rats (called 'slow learners') compared with 3- and 14-month-old rats [29].

Agmatine treatment has generally been found to improve performance of animals in learning and memory paradigms ([123,124] and references therein). Intriguingly, agmatine treatment might lead to even better improvements in learning and memory of aged rats compared with young adults (3-months old) [124]. These effects of agmatine might involve suppressed age-related elevation in NOS activity [125]. All the same, it is important to note that memory and learning tests are inherently stressful to various degrees and, therefore, the observed cognitive effects might owe, in part, to the anxiolytic effects of agmatine [123].

It is no wonder that these positive findings have raised the possibility that agmatine could have utility in Alzheimer's dementia. However, evidence for this remains rudimentary. One study reported [99] that agmatine treatment before intracerebrovascular infusion of a toxic pre-aggregated form of amyloid beta protein ($A\beta_{25-35}$) and for 9 days thereafter, led to correction of memory deficits in rats even when tested weeks later. Note, however, that Aβ aggregate formation is also associated with traumatic brain injuries; therefore, this paradigm is not a unique model of Alzheimer's dementia. Another study showing that agmatine treatment can reduce memory impairments in a diabetic rat model [126], might also be relevant because malfunctions in glucose metabolism have been implicated in Alzheimer's dementia. Thus, the evidence hints that agmatine has cognitive enhancement

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properties and might be a novel nootropic with therapeutic potential in Alzheimer's dementia.

Modulation of cell proliferation: implication for cancer treatment

As alluded to in Box 1, agmatine exerts differential effects on proliferation of various cell types, and its interference with cell proliferation depends on the cell type and its stage of differentiation. Thus, whereas agmatine can enhance proliferation of thymocytes and lymphocytes [127] and of endothelial and neuronal stem cells after brain injury [83,84], it can also inhibit proliferation of vascular smooth muscle cells, macrophages, astrocytes, fibroblasts and tumor cells [42,128]. The mechanisms underlying these differential effects are unclear, but modulation of polyamine metabolism is probably involved (Box 2). Furthermore, several lines of evidence from Haenisch and Molderings indicate that tumor cell growth is associated with modulation of endogenous agmatine metabolism [15,129]. These include: (i) lower cellular agmatine concentrations coupled with reduced ADC expression observed in human colon cancer polyps [129]; (ii) reduced expression of agmatinase and DAO detected in human leukemia cells [129]; (iii) silencing ADC expression by RNA interference (RNAi) in human cancer cell lines led to enhanced proliferation [15]; and (iv) treatment with exogenous agmatine at concentrations normally achieved in blood exerted antiproliferative effects in human cancer cell lines [15]. These findings are in agreement with the observed cytotactic effects of agmatine, implicating downregulation of polyamine metabolism as a possible antiproliferative target [20,128,129]. Unfortunately, no *in vivo* studies have yet been reported, leaving the potential of agmatine for anticancer treatment open for further research.

Agmatine might also be important in mitigating chemotherapy-induced neuropathy (see 'Treatment for neuropathic pain'), a serious neurotoxic effect of most current chemotherapeutic agents. Based on its robust neuroprotective and neuropathic pain-reducing effects, agmatine treatment for cancer might offer this added benefit and thereby provide a useful add-on to contemporary anticancer therapy.

Concluding remarks and future perspectives

Accelerated progress of drug discovery

Nearly a century since its discovery in 1910 [1], and despite entrenched false dogma, new discoveries during the 1990s of endogenous agmatine and its biosynthesis and metabolism in mammals have reignited scientific research and propelled agmatine from relative obscurity to the forefront of today's interest in drug development (Box 1). These recent findings place agmatine as a key modulator of arginine metabolic pathways, notably NO synthesis and polyamine metabolism, thereby playing important roles in physiology and cellular repair mechanisms (Box 2). Ample evidence has also shown that agmatine is a neuromodulator and co-neurotransmitter, with significant implications for nervous system function and reaction to stress and trauma.

Extensive preclinical research has shown that agmatine exerts general cytoprotective effects, not only in neuroprotection, but also in nephroprotection, cardioprotection and gastroprotection. Studies with animal models indicate that exogenous agmatine treatments exert beneficial effects in a host of complex clinical disorders, such as diabetes mellitus, neurotrauma and neurodegenerative disorders, opioid addiction, mood disorders, cognitive disorders and cancer (summarized in Table 2). Research exploring mechanisms of actions shows that agmatine modulates multiple molecular targets, including several neurotransmitter receptors, membrane transporters and key enzymes (Box 3). Agmatine appears to be an exceptional modulatory molecule and it is postulated that for exerting its functions, agmatine modulates synergistically multiple molecular mechanisms, fitting the therapeutic profile of a 'magic shotgun' for complex clinical disorders.

The rapidly accrued preclinical evidence for agmatine therapeutic effects has recently prompted clinical trials supporting oral agmatine as a safe and effective treatment of neuropathic pain and probably of depression. These clinical trials have already substantiated the use of agmatine as a safe and effective nutraceutical.

Drug development: therapeutic potential outweighing risks

There remain constraints on progress towards practical development of agmatine as a drug. First, the lower level of protection against commercial competition afforded by 'usage' patents for new indications of known compounds, such as agmatine with its long known methods of chemical synthesis, is viewed as being much less lucrative by drug developers than that provided by 'composition of matter' patents for new chemical entities. Second, although research of new compounds to modulate endogenous agmatine metabolism holds promise, it is rudimentary and remains speculative. Third, even though agmatine, as a naturally occurring substance, has been developed and introduced to the dietary supplement and nutraceutical market, nutraceutical products in the USA fall under the 'Dietary Supplement Health and Education Act (DSHEA)', which forbids promotion of nutraceuticals for the treatment, cure, or prevention of any disease. Similar regulatory restrictions exist worldwide and severely limit the advertising of nutraceuticals to the medical market.

Despite these constraints, compelling evidence indicates the therapeutic potential of agmatine for a spectrum of diseases. A summary of the advances made and the gaps still remaining for future research are indicated in Table 2. Although comparative efficacy studies with presently available drugs are still required, the broad safety profile of agmatine has been established with no serious adverse effects, either as a stand-alone or as an add-on treatment. This should be a paramount advantage when compared with most existing drugs and certainly to combination therapy. Moreover, its general cytoprotective actions suggest that agmatine should be considered not only as a curative, but also as a preventive therapeutic.

Although it is hard to anticipate which clinical indication will prove most applicable (Table 2), this consortium anticipates that more clinical trials of agmatine will soon be forthcoming. Hopefully, this review will help stimulate more vigorous research, and provide a strong incentive for investments to accelerate drug development efforts to bring agmatine-based treatments to the clinic.

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