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Agmatine: a mastoparan-like activity related to direct activation of heterotrimeric G proteins

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Abstract

We examined agmatine and imidazoline derivatives as putative ligands of trimeric G protein in rat peritoneal mast cells. Agmatine induced a concentration-dependent and pertussis toxin-sensitive secretion of histamine (exocytosis) and arachidonate. Clonidine and idazoxan had no effect. Blockage of Gβγ dimers by a specific anti-Gβ antibody inhibited exocytosis elicited by agmatine and mastoparan. The G protein antagonist [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) prevented both mastoparan- and agmatine-induced exocytosis when it was allowed to reach its intracellular targets by streptolysin-O permeabilisation. In intact cells, this response was prevented by both the removal of sialic acid residues by neuraminidase and by [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) acting at the mast cell surface. Exocytosis was restored by permeabilisation of the plasma membrane with streptolysin-O. These results suggest that agmatine might have several molecular targets, exerting its neurotransmitter function at low concentrations (i.e., with high affinity) through membrane receptors and at high concentrations (i.e., with weak affinity) through direct G protein activation.

Keywords: Agmatine; Imidazoline; Exocytosis; G protein; Mastoparan; Mast cell

1. Introduction

Agmatine is the product of arginine decarboxylation (Fig. 1) and can be hydrolysed by agmatinase to putrescine, the precursor for the higher polyamines, spermidine and spermine (Wu and Morris, 1998). Alternatively, putrescine can be synthesised by ornithine decarboxylase. Agmatine has been considered for many years to be only an intermediate in bacterial polyamine metabolism. However, in 1994, while searching for an endogenous ligand for imidazoline binding sites, Li et al. (1994) isolated a candidate molecule from mammalian brain and identified it as agmatine. Agmatine is stored in neurons, is released in a Ca^{2+}-dependent manner by depolarising stimuli and is taken up by synaptosomes. Hence, agmatine is proposed to be a neurotransmitter in the brain (Reis and Regunathan, 2000).

Several molecular targets have been proposed for this putative neurotransmitter. Agmatine is considered an agonist at imidazoline I_1 receptors (Reis and Regunathan, 1998) and α_2-adrenoceptors (Li et al., 1994). Agmatine modulates NMDA channel activity by blocking the polyamine-binding site (Fairbanks et al., 2000; Yang and Reis, 1999). Agmatine might also decrease the opening of other ligand-gated cationic channels including 5-HT_3 receptors (Molderings et al., 1996) and nicotinic acetylcholine receptors (Santos et al., 2001). Moreover, agmatine can inhibit inducible nitric oxide synthase activity in isolated rat aorta (Auguet et al., 1995), but stimulates nitric oxide synthase in endothelial cells (Morrissey and Klahr, 1997).

Mastoparan is an amphiphilic tetradecapeptide toxin from wasp venom, which mimics receptors by activating directly trimeric GTP-binding proteins (G proteins) (Higashijima et al., 1988). We noticed similarities in the effects of agmatine and of mastoparan, both of which stimulate insulin secretion and inhibit catecholamine secretion from chromaffin cells. Indeed, agmatine was reported as early as 1926 to reduce blood sugar levels (Frank et al., 1926) and was recently shown to elicit a slight stimulation of insulin secretion (Berdeu et al., 1996; Chan, 1998; Shepherd et al., 1996). Such an ability to induce insulin secretion had been described for mastoparan, with the demonstration of its direct activation of pertussis toxin-sensitive G proteins (Hillaire-Buys et al., 1992; Konrad et al., 1995). Furthermore,

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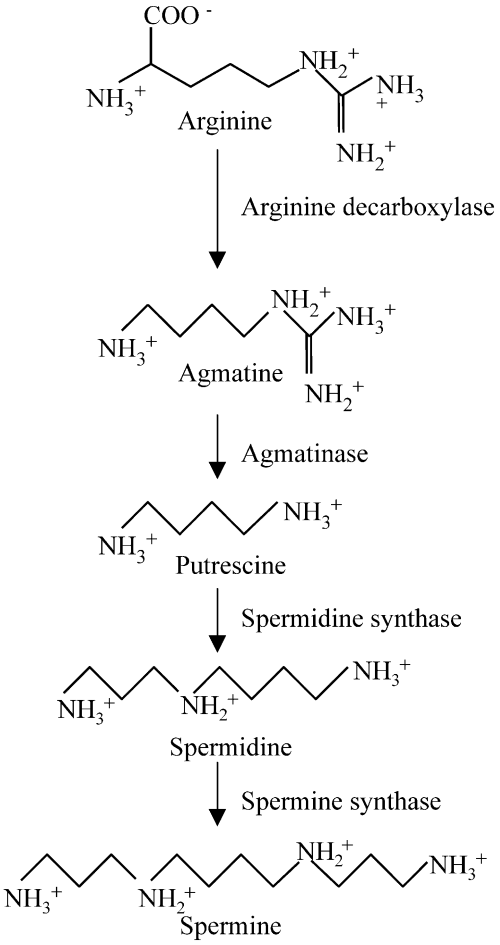

Fig. 1. Metabolic pathway of agmatine.

Santos et al. (2001) recently suggested that agmatine interferes with catecholamine secretion induced by acetylcholine from chromaffin cells by blocking directly nicotinic receptor currents, a phenomenon unrelated to imidazoline receptors. Interestingly, such a property had been proposed in 1989 for mastoparan (Wilson, 1989), but this effect of mastoparan was later demonstrated to be a consequence of the direct activation of trimeric G proteins (Vitale et al., 1993).

Altogether, these observations led us to suggest that agmatine, like mastoparan, might be a ligand for trimeric G proteins. To study this hypothesis, we used rat peritoneal mast cells. These cells respond to mastoparan and other cationic compounds, such as spermine, through a G protein-dependent pathway leading to exocytosis (histamine secretion) and arachidonate release (Ferry et al., 2001). Experiments were designed to demonstrate the requirement of agmatine to cross the plasma membrane in order to reach its intracellular targets. We show that agmatine stimulates mast cells through these pathways, demonstrating that trimeric G proteins are valuable candidates as direct targets for agmatine.

2. Materials and methods

2.1. Materials

Agmatine, arcaine, clonidine, idazoxan, mastoparan, spermine, GTPγS and type V neuraminidase from *Clostridium perfringens* were purchased from Sigma (St. Louis, MO). Pertussis toxin was obtained from List Biological Laboratory (Campbell, CA). [^{3}H]Arachidonic acid was purchased from Amersham Pharmacia Biotech (UK). Anti Gβ antibody (carboxy-terminal 20 amino acids of β1 of mouse origin, with broad specificity to mouse, rat and human Gβ1 to Gβ4) was provided by Santa Cruz Biotechnology (Santa Cruz, CA). [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) and [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) were purchased from Bachem (Paris, France). [D-Pro^4,D-Trp^{7,9,10}] substance P-(4–11), i.e., D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Met-NH_2, has been called SP-A (Piotrowski and Foreman, 1985) or GPAnt-1 (Mukai et al., 1992). [*p*-Glu^5,D-Trp^{7,9,10}]substance P(5–11), i.e., *p*Glu-Gln-D-Trp-Phe-D-Trp-D-Trp-Met-NH_2, has been called GPAnt-2 (Mukai et al., 1992).

2.2. Isolation and treatment of rat peritoneal mast cells

The procedures followed for the care and the euthanasia of the animals were in accordance with the European Committee standards on the care and use of laboratory animals (European Directive 86/609/CEE Nov. 26, 1986). Male Wistar rats weighing 300–500 g were stunned and bled. Twelve millilitres of balanced salt solution (HEPES buffer) containing (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 0.3, $MgCl_2$ 1.0, NaH_2PO_4 0.4, glucose 5.6 and HEPES 10, NaOH to pH 7.4, supplemented with 0.1% bovine serum albumin, was injected into the peritoneal cavity. The peritoneal fluid was collected and centrifuged for 3 min at 180 × *g*. The pellet was suspended in the same buffer and mast cells were purified on a discontinuous bovine serum albumin gradient (30% and 40% w/v) as previously described (Mousli et al., 1989). The pellet was resuspended in HEPES buffer, and cells were examined under a light microscope for purity (more than 97%). The Trypan blue-exclusion test indicated viability greater than 95%.

Purified mast cells (3×10^4 cells/assay) were pre-treated with pertussis toxin (50 ng/ml) or with neuraminidase (1 U/ml) at 37 °C for the indicated time. Cells were pre-incubated in the HEPES buffered solution at 37 °C for 5 min before adding triggers. Reactions were terminated 10 min later by the addition of ice-cold buffer. The amount of histamine secreted was determined fluorimetrically, according to the method of Shore et al. (1959), but without the extraction step. Histamine release from the cells in the absence of any stimulus (spontaneous release) was less than 5% of the total histamine content.

2.3. Mast cells permeabilisation

Purified mast cells (3×10^4 cells/assay) were pre-incubated for 5 min at 37 °C and permeabilised by exposure to

streptolysin-O (0.4 U/ml) for 1 min at 37 °C. Then, mast cells were incubated with antibodies or with analogues of substance P for 5 min. Cells were triggered by basic secretagogues (agmatine, mastoparan or spermine) and reactions were terminated 2 min later by the addition of ice-cold buffer. Passive histamine release, in the absence of secretagogue, was less than 10% of the total histamine content. Permeabilisation was controlled in each experiment by monitoring secretion elicited by GTPγS, a non-hydrolysable analogue of GTP that triggers heterotrimeric and small G proteins in permeabilised mast cells (Howell et al., 1987).

2.4. Determination of arachidonic acid release

Purified mast cells were suspended in HEPES buffer (5×10^5 cells/ml) and incubated with 5 μCi/ml [^{3}H]arachidonic acid for 2 h at 37 °C. Cells were washed twice, resuspended in HEPES buffer (1×10^5 cells/assay), pre-incubated for 10 min and triggered for 20 min at 37 °C. The reaction was terminated by adding ice-cold buffer and placing the tubes on ice. Supernatants obtained after centrifugation ($180 \times g$, 3 min, +4 °C) were collected and used to determine, by liquid scintillation counting, the amount of arachidonate released.

3. Results

3.1. Characterization of the secretory response of mast cells to agmatine

Agmatine induced a concentration-related secretion of histamine from rat peritoneal mast cells, with an EC_{50} of about 2 mM (Fig. 2A). The maximal secretion was obtained at 10 mM, corresponding to 48.5 ± 2.1% of the total histamine content. Histamine secretion occurred rapidly, with a maximum observed 20 s after the addition of agmatine. A preliminary permeabilisation with streptolysin-O did not significantly modify the kinetics of exocytosis (Fig. 2B). We previously observed a similar time course for the mast cell response to mastoparan and spermine (Bueb et al., 1992; Mousli et al., 1989). Clonidine, an agonist at imidazoline receptors, and idazoxan, an antagonist of imidazoline receptors, were unable to induce histamine secretion from mast cells. Idazoxan did not prevent agmatine-induced secretion (Table 1).

A crucial characteristic of the effect of mastoparan on mast cells is its sensitivity to pertussis toxin (Mousli et al., 1989), which is known to ADP-ribosylate a cysteine residue in the carboxy terminus of α subunits from G_i, G_o and G_t proteins (Gilman, 1987). Two pertussis toxin substrates have been proposed in rat peritoneal mast cells (Bueb et al., 1990) and identified as being the G_{i2} and G_{i3} proteins (Aridor et al., 1993; Daeffler et al., 1999a). Table 1 shows the effect of pertussis toxin on histamine secretion induced by mastoparan, spermine and agmatine. The treatment of mast cells

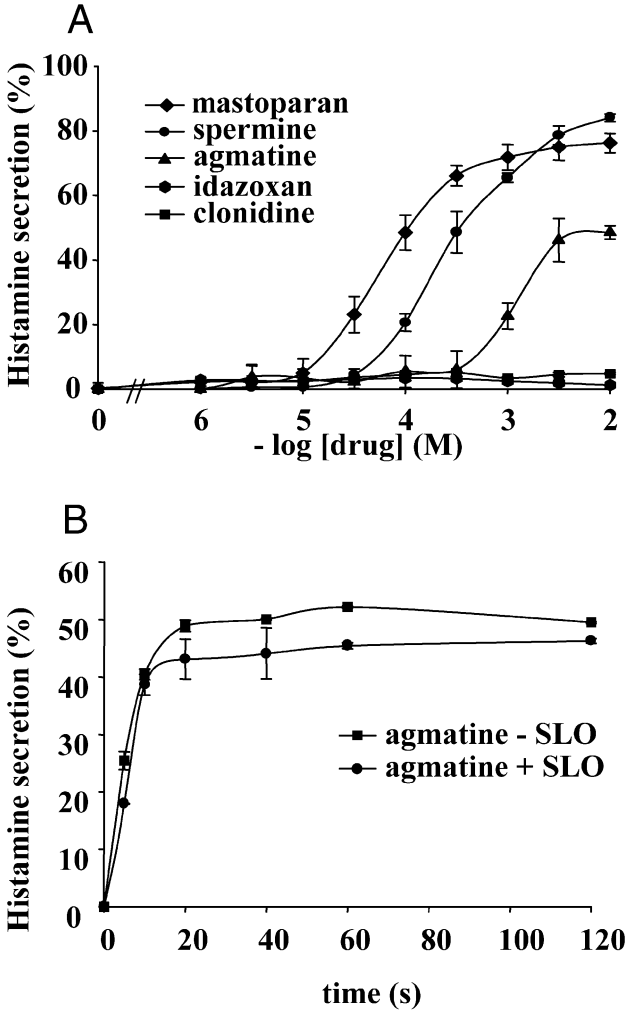

Fig. 2. Concentration-dependent effects of mastoparan, agmatine, spermine and imidazoline derivatives on histamine secretion (A) and time course for histamine secretion induced by agmatine (10 mM) on intact or permeabilised cells (B). Panel (A), purified mast cells (3×10^4/assay) were pre-incubated for 5 min at 37 °C and stimulated by drugs for 10 min at 37 °C. Panel (B), purified mast cells were permeabilised for 1 min with streptolysin-O (SLO) and stimulated by agmatine for the indicated times. Results are means ± S.E.M. of three independent experiments.

with pertussis toxin for 2 h led to almost complete inhibition of the secretory responses.

Both Gα and Gβγ subunits from trimeric G proteins can be responsible for the activation of related signalling pathways (Clapham and Neer, 1997). Gomperts's group showed that βγ subunits introduced into permeabilised mast cells amplified the secretion induced by Ca^{2+} and GTPγS, whereas Gα subunits had no effect (Pinxteren et al., 1998). We confirmed the involvement of Gβγ dimers in response to agmatine by studying the effect of an anti-Gβ antibody with broad specificity for Gβ1 and Gβ4 subunits on histamine secretion. This antibody strongly inhibited histamine secretion elicited by agmatine, mastoparan and GTPγS (Fig. 3).

Previous studies have indicated that compound 48/80, a synthetic polyamine, spermine and mastoparan induce ara-

Table 1
Effect of pertussis toxin, EGTA and idazoxan on histamine secretion induced by mastoparan, spermine and agmatine from rat peritoneal mast cells

Compounds (mM)	Histamine secretion (% of total content)			
	Control	Ptx	EGTA	Idazoxan
Mastoparan				
0.03	23.1 ± 5.6	5.2 ± 3.3	22.4 ± 2.3	–
0.1	48.5 ± 5.4	10.2 ± 4.1	44.0 ± 3.6	–
0.3	66.1 ± 3.1	12.2 ± 2.7	62.0 ± 4.8	–
Spermine				
0.1	17.1 ± 0.5	3.4 ± 2.1	16.7 ± 1.0	–
0.3	50.7 ± 0.6	5.7 ± 3.2	43.1 ± 2.0	–
1	67.5 ± 3.5	9.6 ± 4.6	66.6 ± 1.9	–
Agmatine				
1	18.9 ± 3.0	2.1 ± 2.0	17.9 ± 0.3	17.7 ± 4.0
3	48.0 ± 2.8	3.9 ± 2.1	46.0 ± 4.2	44.8 ± 4.0
10	49.4 ± 0.6	2.7 ± 3.1	50.4 ± 5.2	50.4 ± 4.2

Purified rat peritoneal mast cells were pre-incubated in the absence (control) or presence of pertussis toxin (Ptx, 50 ng/ml) for 2 h at 37 °C or EGTA (2 mM), idazoxan (10^{-6} M) for 10 min at 37 °C and stimulated for 10 min at 37 °C with the indicated concentrations. Values are means ± S.E.M. of three independent experiments each in duplicate.

chidonate release from mast cells (Nakamura and Ui, 1985; Ferry et al., 2001). We therefore examined whether agmatine might induce arachidonic acid release from rat peritoneal mast cells (Fig. 4). Indeed, agmatine, like mastoparan and spermine, caused a concentration-related release of arachidonic acid from rat peritoneal mast cells with an EC_{50} of about 2 mM (agmatine), 0.1 mM (mastoparan), and 0.6 mM (spermine). Pertussis toxin pre-treatment inhibited arachidonic acid release induced by agmatine (Fig. 4).

Another characteristic of the exocytosis elicited by basic compounds, such as mastoparan, is its independence from extracellular Ca^{2+} (Mousli et al., 1989). To assess the importance of extracellular Ca^{2+} in activation–secretion coupling by agmatine, mast cells were stimulated in the presence or absence of 2 mM of EGTA. Table 1 shows that extracellular Ca^{2+} chelation with 2 mM of EGTA had no significant effect on the mast cell response induced by mastoparan, spermine or agmatine.

3.2. Agmatine acts intracellularly

At this point of our study, the above results indicated that the response of mast cells to agmatine is related to the activation of trimeric G proteins and does not involve extracellular Ca^{2+}. Thus, the target of agmatine might be some membrane G protein-coupled receptor or trimeric G protein. In rat peritoneal mast cells, pertussis toxin-sensitive G proteins are located in the inner face of the plasma membrane and on Golgi and granules membranes (Aridor et al., 1993). Thus, G protein ligands have to cross the plasma membrane to act on their targets, but this has been demonstrated to be dependent on the preliminary binding of mast cell secretagogues to sialic acid residues of the cell surface (Mousli et al., 1989).

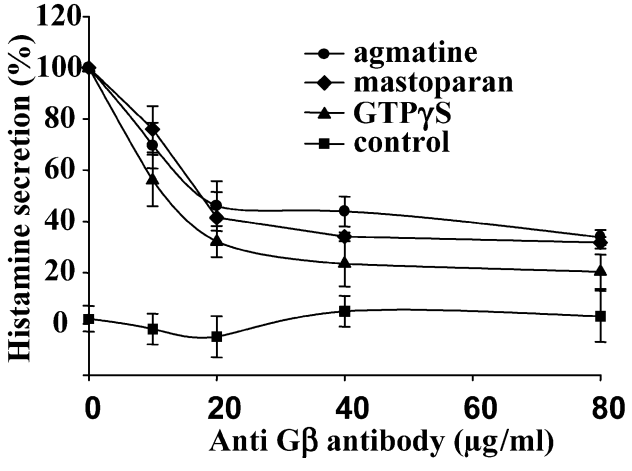

Fig. 3. Effect of anti Gβ antibody on histamine secretion induced by agmatine, mastoparan and GTPγS. Cells were incubated with streptolysin-O (0.4 U/ml) for 1 min at 37 °C and the antibody was added. Mast cells were stimulated by agmatine (3 mM), mastoparan (0.1 mM) or GTPγS (0.1 mM) for 2 min at 37 °C and reactions were stopped by addition of ice-cold buffer. Histamine secretion (100%) in the absence of antibody corresponded to 39.7 ± 12.7% (mastoparan), 44.8 ± 1.9% (agmatine) or 42.1 ± 3.8% (GTPγS) of the total histamine content. Controls were unstimulated permeabilised cells incubated with antibody. Values are means ± S.E.M. of four independent experiments.

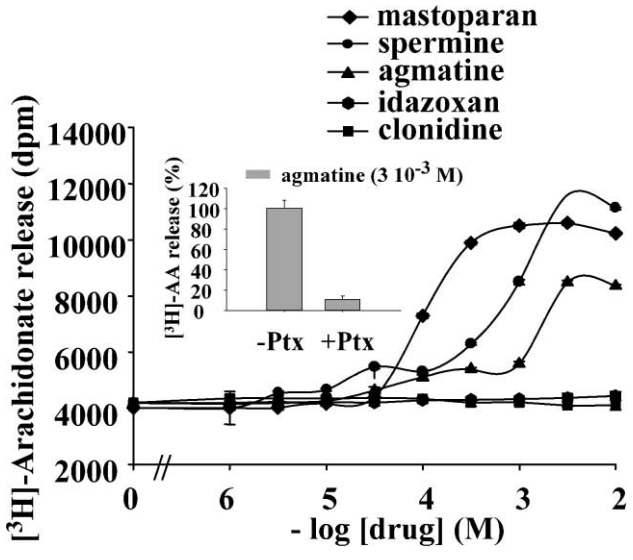

Fig. 4. Concentration-dependent effects of mastoparan, agmatine, spermine and imidazoline derivatives on arachidonate release from rat peritoneal mast cells. Purified mast cells were labelled with [^{3}H]arachidonic acid as described under Materials and methods, washed twice in HEPES buffer, resuspended at 1×10^5/assay and stimulated with the indicated concentrations of drugs for 20 min at 37 °C. Insert shows the effect of pertussis toxin pretreatment (50 ng/ml) for 2 h at 37 °C on arachidonic acid release induced by agmatine (3 mM); 100% value stands for arachidonate release induced by agmatine (8480 dpm ± 77). Results are means ± S.E.M. of three independent experiments.

Accordingly, Fig. 5 shows that pre-treatment of mast cells with neuraminidase, which hydrolyses sialic acid residues at the cell surface, induced an inhibition (>60%) of histamine secretion elicited by different concentrations of agmatine. The secretory response to agmatine was restored when neuraminidase-treated mast cells were permeabilised with streptolysin-O, which demonstrates that agmatine has to cross the plasma membrane to induce mast cell secretion. In these experiments, the efficiency of the permeabilisation process was controlled through the secretory effect of GTPγS, previously shown to require membrane permeabilisation to elicit secretion (Howell et al., 1987).

To further assess the role of the cell membrane and trimeric G proteins in the effect of agmatine, we used two substance P analogues. [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) has been previously shown to inhibit the mast cell response to basic secretagogues (Piotrowski and Foreman, 1985) by interfering with their preliminary binding at the cell surface (Chahdi et al., 1998b). [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) is an antagonist ligand of G proteins (Mukai et al., 1992) which require mast cell permeabilisation to block exocytosis (Chahdi et al., 1998b). Incubation with increasing concentrations of [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) inhibited agmatine-induced exocytosis with an IC_{50} of about 7 μM, whereas in permeabilised cells, [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) had no effect (Table 2). Table 3 shows that pre-incubation of permeabilised mast cells with increasing concentrations of [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) inhibited the secretory response to agmatine with an IC_{50} of about 10 μM. However, in intact mast cells, [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) had no effect.

These results, obtained with substance P analogues, demonstrate that agmatine first binds to sialic acid residues at the mast cell surface and then crosses the plasma membrane to stimulate pertussis toxin-sensitive G proteins.

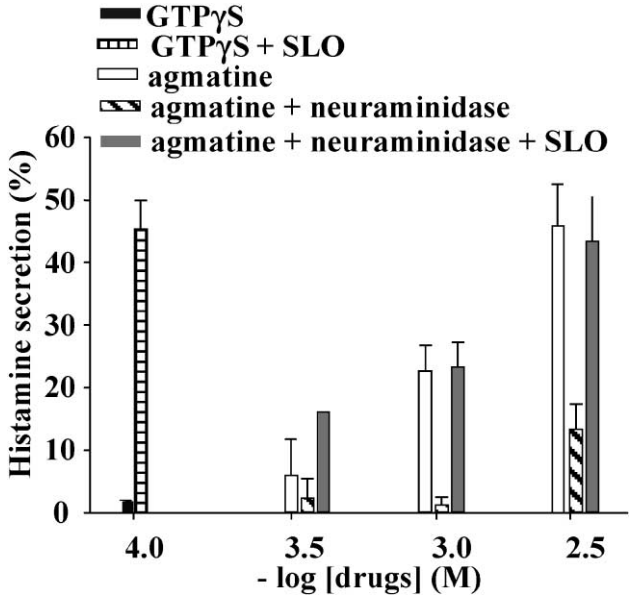

Fig. 5. Effect of sialic acid depletion and permeabilisation on histamine secretion induced by agmatine. Mast cells were pre-incubated with neuraminidase (1 U/ml) for 1 h at 37 °C and were then permeabilised with streptolysin-O (SLO, 0.4 U/ml) for 1 min at 37 °C. Alternatively, these pre-treatments were omitted. Cells were then stimulated with different concentrations of agmatine and cell responses were stopped 2 min later by the addition of ice-cold buffer. GTPγS (0.1 mM) was used as a control for permeabilisation. Values are means ± S.E.M. of four independent experiments.

Table 2
Effect of [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11), a cell surface inhibitor of mast cell activation, on histamine secretion induced by mastoparan and agmatine from mast cells

Compound	Histamine secretion (% of total content)				
	[D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) (μM)				
	0	2.5	5	10	20
Mastoparan					
Intact cells	59.0 ± 3.0	53.0 ± 3.1	42.0 ± 3.5	30.0 ± 4.0	22.1 ± 4.8
Permeabilised cells	57.0 ± 5.0	58.0 ± 3.0	56.0 ± 4.0	55.0 ± 4.5	54.0 ± 4.8
Agmatine					
Intact cells	40.6 ± 2.0	31.7 ± 1.4	35.2 ± 2.6	25.5 ± 1.9	17.4 ± 1.2
Permeabilised cells	44.3 ± 2.0	44.7 ± 0.2	42.8 ± 3.1	42.7 ± 2.8	39.9 ± 4.0

Purified rat peritoneal mast cells (3×10^4/assay) were permeabilised with streptolysin-O (1 U/ml) for 1 min at 37 °C or left intact. Cells were then pre-incubated for 2 min at 37 °C with the indicated concentrations of [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) and were stimulated with mastoparan (0.1 mM) or with agmatine (3 mM) for 2 min at 37 °C. Values are means ± S.E.M. of four independent experiments.

Table 3
Effect of [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11), an antagonist of trimeric G proteins, on histamine secretion induced by mastoparan and agmatine from mast cells

Compound	Histamine secretion (% of total content)				
	[*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) (μM)				
	0	5	10	50	100
Mastoparan					
Intact cells	58.0 ± 3.0	55.0 ± 3.8	56.0 ± 4.5	53.0 ± 3.0	54.0 ± 3.6
Permeabilised cells	58.0 ± 4.0	56.0 ± 3.1	44.0 ± 4.5	36.0 ± 4.2	28.0 ± 5.2
Agmatine					
Intact cells	42.6 ± 3.6	42.5 ± 2.3	41.9 ± 1.3	42.4 ± 1.1	42.7 ± 2.5
Permeabilised cells	43.4 ± 3.1	29.5 ± 6.2	23.2 ± 2.6	16.6 ± 1.8	12.6 ± 1.5

Purified rat peritoneal mast cells (3×10^4/assay) were permeabilised with streptolysin-O (1 U/ml) for 1 min at 37 °C or left intact. Cells were then pre-incubated for 2 min at 37 °C with the indicated concentrations of [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) and were stimulated with mastoparan (0.1 mM) or with agmatine (3 mM) for 2 min at 37 °C. Values are means ± S.E.M. of four independent experiments.

4. Discussion

The present results show that agmatine, a putative neurotransmitter, induced concentration-dependent exocytosis (histamine secretion) and arachidonate release from rat peritoneal mast cells. This response to agmatine was similar to that elicited by mastoparan or spermine: it was independent of extracellular Ca^{2+}, sensitive to pertussis toxin, and inhibited by the G protein antagonist [*p*-Glu^5,D-Trp^{7,9,10}]substance P-(5–11) and by anti-Gβ antibody. Thus, we propose agmatine as a direct activator of G protein.

The necessity for agmatine to bind to the cell surface and to penetrate into mast cells to elicit exocytosis is illustrated by the experiments with neuraminidase (Fig. 5) and with [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11) (Table 2). Pre-treatment of mast cells with neuraminidase, which removes sialic acid residues from the plasma membrane, and [D-Pro^4,D-Trp^{7,9,10}] substance P-(4–11), which binds to the cell surface (Chahdi et al., 1998b), inhibited agmatine-induced exocytosis. This observation confirms the role of sialic acid residues belonging to membrane glycoproteins or gangliosides in the initial binding of cationic secretagogues to the mast cell membrane. This proposal implies that permeabilisation of mast cells pretreated with neuraminidase should overcome the inhibitory effect of sialic acid residue depletion. Accordingly, the treatment of mast cells with streptolysin-O restored the secretory response to agmatine previously inhibited by neuraminidase or by [D-Pro^4,D-Trp^{7,9,10}]substance P-(4–11).

Thus, as demonstrated for substance P (Lorenz et al., 1998), and methoctramine (Chahdi et al., 1998a), agmatine has to reach G proteins to elicit mast cell degranulation. The mechanism by which it crosses the plasma membrane remains unknown. In yeast, multiple polyamine uptake systems have been demonstrated and belong to the ATP binding cassette (ABC) transporters (Igarashi and Kashiwagi, 1999; Tomitori et al., 2001). In mammalian cells, two distinct agmatine uptake systems have been proposed in rat brain synaptosomes (Sastre et al., 1997) and in a few mammalian cells lines such as human glioma cell line SK-MG-1 (Molderings et al., 2001). Furthermore, a recent report shows that agmatine is dependent on the polyamine transporter for cellular uptake (Satriano et al., 2001). Such systems have not yet been described in mast cells.

Molderings et al. (1999) showed that agmatine, in the millimolar concentration ranges, can induce histamine secretion from histamine-storing cells in rat stomach via the involvement of imidazoline receptors. In the present report, we show that agmatine induced histamine secretion over the same range of concentrations but independently of imidazoline receptors. The discrepancy between our results and those reported by Molderings et al. (1999) might predominantly arise from the different sources of cells. The present experiments were performed with purified rat peritoneal mast cells. In contrast, Molderings et al. (1999) used a rat stomach preparation in which histamine is stored mainly in enterochromaffin-like (ECL) cells and in a few mast cells (Doi et al., 1998). ECL cells and mast cells have distinct mechanism of exocytosis. Table 1 shows that histamine secretion from rat peritoneal mast cells elicited by agmatine is independent of the extracellular Ca^{2+} pool. In contrast, in ECL cells, the influx of extracellular Ca^{2+} across the plasma membrane is necessary to activate the exocytosis of histamine (Prinz et al., 1993, 1999). Thus, the main difference between the exocytotic processes of ECL and mast cells might be their dependence on extracellular Ca^{2+}, corresponding to different signalling pathways. Furthermore, clonidine (an imidazoline receptor agonist) does not elicit a secretory effect in rat mast cells (Fig. 2). In addition, the imidazoline antagonist idazoxan was not able to reverse the secretory effect of agmatine (Table 1). The concentration of agmatine needed to induce histamine secretion from both rat stomach preparations and rat peritoneal mast cells is in the millimolar range. The question has to be addressed whether this effect is functionally relevant to receptor binding. Indeed, the binding characteristics of imidazoline I_1 receptor agonists (for agmatine) are in the nanomolar range of concentrations (Eglen et al., 1998).

An effect of agmatine at high concentrations has been observed in pancreatic β cells (Berdeu et al., 1996; Chan, 1998; Shepherd et al., 1996). In these cells, agmatine evokes an insulin secretion response in the range of 0.1–3 mM. In contrast, at lower concentrations, agmatine is considered a ligand for several receptors (see Introduction). Taken together, these observations suggest that agmatine might have several targets: at low concentrations (i.e., showing high affinity) it may exert its action through several membrane receptors, and at high concentrations (i.e., showing low affinity) it may act via G protein.

Altogether, the present results indicate that agmatine belongs to the large group of basic secretagogues of mast cells (for reviews, Paton, 1957; Lagunoff et al., 1983; Mousli et al., 1990, 1994). This group includes cationic neuropeptides such as substance P (Mousli et al., 1989), neuropeptide Y (Mousli et al., 1995), peptidic hormones such as bradykinin (Bueb et al., 1990), various venom peptides such as mastoparan (Higashijima et al., 1987) and mast-cell degranulating (MCD) peptide (Fujimoto et al., 1991; Mousli et al., 1991). Synthetic polyamines such as compound 48/80, methoctramine and arcaine (Paton, 1951; Aridor et al., 1993; Chahdi et al., 1998a; Daeffler et al., 1999b), and natural polyamines such as spermidine and spermine (Bueb et al., 1992), are also basic secretagogues, as are a large number of various drugs such as cannabinoids (Bueb et al., 2001), morphine (Klinker and Seifert, 1997), levofloxacin (Mori et al., 2000) and others (for review, Klinker et al., 1995). Their main characteristic is their potency to activate directly heterotrimeric G proteins, a receptor-mimetic effect first proposed for mastoparan (Higashijima et al., 1988).

The mechanism of action of basic secretagogues, which has mainly been described in rat peritoneal mast cells, has also been demonstrated in human skin mast cells (Columbo et al., 1996; Emadi-Khiav et al., 1995) and in HL-60 (human

leukemic cells) (Klinker et al., 1995). Such a mechanism might also occur in neuronal cells. Indeed, Lee et al. (1989) showed that in rat cerebral cortex, methoctramine, a synthetic polyamine with acetylcholine muscarinic receptor antagonist properties, was a powerful activator of phosphoinositide hydrolysis at concentrations above 5 μM. These authors concluded that this effect was unrelated to membrane receptors. Methoctramine, at high concentrations, was later shown to directly activate G proteins (Chahdi et al., 1998a; Daeffler et al., 1999a). We propose that the putative neurotransmitter agmatine shares with some peptidic neurotransmitters, such as substance P, and with some cationic drugs, such as methoctramine, the capacity to act at low concentrations on selective membrane receptors and at higher concentrations on trimeric G proteins.

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