Agmatine Selectively Blocks the *N*-Methyl-D-Aspartate Subclass of Glutamate Receptor Channels in Rat Hippocampal Neurons[1]

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ABSTRACT

We investigated in rat hippocampus neurons whether 4-(aminobutyl)guanidine (agmatine), formed by decarboxylation of L-arginine by arginine decarboxylase and metabolized to urea and putrescine, can modulate the function of *N*-methyl-D-aspartate (NMDA) receptor channels. In cultured hippocampal neurons studied by whole-cell patch clamp, extracellular-applied agmatine produced a voltage- and concentration-dependent block of NMDA but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid nor kainate currents. Analysis of the voltage dependence of the block suggests that agmatine binds at a site located within the NMDA channel pore with a dissociation constant of 952 μM at 0 mV and an electric distance of 0.62. We also tested effects of several agmatine analogs. Arcaine (1,4-butyldiguanidine) also produced a similar voltage-dependent block of the NMDA current, whereas putrescine (1,4-butyldiamine) had little effect, suggesting that the guanidine group of agmatine is the active moiety when blocking the NMDA channel. Moreover, spermine (an endogenous polyamine) potentiated the NMDA current even in the presence of blocker agmatine or arcaine, suggesting that the guanidine-containing compounds agmatine and arcaine interact with the NMDA channel at a binding site different from that of spermine. Our results indicate that in hippocampal neurons agmatine selectively modulates the NMDA subclass of glutamate receptor channels mediated by the interaction between the guanidine group and the channel pore. The results support other data that agmatine may function as an endogenous neurotransmitter/neuromodulator in brain.

4-(aminobutyl)guanidine) (agmatine) (Fig. 1) is synthesized by decarboxylation of L-arginine by arginine decarboxylase (ADC) and hydrolyzed to putrescine and urea by agmatinase (agmatine uryl-hydrolase). Agmatine has long been known to be a constituent of bacteria, plants, and a range of invertebrates and has been viewed as a precursor of putrescine (Tabor and Tabor, 1984). Because putrescine is a precursor of spermidine and spermine, agmatine is believed to function in these organisms as a metabolic intermediate in formation of polyamines.

Recently, we discovered that agmatine, ADC, and agmatinase are expressed in mammalian tissues (Li et al., 1994; Raasch et al., 1995; Sastre et al., 1996). In mammalian brain, agmatine is locally synthesized (Li et al., 1994, 1995) and stored regionally in neurons (Otake et al., 1998). In particular, agmatine is associated with synaptic vesicles in axon terminals, which make asymmetric (excitatory) synapses upon pyramidal neurons in hippocampus (Reis et al., 1998).

In rat brain synaptosomes agmatine can be released by depolarization (Reis and Regunathan, 1997), reaccumulated by reuptake (Sastre et al., 1997), and metabolized to putrescine by agmatinase (Sastre et al., 1996). Agmatine is bioactive, releasing several neurotransmitters and hormones including catecholamines from adrenomedullary chromaffin cells (Li et al., 1994), luteinizing hormone in vivo from pituitary and luteinizing hormone-releasing hormone in vitro from hypothalamus (Kalra et al., 1995), and insulin from pancreatic islet cells (Sener et al., 1989). Observations suggest that in mammals agmatine may have actions other than as a metabolic precursor of polyamines. Rather it may have actions of its own and possibly may act as a novel neurotransmitter and/or neuromodulator.

There is inferential evidence that agmatine may modulate the actions of L-glutamate. Agmatine displaces binding to MK-801, an open-channel blocker of the *N*-methyl-D-aspartate (NMDA) receptor channels, in membranes of rat cerebral cortex (Anis et al., 1990). Agmatine also enhances opioid analgesia and prevents tolerance in vivo (Kolesnikov et al., 1996), actions that resemble those produced by NMDA receptor antagonists (Wong et al., 1996; Elliott et al., 1996). The
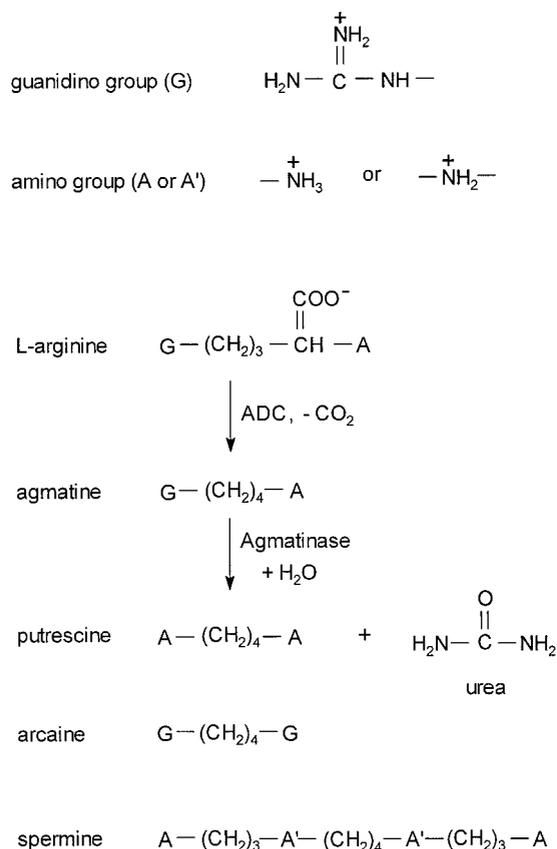
ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ADC, arginine decarboxylase; AP5, ($\pm$)-2-amino-5-phosphonopentanoic acid.

observations raise the possibility that agmatine may act to modulate the function of glutamatergic neurotransmission at receptors of the NMDA subclass.

In this study, we used whole-cell patch clamp to determine whether agmatine modulates the NMDA receptor channel in rat hippocampus neurons, which contain agmatine (Feng et al., 1997). We demonstrate that agmatine selectively blocks the NMDA subclass of glutamate receptor channels. And the blocking action of agmatine is mediated by interactions between its guanidine group and the NMDA channel pore. Some preliminary results were presented in an abstract (Yang and Reis, 1996).

Materials and Methods

Cell Culture. After a pregnant Sprague-Dawley rat was sacrificed by injecting a lethal dose of sodium pentobarbital (100 mg/kg, i.p.), embryonic day 19 (E19) fetuses were removed from the uterus. Hippocampi, dissected out from fetal brains, were digested at 37°C for 30 min with an enzyme mixture containing trypsin (1 mg/ml), collagenase (0.1 mg/ml), and DNAse I (0.01 mg/ml) in Hank's balanced salt solution. Neurons were mechanically dissociated by trituration and plated on polyornithine-coated glass coverslips. Neurons were fed with Dulbecco's modified Eagle's medium plus 10% fetal bovine serum and maintained in a CO_2 (5%) incubator at 37°C.

Electrophysiology. Whole-cell patch clamp recordings (Hamill et al., 1981; Axopatch 200A, Axon Instruments, Foster City, CA) were made at room temperature (21–24°C) from cultured hippocampal neurons 5–15 days after plating. Patch electrodes, pulled from boroscilicate glass tubing, had resistance in the range of 3–5 MΩ. The electrode filling solution contained: 145 mM CsCl, 2 mM MgCl$_2$, 5 mM EGTA, 3 mM ATP, 0.1 mM GTP, 0.1 mM leupeptin, and 10 mM HEPES-CsOH, pH 7.2. The Mg^{++}-free (no added MgCl$_2$) extracellular solution contained: 145 mM NaCl, 5 mM KCl, 0.2 mM CaCl$_2$, 10 mM HEPES-NaOH, and 11 mM glucose, pH 7.4, supplemented with tetrodotoxin 1 μM and bicuculline 10 μM (Donevan et al., 1992; Benveniste and Mayer, 1993). Agmatine (from Research Biochemistry Inc., Natick, MA) was prepared freshly for each experiment. The bath was perfused continuously at 1 to 2 ml/min with the external solution during recording. A fast perfusion system used for drug application consisted of a small manifold with seven inlets and one common outlet made with silica tubing with an inner diameter of 250 μm (courtesy of Steve Griffin, MicroQuartz Sciences, Phoenix, AZ). The dead volume inside of the manifold is approximately 1 μl. The solutions were driven by gravity and controlled by solenoid valves (Lee Company, Westbrook, CT). Drugs and ligands were purchased from Research Biochemistry Inc. and chemicals from Sigma Chemical Co. (St. Louis, MO).

The pClamp system (Axon) was used for data acquisition and analysis on a 486-based IBM-PC. A ramp protocol from −100 to +80 mV in 1 s was used to obtain steady-state current-voltage (I-V)

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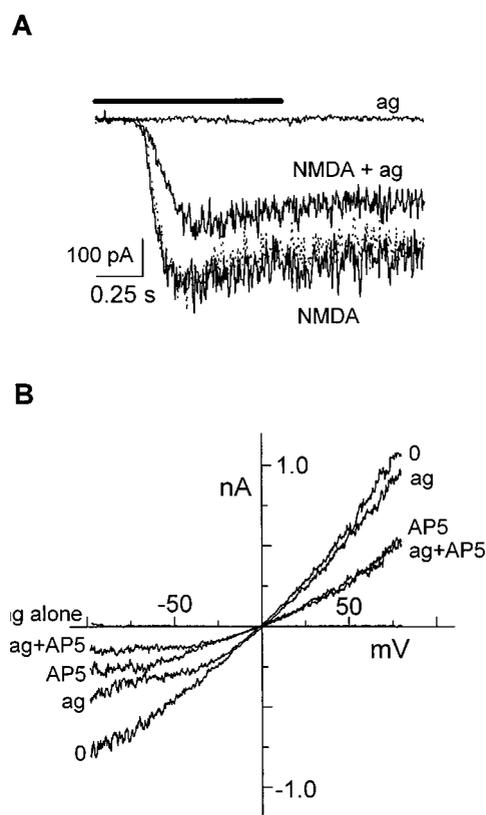
Fig. 2. Agmatine (ag) reversibly blocked the NMDA receptor channel and did not bind the agonist site. A, whole-cell currents elicited by NMDA (50 + 10 μM glycine) without or with 100 μM agmatine (labeled NMDA and NMDA + ag, respectively) in a hippocampal neuron. Agmatine by itself (ag, 100 μM) did not elicit any detectable current in the presence of 10 μM glycine but absence of NMDA. The drug perfusion period is indicated by the heavy line above the current traces. B, steady-state ramp I-V curves obtained from another neuron in the presence of AP5 (10 μM), agmatine (ag, 100 μM), or both (ag + AP5) and in the absence of both AP5 and agmatine (trace 0). AP5 and/or agmatine were added into the external solution containing 50 μM NMDA plus 10 μM glycine, except for the trace labeled ag alone (100 μM).

Fig. 1. Chemical structures of agmatine and related molecules. Agmatine is synthesized from L-arginine by ADC and metabolized to putrescine and urea by agmatinase. Spermine, an endogenous polyamine, and arcaine, a synthetic diguanidine, are agmatine analogs. The molecules contain guanidine (G) and/or amine (A or A′) groups that are positively charged at the physiological pH except for urea. In particular, agmatine, arcaine, and putrescine each has two positively charged groups separated by a butyl chain.

curves. The whole-cell current induced by NMDA (50 μM plus glycine 10 μM), kainate (50 μM), or AMPA (50–200 μM) was determined by subtracting the background current recorded in the absence of the ligands. The steady-state I-V curves in some cases (shown in Figs. 5 and 6) were normalized as (I/I_o)-V, the ratio of currents recorded with and without drugs as function of voltage. The standard errors for normalized I-V curves were obtained by first averaging over voltage, because the errors were voltage independent, and then averaging among different cells. The part of normalized I-V curves around 0 mV was omitted because the normalization procedure introduced singularity in the region. In some cases (shown in Figs. 4 and 6), the steady-state I-V curves were first fitted to polynomials and then data points were extracted at 10 mV increments from −90 mV to +80 mV. A nonlinear least-squares curve-fitting routing (SigmaPlot; Jandel Scientific, San Rafael, CA) was used for curve fitting.

Results

Effect of Agmatine on NMDA Receptor Channel. Whole-cell currents were recorded from cultured hippocampal neurons that had triangular shape. Agmatine (100 μM) reduced the NMDA current by approximately 40% at −60 mV (Fig. 2A), when applied in the external solution containing 50 μM NMDA and 10 μM glycine (a saturating concentration; Johnson and Ascher 1987; Mayer et al., 1989; Benveniste et al., 1990). The effect was reversible upon the washing off of agmatine (Fig. 2A). The block was most potent at hyperpolarizing membrane potentials and less effective at positive voltages (Fig. 2B). By itself, agmatine (100 μM), even in the presence of 10 μM glycine, did not elicit any detectable whole-cell current at all voltages tested, suggesting that it is not an NMDA receptor agonist (Fig. 2).

(±)-2-Amino-5-phosphonopentanoic acid (AP5), an antagonist that competes with NMDA at the agonist binding site, inhibited the NMDA current in both inward and outward directions (Fig. 2B). In the presence of AP5, agmatine produced an additional block of the NMDA current, similar to its effects in the absence of AP5 (Fig. 2B). The observation suggests that, unlike AP5, agmatine is not a competitive NMDA receptor antagonist.

**Voltage Dependence and Affinity of Agmatine Block.** To determine the mechanism of agmatine's action on the NMDA receptor channel, we analyzed the voltage dependence of the blockade by measuring the steady-state I-V relationship at various concentrations of agmatine (Fig. 3A). The dose-response curve for agmatine was shifted to the right as membrane voltage became more positive (Fig. 3B). The apparent dissociation constants, K_Vs, determined at various voltages (V) fitted well to the Woodhull model (Woodhull, 1973), giving rise to $K_{V=0}$ of 952 μM and an effective valence ($z\delta$) of 0.62 (Fig. 3C). For example, the K_d is approximately 200 μM at the resting membrane potential (between −60 and −70 mV). Such analysis suggests that agmatine interacts directly with the NMDA channel pore by binding at a site partway across the membrane electric field.

Actions of Agmatine at Non-NMDA Receptor Channels. We investigated whether agmatine affects non-NMDA glutamate receptor channels activated by AMPA or kainate. Agmatine at 100 μM caused a small (10–15%) decrease of the whole-cell current elicited by AMPA (Fig. 4A) or kainate (Fig. 4B). In contrast to its potent and voltage-dependent actions on the NMDA current, the effect of agmatine on non-NMDA currents was not only small (−80 mV; Fig. 4C) but also voltage-independent in the range of −100 to +80 mV. Even at the concentration of 3 mM, the reduction of non-NMDA currents by agmatine was no more than 20%, whereas the NMDA current was fully blocked at this concentration (Fig. 4C). The results indicate that agmatine selectively acts on the NMDA subclass of glutamate receptor channels.

Is the Active Moiety of Agmatine the Guanidino or Amino Group? Structurally agmatine is a butyl chain with a guanidino group at one end and an amino group at the other (Fig. 1). To determine whether the guanidino or amino moiety of agmatine mediates blockade of the NMDA receptor channel, we examined the actions of several structural analogs of agmatine shown in Fig. 1.

Arcaine (100 μM), a synthetic analog of agmatine with two terminal guanidino groups (Fig. 1), blocked the NMDA receptor more potently than agmatine (Fig. 5). Its blockade was
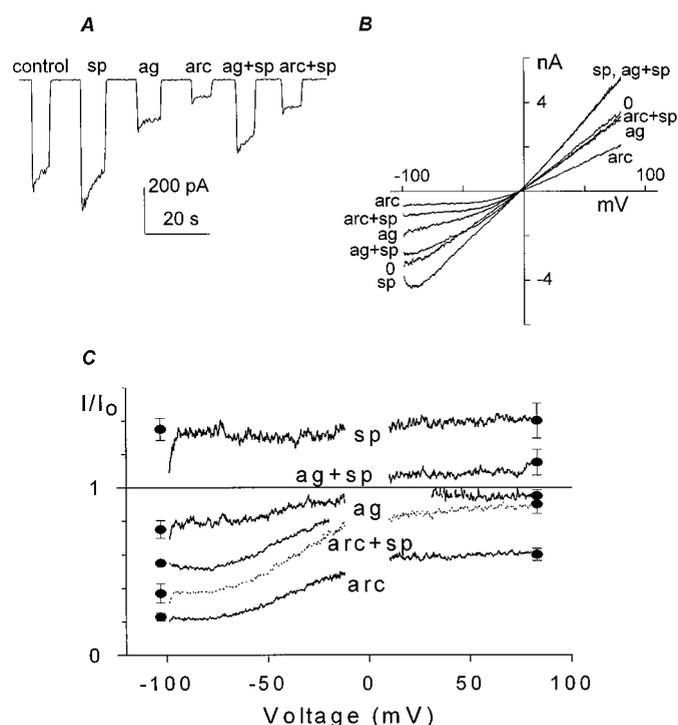
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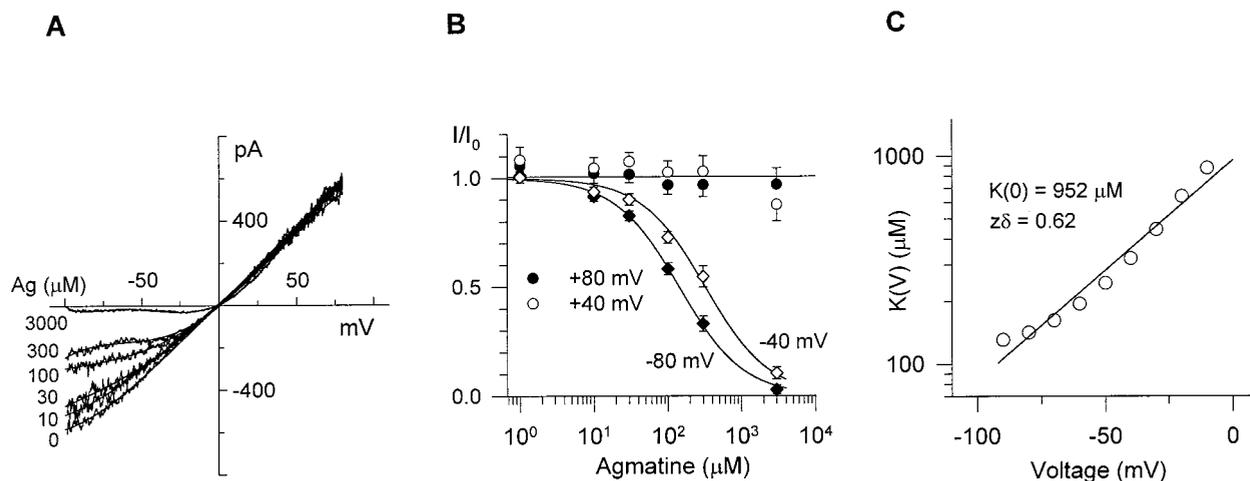
Fig. 3. Concentration- and voltage-dependent block of the NMDA current by agmatine. A, the steady-state ramp I-V curves recorded from a hippocampal neuron at various agmatine (Ag) concentrations. Solid lines are polynomial fits to the data. B, dose-response relations at different membrane voltages (I/I_o, the ratio of currents recorded with and without agmatine with the control level of $I/I_0 = 1$; mean ± S.E.M., 4–10 cells for each point). At negative voltages, the dose-response curves were fitted to the equation (solid lines): $I/I_0 = 1/(1 + X/K_V)$, where X is agmatine concentration and K_V is the apparent dissociation constant at voltage V. C, K_V as function of voltage for V < 0 was fitted to the Woodhull model: $K_V = K_0 \exp(z\delta FV/RT)$, where RT/F = 25.4 mV at 22°C, with the best fit parameters of K_0 = 952 μM and $z\delta$ = 0.62.
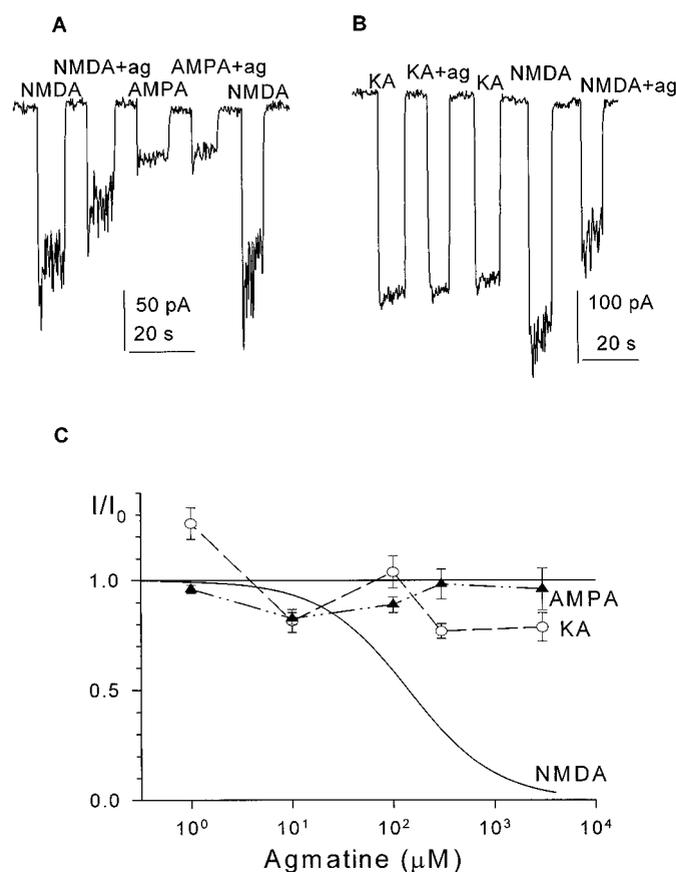
Fig. 4. Agmatine (ag) had little effect on non-NMDA currents. A, whole-cell currents activated by AMPA (200 μM) and NMDA (50 + 10 μM glycine) in a hippocampal neuron. B, whole-cell currents elicited by kainate (KA; 50 μM) and NMDA (50 + 10 μM glycine) in another neuron. For both A and B, agmatine was 100 μM and holding potential was −60 mV. C, dose-response curves at −80 mV (I/I$_o$, the ratio of currents recorded with and without agmatine with the control level of I/I$_o$ = 1; mean ± S.E.M.: AMPA, broken line, 3–16 cells; KA, dashed line, 4–12 cells; NMDA, continuous line, data at −80 mV are same as that shown in Fig. 3C). The horizontal line of I/I$_o$=1 indicates the control level.

Fig. 5. Action of the guanidino versus amino group on the NMDA current. A, spermine (sp; 100 μM) potentiated the NMDA whole-cell current independent of the presence of guanidine-containing molecules [100 μM agmatine (ag) or arcaine (arc)], which both blocked the NMDA current. Holding potential −60 mV. B, steady-state ramp I-V curves obtained from the same neuron in A. The control external solution (trace 0) contained 50 μM NMDA and 10 μM glycine. C, normalized I-V relationships (I/I$_o$, the ratio of currents recorded with and without 100 μM drug; mean ± S.E.M., 10–16 cells except for 57 cells for agmatine). The horizontal line of I/I$_o$=1 indicates the control level.

voltage dependent at negative membrane potentials but voltage independent at positive potentials (Fig. 5, B and C). In contrast, spermine (100 μM), an endogenous polyamine with two terminal and two internal amino groups (Fig. 1), failed to block the channel. Rather it caused a voltage-independent potentiation of the NMDA current at all membrane potentials except those more negative than −90 mV (Fig. 5). The potentiation by spermine in that instance was a glycine-independent type, as has been reported by others (Lerma, 1992; Benveniste and Mayer, 1993), because the NMDA currents were recorded in the presence of 10 μM glycine, a saturation dose for the glycine coagonist site of the NMDA receptor (Johnson and Ascher, 1987; Mayer et al., 1989; Benveniste et al., 1990). The potentiation by spermine occurred in the presence of agmatine (100 μM) or arcaine (100 μM), both of which blocked the NMDA current as shown above. The observation suggests that the binding site(s) for agmatine or arcaine differ from that of spermine, at least at the concentration of 100 μM. Furthermore, putrescine (100 μM), an endogenous polyamine and metabolic product of agmatine (Sastre et al., 1996), slightly enhanced the NMDA current at positive voltages and had little effect at negative voltages (Fig. 6). This result suggests that the guanidino

group of agmatine is the active moiety that interacts with the NMDA channel because putrescine only differs from agmatine by a terminal amino group (Fig. 1) and was ineffective to block the NMDA current.

We also tested two other compounds: urea (100 μM) and L-arginine (100 μM), a metabolic product and an immediate precursor of agmatine (Fig. 1), respectively (Sastre et al., 1996). Urea, not charged at the physiological pH (Fig. 1), potentiated NMDA currents by 20 to 30%, an effect similar in magnitude to that of spermine (30–40%). Arginine, however, slightly enhanced the NMDA current at both positive and negative voltages (Fig. 6).

These results suggest that the moiety of agmatine responsible for blockade of the NMDA receptor channel is the guanidino group because agmatine and arcaine, which contain at least one guanidino group (Fig. 1), blocked the NMDA response, whereas spermine or putrescine, which lack them but contain two or more amino groups (Fig. 1), was ineffective. If this is the case for the single positive charge (z = 1) of the guanidino group, the electric distance (δ) for agmatine block should be 0.62 (Fig. 3), specifically, agmatine directly interacts with the NMDA channel pore at a binding site located within the membrane electric field at about 60% from the extracellular side. The results also suggest that the block of the NMDA receptor by agmatine is unlikely a general surface charge-screening effect because the blocking effect is not correlated with the positive charges carried by the molecules tested (Fig. 1).

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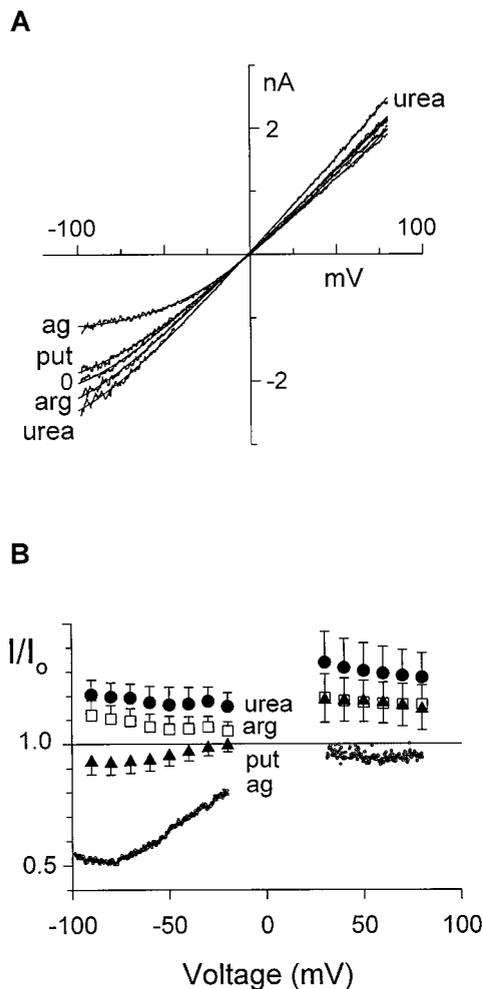
Fig. 6. Effects of precursor and metabolites of agmatine on the NMDA current. A, steady-state ramp I-V curves obtained from a single neuron in the presence of L-arginine (arg), putrescine (put), urea, or agmatine (ag), all at 100 μM and added in the control external solution (50 μM NMDA and 10 μM glycine; trace 0). Solid lines through the traces are polynomial fits to the data. B, normalized currents, I/I_0 (the ratio of currents recorded with and without the drug; mean $\pm$ S.E.M., 5–7 cells), as function of membrane voltage. The data for agmatine are same as that shown in Fig. 5C. The horizontal line of $I/I_0 = 1$ indicates the control level.

Discussion

In the present study we demonstrated that in cultured hippocampal neurons agmatine is a concentration- and voltage-dependent antagonist of channels of the NMDA but not AMPA or kainate subclass of glutamate receptors. The action of agmatine is consistent with the mechanism of open-channel block based on analysis of voltage dependence of the block (Fig. 3). The agmatine binding site determined this way is located within the NMDA channel pore with an electric distance ($z\delta$) of 0.62. This interpretation is consistent with the results from the receptor binding study (Anis et al., 1990), in which agmatine displaces the open-channel blocker MK-801.

The actions of agmatine upon the NMDA channel are unique for an endogenous product of metabolism. Although agmatine is a metabolic precursor of the diamine putrescine and higher polyamines (Tabor and Tabor, 1984), it is not a diamine because it contains one terminal guanidino group (Fig. 1). The blocking effect of agmatine appears to relate to

the guanidino moiety because arcaine, a synthetic biguanidine, also produces a comparable voltage-dependent and receptor-selective blockade of NMDA channels in cultured hippocampal neurons (Donevan et al., 1992). Quantitatively, agmatine has an almost identical electric distance ($\delta = 0.62$ for $z = 1$; Fig. 3) with that of arcaine ($\delta = 0.67$ for $z = 1$; Donevan et al., 1992), suggesting that agmatine shares the same binding site with arcaine in the NMDA channel pore. Although both agmatine and arcaine have two positive charges at the physiological pH (Fig. 1), setting $z = 1$ is justified because the butyl chain of these two linear molecules are not flexible enough to bend over (Romano et al., 1992) to allow both positively charged terminal groups simultaneously entering the channel pore. However, the diamine putrescine that contains no guanidine groups (Fig. 1) was ineffective to block the NMDA current. These results indicate that it is the guanidine group of agmatine that preferably interacts with the NMDA channel.

Furthermore, the effects of agmatine and arcaine are distinct from that of the polyamine spermine. Spermine potentiates the NMDA current even in the presence of the blocker agmatine (Fig. 5) or arcaine (Fig. 5 and Donevan et al., 1992), suggesting that the guanidine-containing molecules do not share the same binding site with spermine, at least at concentrations ≤ 100 μM. Although at higher concentrations spermine blocks the NMDA receptor channel (Rock and Macdonald, 1995), its action differs from that of the guanidine-containing molecules in several aspects. 1) Agmatine is 10- to 100-fold more potent in displacing MK-801 binding than putrescine or spermine, respectively (Anis et al., 1990). 2) Block of the NMDA current by arcaine has a stronger voltage dependence than that by polyamines (Maciver et al., 1991; Donevan et al., 1992; Rock and Macdonald, 1992; Benveniste and Mayer, 1993). 3) Arcaine at 300 μM reduces the activities of single NMDA channels to an undetectable level, whereas endogenous polyamines (putrescine, spermidine, and spermine) at concentrations ≥ 10 mM merely reduce the single-channel amplitude by 60 to 70% (Rock and Macdonald, 1992). Therefore, the guanidino group is preferred to the amino group when blocking the NMDA receptor channel and guanidine-containing molecules, such as agmatine and arcaine, should be distinguished from polyamines based on their different actions on the NMDA receptor channel.

Agmatine has been detected biochemically (Raasch et al., 1995; Stickle et al., 1996; Feng et al., 1997) and localized immunocytochemically in neurons but not glial cells (Otake et al., 1998) in various brain regions. The presence of arginine decarboxylase activity in brain indicates that agmatine is locally synthesized, probably within these neurons themselves (Li et al., 1994; Raasch et al., 1995). Of relevance to this study are our recent observations by light and electron-microscopy (Reis et al., 1998) that in the rat hippocampus immunoreactive agmatine (or agmatine-like immunoreactivity) is stored in the perikarya, the processes and axon terminals of pyramidal neurons and some interneurons. Moreover, immunoreactive agmatine is associated with the vesicles of axons, which make asymmetric (or excitatory) synapses, primarily dendritic, on pyramidal neurons. Because the majority of excitatory neurons innervating pyramidal neurons contain glutamate (Witter, 1993), and because pyramidal neurons express glutamate receptors of the NMDA subclass in apposition to the innervating terminals (Siegel et al.,

1994), the findings suggest that agmatine may be costored with L-glutamate and, as demonstrated here, would be counter regulatory to L-glutamate when coreleased but only at the NMDA receptor.

The concentration of agmatine required to block the NMDA receptor reported in this study is relatively high. Is this blocking effect by agmatine physiologically relevant? The concentration of agmatine is 611 ng/g wet wt. in rat hippocampus (Feng et al., 1997) and ranges from 10 to 760 ng/g wet wt. in whole rat brain (Raasch et al., 1995; Stickle et al., 1996; Feng et al., 1997), comparable with concentrations of other neurotransmitters such as norepinephrine (430 ng/g) (Bacapoulos and Bhatnagar, 1977). Assuming a uniform distribution of agmatine (mw 130) and 1 ml vol/g wet tissue, an estimated molar concentration of agmatine in whole rat brain would be 0.1 to 6 μM. However, agmatine, like norepinephrine (Milner and Bacon, 1989), is not uniformly distributed within neurons; rather it is associated with subcellular structures such as clear synaptic vesicles (Reis et al., 1998) and dense-core vesicles (Otake et al., 1998) as demonstrated by electron microscopy. Thus the concentrations of agmatine at some subcellular sites could be approaching concentrations required to block the NMDA channel. Although agmatine can be released from rat brain synaptosomes (Reis and Regunathan, 1997), it remains to be directly demonstrated that agmatine could be released from nerve terminals in amounts sufficient to block NMDA channel function.

The findings reported here add further evidence in support of our hypothesis (Reis and Regunathan, 1997) that agmatine meets many of the criteria for an endogenous neurotransmitter/neuromodulator in brain: 1) it is synthesized in the central nervous system (Li et al., 1994, 1995), 2) it expressed specific populations of central neurons (Otake et al., 1998), 3) it is stored in axon terminals in association with storage vesicles and in apposition to synaptic specializations (Reis et al., 1998), 4) it can be released from synaptosomes by depolarization (Reis and Regunathan, 1997), 5) it can be inactivated by enzymatic conversion and/or by reuptake (Sastre et al., 1996, 1997), and 6) it can bind to (Anis et al., 1990) and modulate the actions of (this report) the NMDA subclass of glutamate receptors. Although the physiological role of agmatine at NMDA receptors in hippocampus and other regions of the central nervous system is unknown at present, it is of interest, particularly because agmatine enhances opioid analgesia and prevents tolerance in vivo (Kolesnikov et al., 1996), actions that resemble those of NMDA receptor antagonists (Wong et al., 1996; Elliott et al., 1996).

In summary, we demonstrate in the present study that agmatine is a selective blocker of the NMDA subclass of glutamate receptor channels. The blocking effect of agmatine is mediated by interaction between the guanidine group of agmatine and the pore of the NMDA receptor channel. The results support other data that agmatine may function as an endogenous neurotransmitter/neuromodulator in brain.

Acknowledgments

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