Biochem. J. (1992) 282, 545–550 (Printed in Great Britain)

Natural polyamines stimulate G-proteins

Jean-Luc BUEB,* Angelo DA SILVA, Mouafak MOUSLI and Yves LANDRY†
Laboratoire de Neuroimmunopharmacologie, Université Louis Pasteur-Strasbourg I, B.P. 24, 67401 Illkirch Cedex, France

The natural polyamines spermine and spermidine, the biosynthetic precursor putrescine and their analogues cadaverine and tyramine stimulate the GTPase activity of purified GTP-binding proteins (G_o/G_i) from calf brain reconstituted into phospholipid vesicles. The order of potency was spermine > spermidine > putrescine = cadaverine > tyramine. The physiological relevance of this observation was assessed, showing the same order of potency of polyamines in the stimulation of peritoneal and tracheal rat mast cells. The activation of rat mast cells by polyamines was inhibited by benzalkonium chloride or by a 2 h pretreatment of the cells with pertussis toxin. The increase in inositol phosphates evoked by polyamines was also inhibited by pertussis toxin. Therefore we propose that intracellular polyamines might control the basal level of second messengers and modulate extracellular signals transduced through G-protein-coupled receptors.

INTRODUCTION

The polyamines putrescine (butan-1,4-diamine), spermidine [*N*-(3-aminopropyl)butane-1,4-diamine] and spermine [*NN'*-bis-(3-aminopropyl)butane-1,4-diamine] are polycationic metabolites widely distributed in prokaryotic and eukaryotic cells. Their concentrations within cells are strongly regulated, with a predominant role of ornithine decarboxylase (for reviews see Seiler, 1990; Pegg & McCann, 1982). Polyamines play a role in numerous cell functions and are specially required for optimal cell growth, division and differentiation (Pegg & McCann, 1982; Tabor & Tabor, 1984, 1985; Schuber, 1989). Recent reports show that polyamines also play a facilitatory role in glucose-stimulated insulin-gene expression (Welsh, 1990), depress macrophage tumoricidal activity (Bowlin *et al.*, 1990), and repair gastric-mucosal stress ulcer (Wang & Johnson, 1990). Polyamines are greatly increased in tissues, serum and urine of patients suffering from gastrointestinal diseases (Löser *et al.*, 1989). The levels of polyamines and of ornithine decarboxylase activity in the brain have been reported to be increased following transient cerebral ischaemia (Paschen *et al.*, 1988; Dempsey *et al.*, 1988).

In spite of the numerous reports describing the various effects of polyamines, the mode of action of these aliphatic cations at a molecular level remains a matter of speculation. Electrostatic interactions between the positively charged amino groups of polyamines (at physiological pH values) and the various negatively charged moieties of the cells constitute the most widely accepted basis for the mechanism of action of intracellular polyamines. Indeed, polyamines are known to bind to negatively charged cellular macromolecules such as nucleic acids, and numerous cytosolic enzymes (Schuber, 1989).

Various arguments have been proposed suggesting a role for polyamines in the transduction of extracellular messages. Koenig *et al.* (1983) showed that polyamines can act as intracellular signals enhancing Ca^{2+} influx across the plasma membrane and Ca^{2+} efflux from the mitochondria. More recent McCormack (1989) showed that spermine has a direct effect on mitochondrial Ca^{2+} transport. Fan & Koenig (1988) observed that depletion of intracellular polyamines in heart cells abrogated the stimulation of Ca^{2+} influx, hexose and amino acid transport evoked by isoprenaline, an agonist of β-receptors. Charlton & Baylis (1990) suggested that polyamines are involved in the stimulation of rat renal medullary cells by [arginine] vasopressin, leading to the stimulation of the sodium pump. Polyamines also inhibit adenylate cyclase (Clo *et al.*, 1979, 1988; Khan *et al.*, 1990) and may enhance the phosphorylation of cell proteins following the activation of polyamine-dependent protein kinases (Cochet & Chambaz, 1983). Polyamines have also a modulatory effect on the *N*-methyl-D-aspartate-receptor/ion-channel complex, exerted through a recently suggested recognition site located on the complex itself (for review see Williams *et al.*, 1991). Such interactions would be more complex than simply electrostatic ones, and here again the molecular mechanism of action of these natural cationic molecules on the polyamine site(s) of the *N*-methyl-D-aspartate-receptor complex has to be explored. Recently, the synthetic polyamine compound 48/80, well known to induce histamine release from rat peritoneal mast cells, and cationic amphiphilic peptides such as mastoparan, substance P and bradykinin, have been shown to interact directly with GTP-binding proteins (G proteins) (Aridor *et al.*, 1990; Higashijima *et al.*, 1990; Mousli *et al.*, 1989, 1990*a*,*b*; Bueb *et al.*, 1990). The only common feature between these latter molecules being their postively charged clusters of amino acid residues, we questioned whether natural positively charged polyamines could not share their ability to modulate the activity of G-proteins

In view of the putative role of polyamines in the transduction of extracellular messages, G-proteins may represent an important intracellular target for polyamines. As the stimulation of rat peritoneal mast cells by cationic molecules, involving G-proteins, has been much studied, these cells together with purified G-proteins constitute an interesting model to study this hypothesis.

MATERIALS AND METHODS

GTPase activity of purified G-proteins

A mixture of purified G_o and G_i proteins (G_o/G_i), containing mostly G_o (Homburger *et al.*, 1987; Brabet *et al.*, 1988), was obtained from calf brain membranes by successive elution from DEAE-Sephacel (Pharmacia, Uppsala, Sweden), AcA34 (LKB, Uppsala, Sweden) and heptylamine–Sepharose columns as described by Sternweis & Robishaw (1984). This purified preparation of G-proteins, freed of any receptor system, was reconstituted into phospholipid vesicles (Sternweis, 1986). Briefly, G-proteins (about 120 pmol) in 500 μl of buffer A (50 mM-

* Present address: Centre de Recherche Public – Santé, Neuroimmunologie et Inflammation, 120 route d'Arlon, L-1150 Luxembourg.
† To whom correspondence should be addressed.

NaHepes, pH 8, 1 mM-EDTA, 1 mM-dithiothreitol) plus 0.02% Lubrol 12A9 were mixed with 100 μl of buffer A, containing 0.84% sodium cholate, 0.05% dioleoylphosphatidycholine, 0.05% bovine brain phosphatidylethanolamine and 0.067% bovine brain phosphatidylserine (Sigma). GTPase activity was then determined as described by Brandt *et al.* (1983) and Higashijima *et al.* (1987) with [γ-^{32}P]GTP (Amersham). The assay was performed in buffer A containing Mg^{2+} and polyamines at the indicated concentrations, for 5 min at 25 °C. Reactions were stopped by adding ice-cold activated charcoal to the samples. The γ-^{32}P content of the supernatant was detemined for the freed phosphate groups by scintillation counting, by using Aquasol-2 (New England Nuclear–Du Pont de Nemours).

Mast cell purification

Mast cells were purified from male albino Wistar rats (Iffa Credo, L'Arbresle, France) weighing 300–350 g. The rats were killed by stunning and bleeding. Peritoneal mast cells were harvested in 10 ml of balanced salt solution containing: (mM) NaCl, 137; KCl, 2.7; $CaCl_2$, 0.3; $MgCl_2$, 1; NaH_2PO_4, 0.4; glucose, 5.6; Hepes, 10 and NaOH to pH 7.4, supplemented with 0.2% BSA (Sigma); 10 ml of balanced salt solution was injected intraperitoneally. The peritoneal cavity was opened after gentle abdominal massage for 2 min, and the peritoneal fluid was collected and centrifuged for 2 min at 180 ***g***. The pellet of mixed peritoneal cells was resuspended in the same buffer, and the mast cells were purified by centrifugation for 10 min at 220 ***g*** on a BSA step gradient (40 and 30%, w/v, in the same buffer). The pellet was resuspended and the mast cells were examined under a microscope for purity (> 97%).

Histamine release

After a 5 min preincubation of the mast cells (15000–20000 purified mast cells/assay) at 37 °C in the same balanced salt solution, the cells were challenged for 5 min with the various polyamines. The reactions were stopped by adding ice-cold buffer to the samples. Cells were sedimented for 3 min at 180 ***g***, washed in cold buffer and sedimented again. Histamine remaining in the pellet was released by disrupting the cells in 5% (w/v) trichloracetic acid. Histamine secretion was calculated by subtracting the remaining histamine of treated cells from the total histamine content determined in control cells. Histamine was assayed fluorimetrically by the method of Shore *et al.* (1959) without extraction steps, and expressed as a percentage of the total histamine content of the cells. Spontaneous histamine release, in the absence of any stimulus, never exceeded 8% and was subtracted from the induced releases. Pretreatment of the mast cells with 2-deoxy-D-glucose and/or dinitrophenol (Sigma) was done in balanced salt solution supplemented with 0.2% BSA [with (control) or without 5.6 mM-glucose] for 30 min at 37 °C. The effect of benzalkonium chloride (Sigma), a mixture of quaternary benzyldimethylalkylammonium chlorides, or pertussis toxin (Sigma) on histamine release was assessed after preincubation of the mast cells for 5 min or 2 h, respectively, at 37 °C in balanced salt solution supplemented with 0.2% BSA. Pretreatments with other compounds are indicated in the legend of the corresponding Figure or Table.

[^{3}H]Inositol phosphates

The generation of [^{3}H]inositol phosphates was measured by the method of Berridge *et al.* (1983) as described for mast cells by Nakamura & Ui (1985). Purified mast cells (10^7 cells/ml) were pulse-labelled with 100 μCi of *myo*-[^{3}H]inositol (New England Nuclear–Du Pont, Boston, MA, U.S.A.)/ml for 2 h, with or without pertussis toxin, in Hepes-buffered solution, at 37 °C. The cells were then washed twice and resuspended for 30 min in the buffer containing 10 mM-*myo*-inositol. After further washing with Hepes-buffered solution, the mast cells were distributed (1.5×10^6 cells/0.2 ml) and incubated for 5 min at 37 °C before the drugs were added. The induction was stopped 5 s later with 5% trichloroacetic acid. Supernatants were removed and washed four times with diethyl ether, neutralized with 5.6 mM-sodium tetraborate, deposited on anion-exchange resin columns (AG 1-X8, 200–400 mesh; formate form; Bio-Rad Laboratories, Richmond, CA, U.S.A.), eluted successively and counted for radioactivity. The elution was done successively with (*a*) 2 × 6 ml of water; (*b*) 2 × 6 ml of 60 mM-ammonium formate/5 mM-sodium tetraborate; (*c*) 2 × 6 ml of 0.2 M-ammonium formate/0.1 M-formic acid; (*d*) 3 × 6 ml of 0.4 M-ammonium formate/0.1 M-formic acid; and (*e*) 4 × 3 ml of 1 M-ammonium formate/0.1 M-formic acid (Sigma). The ^{3}H content in the eluates was determined for inositol mono- (*c*) and bis-phosphates (*d*) and other inositol polyphosphates (*e*) by scintillation counting in gel phase using Aquasol-2. Parallel assays with [^{3}H]inositol 1-phosphate, [^{3}H]inositol 1,4-bisphosphate and [^{3}H]inositol 1,4,5-trisphosphate standards (Amersham International, Little Chalfont, Bucks., U.K.) confirmed the accuracy of the separation protocol.

Organ-bath studies

Tracheas of male Wistar rats were dissected out, cleaned of connective tissue, and longitudinally opened by cutting through the cartilage. Three pieces of four cartilaginous ring-segments were carefully cut from the middle of the trachea. The luminal surface of the tracheal segments was gently rubbed with a cotton-wool swab to remove the epithelial layer (Frossard & Muller, 1986), and the segments were mounted in 10 ml organ baths containing modified Krebs solution of composition (mM): NaCl, 120; KCl, 4.75; $CaCl_2$, 1.25; $MgSO_4$, 1.2; glucose, 10; KH_2PO_4, 1.15; $NaHCO_3$, 25; bubbled with O_2/CO_2 (19:1). Tracheal smooth-muscle contractions were measured isometrically with Narco F60 force-displacement transducers connected to Narco Physiograph NKIII recorders. Tissues were washed three times at 15 min intervals. The tension was then adjusted to 1 g ('basal tension'), which has been shown to give the optimal length–active-tension relationship. After a 30 min equilibration period, during which pretreatments can be done, experiments were begun. Airway constriction was induced by a single concentration of drug in all experiments. Methysergide, an antagonist of 5-hydroxytryptamine, was generously given by Sandoz.

Statistics

If not especially indicated, values are means ± S.E.M. of three duplicate experiments. Statistical analyses of data were established by Student's *t* test (*$P < 0.01$, significantly different from control).

RESULTS

Fig. 1 shows that natural polyamines are able to stimulate the GTPase activity of purified G_o/G_i proteins reconstituted into phospholipid vesicles. The order of potency is spermine > spermidine > putrescine = cadaverine > tyramine. The presence of 0.3 mM-Mg^{2+} was required to obtain the activation of G-proteins by polyamines, as shown previously with kinins (Bueb *et al.*, 1990). This concentration was optimal to give maximal activation of the batch of G-proteins used.

Spermine, spermidine and putrescine, but also other polyamines such as cadaverine (pentane-1,5-diamine), an analogue

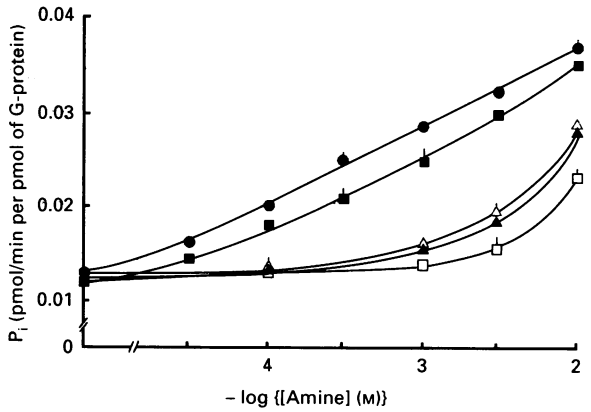

Fig. 1. Effect of polyamines on the GTPase activity of G-proteins purified from calf brain (G_o/G_i)

G-proteins were purified and reconstituted into phospholipid vesicles as stated in the Materials and methods section. Reconstituted G-proteins were incubated at 25 °C, for 5 min, with various concentrations of spermine (●), spermidine (■), putrescine (▲), cadaverine (△) and tyramine (□). Values are means ± S.E.M. of four duplicated experiments: S.E.M. values not shown are included within the symbols.

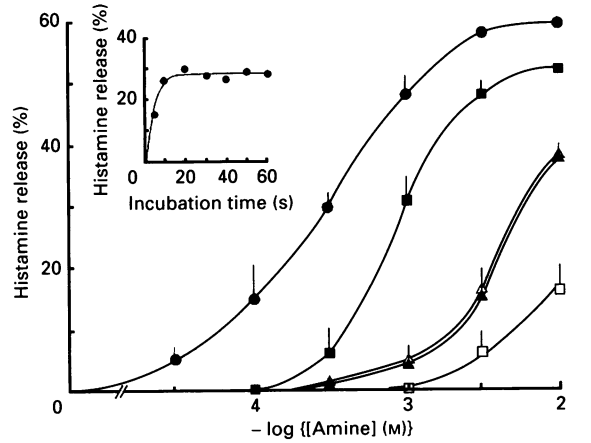

Fig. 2. Effect of polyamines on histamine release from rat peritoneal mast cells

Purified mast cells in balanced salt solution supplemented with 0.2% BSA were stimulated for 5 min at 37 °C with various concentrations of spermine (●), spermidine (■), putrescine (▲), cadaverine (△) and tyramine (□). Inset: time course for histamine secretion (ordinate) induced by 0.3 mM-spermine. Values are means ± S.E.M. of three duplicate experiments: S.E.M. values not known are included within the symbols.

of putrescine, or tyramine [4-(2-aminoethyl)phenol] induced a dose-dependent histamine release from rat peritoneal mast cells (Fig. 2). Cadaverine and putrescine evoked the same responses. The time course of histamine release induced by the polyamines is very rapid and complete within 10 s, as shown in the inset of Fig. 2 with 0.3 mM-spermine. The order of potency of the natural polyamines is the same as above (spermine > spermidine > putrescine = cadaverine > tyramine), tyramine inducing only 16.2 ± 4.3% of histamine release at 10 mM. This order of potency is proportional to the number of positively

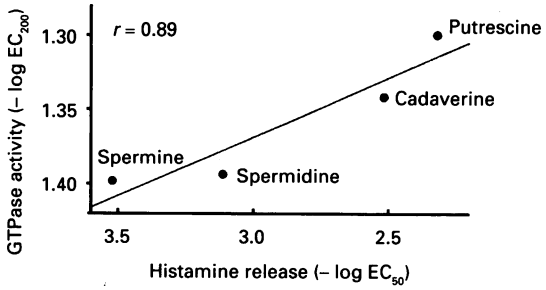

Fig. 3. Correlation between the ability of polyamines to induce histamine release and to stimulate GTPase activity

A linear correlation was calculated between the mean of the EC_{50} values (M) for the various polyamines on histamine release, and the mean of EC_{200} [concn. (M) inducing a 100% increase in basal activity] on GTPase activity.

Table 1. Contraction induced by polyamines on rat tracheal strips

Rat tracheal strips, prepared as described in the Materials and methods section, were challenged by polyamines without (control) or with pretreatment with 0.1 μM-methysergide. Values are means ± S.E.M. of six experiments: * $P < 0.01$ significantly different from control.

	Tension (mg)	
Polyamines	Control	+Methysergide (0.1 μM)
48/80 (50 μg/ml)	355 ± 85	12 ± 4*
Spermine (1 mM)	295 ± 80	15 ± 5*
Spermidine (1 mM)	108 ± 35	5 ± 4*
Putrescine (1 mm)	6 ± 5	0

charged amino groups of the various polyamines. Moreover, there is a good correlation between the EC_{50} values, the concentration of polyamines inducing 50% of histamine release and EC_{200}, the concentrations of polyamines inducing a 100% rise in the basal GTPase activity (Fig. 3).

The high concentrations of polyamines used to induce histamine release may suggest a lytic process, i.e. an energy-independent phenomenon. The cells were therefore treated for 30 min in the absence of glucose with 2-deoxy-D-glucose (5.6 mM), an anti-metabolite of glucose, and/or dinitrophenol (0.1 mM), which uncouples the synthesis of ATP in mitochondria. We showed that the mechanism of action of polyamines on mast cells is energy-dependent, since the basal histamine release (46.8 ± 9.4% of the total histamine content of the cells) was decreased by the pretreatment with 2-deoxy-D-glucose (to 40.6 ± 8.4%), and significantly inhibited by the pretreatment with dinitrophenol (23.0 ± 7.3%*) or dinitrophenol and 2-deoxy-D-glucose (8.1 ± 6.2%*).

Polyamines also induced contraction of rat tracheal smooth muscle (Table 1). At the maximum of the contraction process, we observed that the order of potency of the polyamines found on peritoneal mast cells were the same (spermine > spermidine > putrescine), putrescine being quite inactive. The contraction evoked by the synthetic polyamine compound 48/80 was similar to that induced by the natural polycations. The relationship between the contraction evoked by compound 48/80 and the stimulation of mast cells present in the rat trachea has been detailed previously (Saria *et al.*, 1984). Pretreatment of the trachea during the equilibration period with 0.1 μM of the anti-serotonergic agent methysergide (Joiner *et al.*, 1974) totally

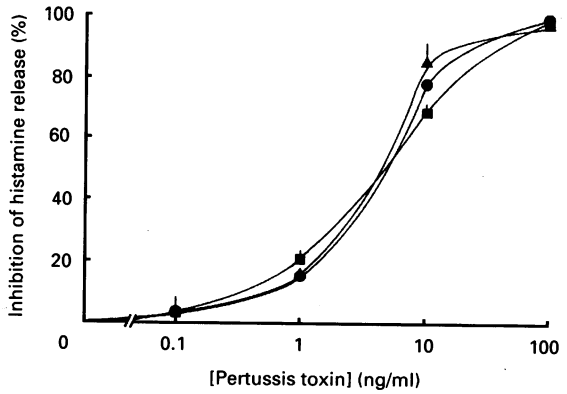

Fig. 4. Effect of pertussis toxin on mast-cell histamine release induced by polyamines

Cells were pretreated with various doses of pertussis toxin for 2 h and stimulated with 0.3 mM-spermine (●), 1 mM-spermidine (■) and 5 mM-putrescine (▲). Results are expressed as percentage inhibition compared with the corresponding controls (i.e. histamine secretion in the absence of pertussis toxin). These control values were 40.5±3.0% of the total histamine content for spermine, 43.6±7.4% for spermidine and 43.6±7.8% for putrescine. Values are means±S.E.M. of three duplicate experiments.

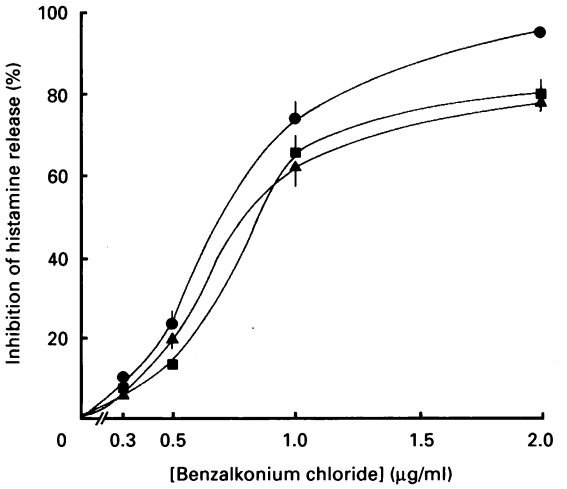

Fig. 5. Effect of benzalkonium chloride on mast-cell histamine release induced by polyamines

Cells were pretreated with various doses of benzalkonium chloride for 5 min and stimulated with 0.3 mM-spermine (●), 1 mM-spermidine (■) and 5 mM-putrescine (▲). Results are expressed as percentage inhibition compared with the corresponding controls (i.e. histamine secretion in the absence of benzalkonium chloride). These control values were 37.1±2.3% of the total histamine content for spermine, 39.1±2.8% for spermidine and 37.9±2.7% for putrescine. Values are means±S.E.M. of three duplicate experiments.

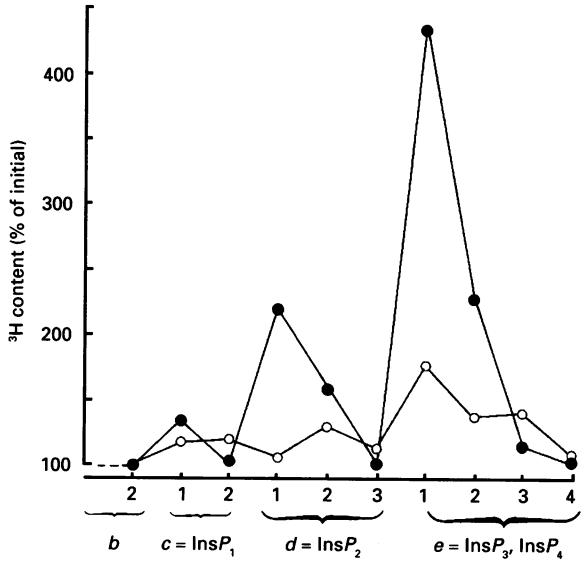

Fig. 6. Generation of [^{3}H]inositol phosphates in response to spermine: elution profiles of pertussis-toxin-treated (○) and control (●) mast cells

Purified rat peritoneal mast cells were labelled with [^{3}H]inositol and treated or not with 300 ng of pertussis toxin/ml for 2 h. The cells were then stimulated for 5 s with 10 mM-spermine. The ^{3}H contents of the eluted fractions *c*, *d*, and *e* (see the Materials and methods section) corresponding to inositol 1-monophosphate (InsP_1), inositol bisphosphates (InsP_2) and inositol tris- and tetrakis-phosphates (InsP_3, InsP_4) respectively are shown as percentages of initial (zero-time) values obtained immediately before the addition of spermine. Data shown are representative of four experiments. The initial values (d.p.m./1.5 × 10^6 cells) for control and pertussis-toxin-treated cells respectively were: InsP_1, 768, 683; InsP_2, 281, 231; InsP_3, 77, 64.

abolished the responses induced by the synthetic and natural polyamines (Table 1). This demonstrates the involvement of mast-cell stimulation, if one considers 5-hydroxytryptamine as the main active mediator of rat tracheal mast cells.

We reported recently that histamine release from rat peritoneal mast cells induced by polycationic amphiphilic peptides or compound 48/80 involves the activation of a G_i-like protein (Bueb *et al.*, 1990; Mousli *et al.*, 1990*a*,*b*,*c*). Fig. 4 shows that pretreatment of mast cells for 2 h with 100 ng of pertussis toxin/ml also totally inhibited the secretion induced by the positively charged polyamines. The concentrations of polyamines were chosen to give the same basal secretion. The dose-dependent effect of pertussis toxin was similar for all polyamines, clearly indicating that a pertussis-toxin-sensitive G-protein is involved in the exocytotic process induced by these polyamines. In the presence of benzalkonium chloride (0.1–3 μg/ml), we observed a dose-dependent inhibition of the histamine release induced by the various polyamines (Fig. 5). Concentrations higher than 3 μg/ml were toxic for rat peritoneal mast cells (J. L. Bueb, unpublished work; Read & Keifer, 1979), inducing a lytic histamine release. Benzalkonium chloride is a cationic surface agent, used as a topical antiseptic, and described by Read & Kiefer (1979) to be an inhibitor of histamine release induced on rat peritoneal mast cells by compound 48/80. More recently, however, benzalkonium chloride was shown to have an inhibitory effect on purified G_i proteins (Higashijima *et al.*, 1990).

Incubation of [^{3}H]inositol-labelled rat peritoneal mast cells with polyamines induced a transient increase in inositol phosphates. This increase was maximal within 5 s. Fig. 6 shows the elution profile of the supernatant of mast cells, which have been stimulated for 5 s with 10 mM-spermine: generation of inositol 1-monophosphate, inositol bisphosphates and inositol triphosphates and/or tetrakisphosphates was significantly raised above the 100% basal level. The spermine-induced increase in the metabolism of phosphoinositides was particularly evident for inositol bisphosphates and inositol tris- and/or tetrakis-phosphates. Fig. 6 also shows that the prior exposure of the purified mast cells to pertussis toxin for 2 h inhibited the increase in inositol phosphates elicited by spermine. This generation of

inositol phosphates within 5 s coincided with rapid release of histamine by mast cells in the presence of the polyamines (Fig. 2).

DISCUSSION

We show here that millimolar levels (0.1–10 mM) of polyamines are able to stimulate purified G-proteins. The concentrations required are rather high, but are in agreement with numerous reports concerning other systems (Sacaan & Johnson, 1989; Walz & Kitareewan, 1990; Charlton & Baylis, 1990; Williams *et al.*, 1991). Intracellular millimolar concentrations have been reported in pancreatic β-cells (Welsh, 1990). Polyamines are also found in high concentration in the central nervous system, with regional variations (Williams *et al.*, 1991). However, the physiological relevance of the effect of polyamines on G-proteins *in vitro* had to be assessed. Mast cells offered a valuable model for this, in view of their sensitivity to the synthetic polyamine compound 48/80, demonstrated to act directly on G-proteins (Mousli *et al.*, 1990*a*,*b*,*c*; Aridor *et al.*, 1990; Tomita *et al.*, 1991). We observed that natural polyamines, applied exogenously on purified peritoneal mast cells, induce an energy-dependent histamine release. The concentration of polyamine required to evoke histamine release is similar to that activating purified G-proteins, the stimulation of the two systems being well correlated. To strengthen this observation, done with isolated rat mast cells, we show that polyamines also induce the contraction of isolated rat trachea. This tissue does not respond to histamine, but responds to 5-hydroxytryptamine, which is also released by rat mast cells. We observed that pretreatment of peritoneal mast cells with pertussis toxin inhibited the secretory response to polyamines as well as the elicited increase in inositol polyphosphates. This demonstrates that polyamines activate phospholipase C through pertussis-toxin-sensitive G-proteins. The target of polyamines could have been the phospholipase C itself, as described for other cell types (for review, see Schuber, 1989), but in this case pertussis toxin would not have had any inhibitory effect. Thus, considering the effects on purified G-proteins, we can reasonably propose that polyamines act directly on these targets in mast cells. The inhibitory effect of benzalkonium chloride shows that these G-proteins belong to the G_i-type, in view of the selective effect of this drug on purified G_i-proteins (Higashijima *et al.*, 1990) and the molecular mass of mast-cell G-protein α-subunits ADP-ribosylated by pertussis toxin (Bueb *et al.*, 1990).

We previously showed that the synthetic polyamine compound 48/80 interacts with the *C*-terminus of the α-subunit of G-proteins (Mousli *et al.*, 1990*c*). This locus also interacts with the venom peptide mastoparan and the neuropeptide substance P (Mousli *et al.*, 1990*a*; Tomita *et al.*, 1991), as well as with intracellular parts of the seven helices receptors, as first proposed by Higashijima *et al.* (1988). Natural polyamines are the only intracellular compounds which might play a direct regulatory role in the interaction of G-proteins with receptors. The intracellular level of polyamines is highly regulated, through the activity of ornithine decarboxylase. This regulation may maintain a level of polyamine below that required for interfering with G-proteins, but a regulatory role of intracellular polyamines cannot be excluded, in view of their ability to reach high intracellular levels, as shown in pancreatic β-cells (Welsh, 1990). Moreover, different pathological situations have been described to be associated with a large increase in polyamines (Paschen *et al.*, 1988; Dempsey *et al.*, 1988; Löser *et al.*, 1989; Bowlin *et al.*, 1990). Thus we propose that polyamines, besides their modulatory role of *N*-methyl-D-aspartase receptor (Williams *et al.*, 1991) and their putative role in the regulation of intracellular Ca^{2+} (Koenig *et al.*, 1983; Fan & Koenig, 1988; McCormack, 1989), might also control the basel level of other second messengers by activating G-proteins. This might also modulate extracellular receptor-dependent signals.

This work was supported by the INSERM (CRE 89–5009 and CJF 91–05) and by the Fondation pour la Recherche Médicale.

REFERENCES

Aridor, M., Traub, L. M. & Sagi-Eisenberg, R. (1990) J. Cell. Biol. **111**, 909–917

Berridge, M. J., Dawson, M. C., Downes, C. P., Heslop, J. P. & Irvine, R. F. (1983) Biochem. J. **212**, 473–482

Bowlin, T. L., Hoeper, B. J. Rosenberger, A. L., Davis, G. F. & Sunkara, P. S. (1990) Cancer Res. **50**, 4510–4514

Brabet, P., Pantaloni, C., Rouot, B., Toutant, M., Garcia-Sainz, A., Bockaert, J. & Homburger, V. (1988) Biochem. Biophys. Res. Commun. **152**, 1185–1192

Brandt, D. R., Asano, T., Pedersen, S. E. & Ross, E. M. (1983) Biochemistry **22**, 4357–4362

Bueb, J.-L., Mousli, M., Bronner, C., Rouot, B. & Landry, Y. (1990) Mol. Phamacol. **38**, 816–822

Charlton, J. A. & Baylis, P. H. (1990) J. Endocrinol. **127**, 377–382

Clo, C., Cardarera, C. M., Tantini, B., Benalal, D. & Bachrach, U. (1979) Biochem. J. **182**, 641–649

Clo, C., Tantini, B., Piganatti, C., Marmiroli, S. & Caldarera, C. M. (1988) in Perspectives in Polyamine Research (Perin, A., Scalabrino, G., Sessa, A. & Ferioli, M. E., eds.), pp. 45–48, Wichtig Editore, Milano

Cochet, C. & Chambaz, E. M. (1983) Mol. Cell. Endocrinol. **30**, 247–266

Dempsey, R. J., Maley, B. E., Cowen, D. & Olson, J. W. (1988) J. Cereb. Blood Flow Metab. **8**, 843–847

Fan, C. C. & Koenig, H. (1988) J. Mol. Cell. Cardiol. **20**, 789–799

Frossard, N. & Muller, F. (1986) J. Appl. Physiol. **61**, 1449–1456

Higashijima, T., Ferguson, K. M., Smigel, M. D. & Gilman, A. G. (1987) J. Biol. Chem. **262**, 757–761

Higashijima, T., Uzu, S., Nakajima, T. & Ross, E. M. (1988) J. Biol. Chem. **263**, 6491–6494

Higashijima, T., Burnier, J. & Ross, E. M. (1990) J. Biol. Chem. **265**, 14176–14186

Homburger, V., Brabet, P., Audigier, Y., Pantaloni, C., Bockaert, J. & Rouot, B. (1987) Mol. Pharmacol. **31**, 313–319

Joiner, P. D., Wall, M., Davis, L. B. & Hahn, F. (1974) J. Allergy Clin. Immunol. **53**, 261–270

Khan, N. A., Quemener, V. & Moulinoux, J.-Ph. (1990) Life Sci. **46**, 43–47

Koenig, H., Goldstone, A. & Lu, C. Y. (1983) Nature (London) **305**, 530–534

Löser, C., Fölsch, U. R., Paprotny, C. & Creutzfeldt, W. (1989) Scand. J. Gastroenterol. **24**, 1193–1199

McCormack, J. G. (1989) Biochem. J. **264**, 167–174

Mousli, M., Bronner, C., Bueb, J.-L., Tschirhart, E., Gies, J.-P. & Landry, Y. (1989) J. Pharmacol. Exp. Ther. **250**, 329–335

Mousli, M., Bronner, C., Landry, Y., Bockaert, J. & Rouot, B. (1990*a*) FEBS Lett. **269**, 260–262

Mousli, M., Bueb, J.-L., Bronner, C., Rouot, B. & Landry, Y. (1990*b*) Trends Pharmacol. Sci. **11**, 358–362

Mousli, M., Bronner, C., Bockaert, J., Rouot, B. & Landry, Y. (1990*c*) Immunol. Lett. **25**, 355–358

Nakamura, T. & Ui, M. (1985) J. Biol. Chem. **260**, 3584–3593

Paschen, W., Röhn, G., Meese, C. O., Djuricic, B. & Schmidt-Kastner, R. (1988) Brain Res. **453**, 9–16

Pegg, A. E. & McCann, P. P. (1982) Am. J. Physiol. **243**, C212–C221

Read, G. W. & Kiefer, E. F. (1979) J. Pharmacol. Exp. Ther. **211**, 711–715

Sacaan, A. I. & Johnson, K. M. (1989) Mol. Pharmacol. **36**, 836–839

Saria, A., Hua, X., Skofitsch, G. & Lundberg, J. M. (1984) Naunyn-Schmiedebergs Arch. Pharmacol. **328**, 9–15

Schuber, F. (1989) Biochem. J. **260**, 1–10

Seiler, N. (1990) Digestion **46**, 319–330

Shore, P. A., Burkhalter, A. & Cohn, V. (1959) J. Pharmacol. Exp. Ther. **127**, 182–186

Sternweis, P. C. (1986) J. Biol. Chem. **261**, 631–637

Sternweis, P. C. & Robishaw, J. D. (1984) J. Biol. Chem. **259**, 13806–13813

Tabor, C. W. & Tabor, H. (1984) Annu. Rev. Biochem. **53**, 749–790

Tabor, C. W. & Tabor, H. (1985) Microbiol. Rev. **49**, 81–99
Tomita, U., Inanobe, A., Kobayashi, I., Takahashi, K., Ui, K. & Katada, T. (1991) J. Biochem. (Tokyo) **109**, 184–189
Walz, F. G. & Kitareewan, S. (1990) J. Biol. Chem. **265**, 7127–7137
Wang, J.-Y. & Johnson, L. R. (1990) Am. J. Physiol. **259**, G584–G592
Welsh, N. (1990) Biochem. J. **271**, 393–397
Williams, K., Romano, C., Dichter, M. A. & Molinoff, P. B. (1991) Life Sci. **48**, 469–498

Received 20 May 1991/10 October 1991; accepted 16 October 1991