ELSEVIER

European Journal of Pharmacology 352 (1998) 343–350

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Pituitary adenylate cyclase activating polypeptide induces multiple signaling pathways in rat peritoneal mast cells

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Received 29 January 1998; revised 7 May 1998; accepted 12 May 1998

Abstract

Pituitary adenylate cyclase activating polypeptide (PACAP) is a high-affinity ligand for at least two types of G-protein coupled receptors, the PACAP type 1 and type 2 receptor. In this study it is demonstrated that the C-terminal PACAP-fragment PACAP(6–27) stimulates serotonin release from rat peritoneal mast cells with higher potency (EC_{50}: 0.2 vs. 2.0 μM) than the PACAP receptor ligand PACAP(1–27). PACAP-induced degranulation of rat peritoneal mast cells was abolished by pertussis toxin and by benzalkonium chloride (IC_{50}: 9.1 μg/ml) indicating the involvement of heterotrimeric G-proteins of the G_i-type. The PACAP effect was also reduced by inhibitors of the phosphatidylinositol specific phospholipase C ((U73122), IC_{50}: 4 μM; (ET-18-O-CH_3), IC_{50}: 18 μM), by D609, a specific inhibitor of the phosphatidylcholine specific phospholipase C (IC_{50}: 41 μM), by the protein kinase C-inhibitor staurosporine (IC_{50}: 0.6 μM) and by the lipoxygenase inhibitor nordihydroguaiaretic acid (NGDA) but not by indomethacin. It is concluded that PACAP peptides stimulate secretion in rat peritoneal mast cells in a PACAP receptor-independent manner, probably via direct activation of heterotrimeric G-proteins of the G_i-type; these G-proteins may lead to a sequential activation of different signaling cascades (see above), which may converge at the level of one or more staurosporine-sensitive protein kinase.

Keywords: Mast cell; G-protein; Phospholipase C; PACAP (pituitary adenylate cyclase activating polypeptide); Benzalkonium chloride; Pertussis toxin; U73122; ET-18-OCH_3; D609; Nordihydroguaiaretic acid; Staurosporine

1. Introduction

The neuropeptide PACAP (pituitary adenylate cyclase activating peptide) exists in two molecular forms, PACAP(1–27) and PACAP(1–38), which have been found throughout the peripheral and central nervous system (Arimura et al., 1991). Two types of high-affinity PACAP receptors (type 1 and type 2) were initially characterized by their ability to recognize PACAP and/or vasoactive intestinal peptide in the nanomolar range (Buscail et al., 1990). These receptors were consecutively cloned and identified as members of the large superfamily of G-protein coupled receptors with seven transmembrane domains (Spengler et al., 1993; Harmar and Lutz, 1994). Structure–activity studies revealed that the N-termini of PACAP peptides are essential for their specific interaction with these high affinity receptors (Schäfer et al., 1991; Schmidt et al., 1993). Recently, Mori et al. (1994) demonstrated that PACAP(1–27) and PACAP(1–38) but also the N-terminally truncated PACAP-fragment PACAP(16–38) stimulated histamine release from rat peritoneal mast cells with low potency but high efficacy. This effect is appar-

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0014-2999/98/$19.00

ently not mediated via a high-affinity PACAP receptor, but may result from a direct activation of heterotrimeric G-proteins.

Comparable high affinity receptor-independent atypical responses in peritoneal mast cells have been documented for a number of other neuropeptides and peptide hormones, e.g., substance P, neuropeptide Y, kinins and others (Lagunoff and Martin, 1983; Mousli et al., 1990; Higashijima et al., 1990). The structural feature that correlates best with the ability of these peptides to induce degranulation in rat peritoneal mast cells is the number of positively charged amino acid residues. The observation that PACAP(1–38) (10 basic amino acid residues) is more potent than (PACAP(16–38) (eight basic amino acid residues), which is more potent than PACAP(1–27) (four basic amino acid residues) therefore fits well into this concept. Other factors like α-helicity and amphipathicity seem to be of lower importance (Cross et al., 1995). The mechanism by which these basic molecules exert their biological action in rat peritoneal mast cells involves a direct activation of at least one pertussis toxin (ptx)- and benzalkonium chloride (BAC)-sensitive, heterotrimeric G-protein of the G_i-type, that is positively coupled to the inositide specific phospholipase C signal transduction cascade (Nakamura and Ui, 1985; Mousli et al., 1989; Higashijima et al., 1990; Bueb et al., 1990; Fischer et al., 1993). The functional significance of this signaling cascade for exocytosis was supported by the finding that an introduction of the phospholipase C inhibitor neomycin into the cytosol of rat peritoneal mast cells via a patch-pipette or via ATP-induced membrane perforations completely prevents exocytosis stimulated by the basic non-peptide molecule compound 48/80 (Penner, 1988; Aridor and Sagi-Eisenberg, 1990). However, suppression of the lipoxygenase signal transduction pathway by the lipoxygenase-inhibitor nordihydroguaiaretic acid (NGDA) has also been shown to completely prevent the compound 48/80 induced exocytotic response in rat peritoneal mast cells (Kuno et al., 1993). The sequence of activation of both signaling cascades is currently unknown. In contrast to the immunoglobulin E (IgE)-induced exocytotic response in mast cells, which has been shown to involve the phospholipase D but not the phosphatidylcholine specific phosholipase C signaling cascade (Gruchalla et al., 1990; Dinh and Kennerly, 1991), the role these enzymes play within the peptidergic pathway of mast cell activation is yet not clear.

Therefore, the aim of the present study was to assess the role of the above mentioned signaling cascades in the secretory response of rat peritoneal mast cells when challenged with the basic peptide PACAP(1–27). To achieve this, two G-protein inhibitors (ptx and BAC) and various cell-permeant signal-transduction inhibitors for the phosphatidylinositol-specific phospholipases C (U73122, ET-18-O-CH_3), the phosphatidylcholine-specific phospholipase C (D609), the lipoxygenase (nordihydroguaiaretic acid) and the protein kinase C (staurosporine) were tested for their ability to influence PACAP(1–27)-induced [^{3}H]serotonin-release in rat peritoneal mast cells.

2. Materials and methods

2.1. Preparation of rat peritoneal mast cells

Rat peritoneal mast cells were obtained by peritoneal lavage and purified using a bovine serum albumin gradient (0.2/40% (wt./vol.)) centrifugation procedure as recently described (Mousli et al., 1989). This method yields mast cells at an average purity of more than 90%. In general, lavages from three rats were combined for one experiment consisting of 20–40 samples. The buffer used for lavage and secretion experiments contained 0.2% bovine serum albumin (γ-globulin and protease-free; Sigma A-3059) and [mM]: 137 NaCl, 2,7 KCl, 0,3 $CaCl_2$, 1 $MgCl_2$, 0.4 NaH_2PO_4, 10 HEPES and 5.6 glucose. Mast cells could easily be identified by their characteristic small nucleus/plasma-relation and their highly contrasted, granule-filled three dimensional appearance when observed by phase-contrast microscopy. These features were enhanced by Alcian blue staining. For this purpose, cells were fixed on cover slips by addition of ice cold methanol for at least 20 min. Subsequently, cover slips were transferred into 1% acetic acid for another 10 min. Alcian blue staining was carried out using a 1% aqueous solution. After a brief wash in water, cells were transferred to 1% alkaline ethanol for 10 min (colour fixation). Finally, cells were counterstained with 1% aqueous Kernechtrot, dehydrated and mounted.

2.2. Secretion experiments

Purified mast cells were incubated for 2 h at 37°C with 1 μCi/ml hydroxytryptamine creatine sulfate,5-[1,2-^{3}H(N)] (Serotonin). Subsequently, cells were washed by centrifugation (5 min, 4°C, $100 \times g$) and resuspended in buffer (final volume: 300 μl/vial). Secretion was usually induced by incubating the cells for 10 min in the presence 5 μM PACAP(1–27). The different signal-transduction inhibitors were preincubated for the time periods indicated. Incubations were stopped on ice followed by centrifugation. The activity of tracer released into the supernatant divided by the total tracer activity in each individual sample was taken as a measure of secretion. All concentrations were determined in duplicate. Only experiments with a basal release of [^{3}H]5-HT below 5 (percent of total) were used. Results are given as means $\pm$ S.E.M. Statistical evaluations were made by means of the Wilcoxon test for matched pairs ($* P < 0.05$, $** P < 0.005$).

D609 (tricyclodecan-9-yl xanthate potassium), ET-18-OCH_3 (1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphorylcholine), U73122 (1-6-(17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1*H*-pyrrole-2,5-dione)

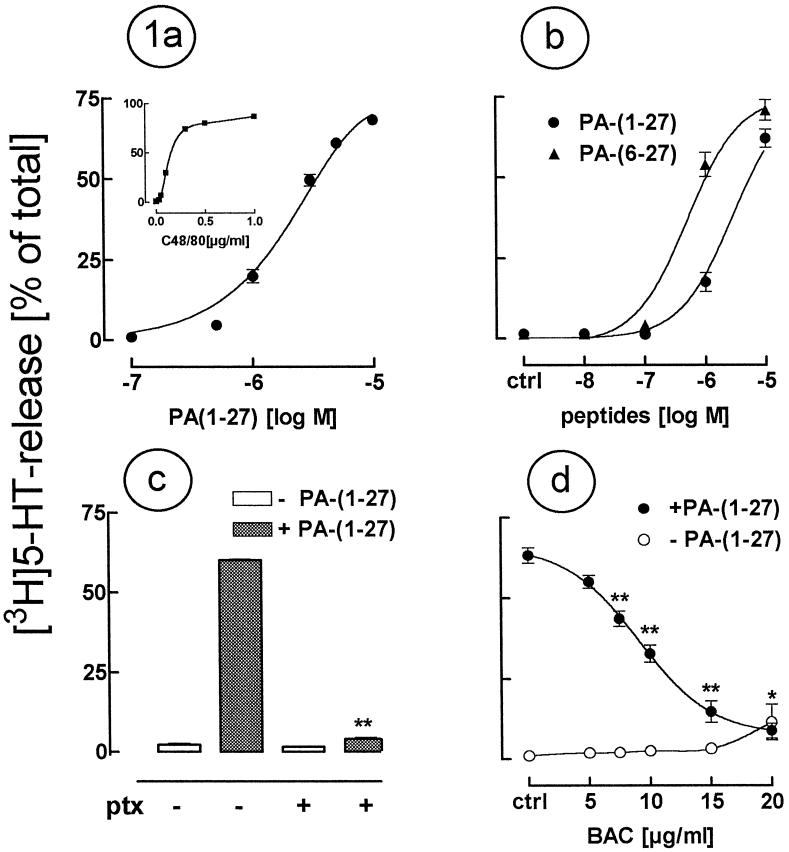

Fig. 1. Effects of different secretagogues or inhibitors on [^{3}H]serotonin-release in rat peritoneal mast cells (mean ± S.E.M.): (a) Single representative experiment, comparing the dose–response relationship of compound 48/80 (inset) and PACAP(1–27). (b) Dose–response curves of PACAP(1–27) and PACAP(6–27) ($n = 4$). (c) Preincubation of rat peritoneal mast cells with 1 µg/ml ptx for 120 min ($n = 8$; $**$ $P \leq 0.005$). (d) Effect of benzalkonium chloride on 5 µM PACAP(1–27) induced [^{3}H]serotonin-release in rat peritoneal mast cells ($n = 8$).

and U73343 were obtained from Biomol (Hamburg, Germany). Staurosporine, benzalkonium chloride (B6295) and pertussis toxin were purchased from Sigma (Deisenhofen, Germany). PACAP(1–27) and PACAP(6–27) were from Bachem (Heidelberg, Germany).

3. Results

3.1. Secretory effect of PACAP(1–27), PACAP(6–27) and compound 48 / 80 in RPMCs

PACAP(1–27) (10^{-7}–10^{-5} M) induced a dose-dependent release of [^{3}H]5-HT (EC_{50}: 2×10^{-6} M, basal: 1.1 ± 0.8, maximum: 72 ± 2.8% of total, Fig. 1a) in rat peritoneal mast cells. Compound 48/80 (0.03–1 µg/ml) stimulated [^{3}H]5-HT-release dose-dependently, the maximal effect was 86.7% of total. Compared to PACAP(1–27), PACAP(6–27) induced secretion with a slightly higher potency (EC_{50}: 0.2 µM; $n = 4$; Fig. 1b). When incubated at 37°C the secretory response of peritoneal mast cells challenged with 5 µM PACAP(1–27) reached a maximum after 10 s of incubation. At 22°C [^{3}H]serotonin-release reached a maximum within 60 s ($n = 2$; data not shown).

3.2. Effects of different signal-transduction inhibitors on PACAP-induced secretion

3.2.1. Pertussis toxin

Preincubation (120 min) of rat peritoneal mast cells with 1 µg/ml ptx significantly ($P < 0.004$) reduced the secretory response to 5 µM PACAP(1–27) (PA(1–27): 58.4 ± 3.0, basal: 1.7 ± 0.2, PA(1–27) + ptx: 4.2 ± 0.2, ptx control: 2.1 ± 0.3; $n = 8$; Fig. 1c; Table 1).

Table 1
Effects of different signal-transduction inhibitors on 5 µM PACAP(1–27)-induced [^{3}H]serotonin release in rat peritoneal mast cells

Substance	Site of action	IC_{50}	Inhibition	n
Pertussis toxin	G-proteins	–	yes	8
Benzalkonium chloride	G-proteins (G_i / G_o)	9.1 µg/ml	yes	8
U73122	PI-PLC	4×10^{-6} M	yes	4
ET-18-O-CH_3	PI-PLC	1.8×10^{-5} M	yes	4
D609	PC-PLC/PLD	4.1×10^{-5} M	yes	4
Staurosporine	PKC	6×10^{-7} M	yes	4
Indomethacin	Cyclooxygenase	–	no	4
NGDA	Lipoxygenase	–	yes	4

3.2.2. Benzalkonium chloride (BAC_{12})

The secretory response induced by 5 μM PACAP(1–27) could dose-dependently be lowered (IC_{50}: 9.1 μg/ml) by preincubation (10 min) with the alkylamine, benzalkonium chloride (BAC_{12}); $n = 8$; Fig. 1d; Table 1. At concentrations above 15 μg/ml benzalkonium chloride by itself stimulated the release of [^{3}H]serotonin.

3.2.3. U73122, U73343

Preincubation of rat peritoneal mast cells for 10 min with the phospholipase C inhibitor U73122 dose-dependently reduced the secretory effect of 5 μM PACAP(1–27) with an IC_{50} of 4 μM ($n = 4$). Maximal inhibition occurred at 10 μM U73122 (PA-(1–27): 53.3 $\pm$ 8.5; PA-(1–27) + 10 μM U73122: 10.9 $\pm$ 5.5). At concentrations above 30 μM U73122 by itself stimulated the release of [^{3}H]5-HT (Fig. 2a). U73343, a derivative of U73122, showed a similar pattern of action. At concentrations up to 5×10^{-5} M U73343 dose-dependently reduced the secretory effect of 5 μM PA-(1–27) (IC_{50}: 20 μM; $n = 4$), at higher concentrations U73343 stimulated degranulation of rat peritoneal mast cells (Fig. 2b; Table 1). Stimulation of rat peritoneal mast cells with 10^{-4} M U73122 or U73343, respectively, showed an effect-maximum after an incubation time (37°C) of 5 min (data not shown).

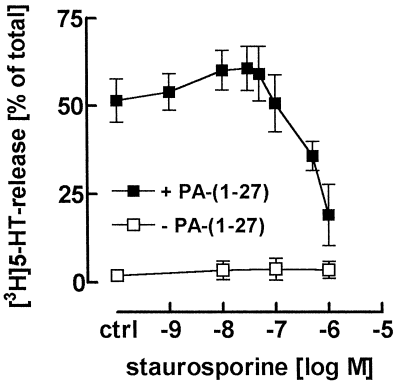

Fig. 3. Dose-dependent effect of staurosporine (20 min preincubation time) on 5 μM PACAP(1–27)-induced [^{3}H]serotonin-release (mean $\pm$ S.E.M.) in rat peritoneal mast cells ($n = 4$).

3.2.4. ET-18-OCH_3

Preincubation of rat peritoneal mast cells for 20 min with the ether lipid ET-18-OCH_3 dose-dependently re-

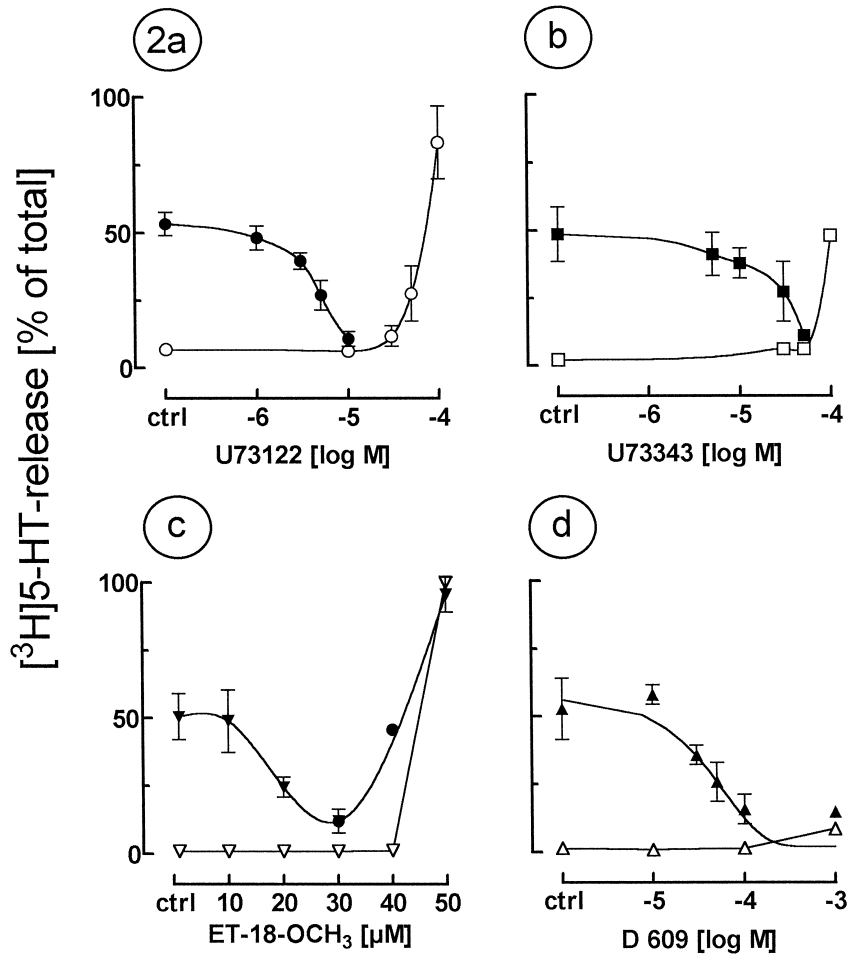

Fig. 2. Effects of different phospholipase inhibitors on unstimulated (open symbols) and 5 μM PACAP(1–27) stimulated (closed symbols) [^{3}H]serotonin-release in rat peritoneal mast cells (mean $\pm$ S.E.M.). Each plot represent four independent experiments ($n = 4$).

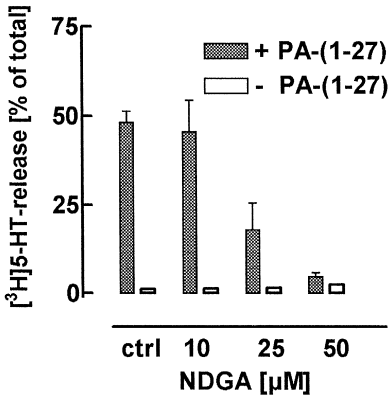

Fig. 4. Effect of NGDA on 5 μM PACAP(1–27) induced [^{3}H]serotonin release in rat peritoneal mast cells ($n = 4$).

duced the secretory response when challenged with 5 μM PACAP(1–27) (IC_{50}: 18 μM; $n = 4$; Fig. 2c; Table 1). At concentrations above 40 μM the phospholipase C-inhibitor by itself stimulated the release of [^{3}H]5-HT.

3.2.5. D609

D609, a selective inhibitor of phosphatidylcholine-specific phospholipase C exerted dose-dependently an inhibitory influence on PACAP-induced degranulation of rat peritoneal mast cells (preincubation time: 20 min; IC_{50}: 4.1×10^{-5} M or 10.9 μg/ml; $n = 4$; Fig. 2d; Table 1).

3.2.6. Staurosporine

Preincubation of rat mast cells for 20 min with staurosporine dose-dependently inhibited PACAP-induced degranulation (IC_{50}: 6×10^{-7} M; $n = 4$; Fig. 3; Table 1). The highest concentration of staurosporine tested (1 μM) reduced the effect of 5μM PA(1–27) (51 ± 3.1) to a value of 19.1 ± 4.3. Low concentrations of staurosporine (1.0×10^{-8} M) lead to a small, insignificant augmentation of the secretory PACAP effect (PA(1–27) + 10 nM staurosporine: 60.1 ± 2.8, staurosporine control: 3.5 ± 1.9).

3.2.7. NGDA, indomethacin

Preincubation of rat peritoneal mast cells for 60 min with NGDA (10–50 μM) significantly reduced the PACAP-response in a dose-dependent manner ($n = 4$; Fig. 4; Table 1). In the same series of experiments preincubation of mast cells (60 min) with 10^{-4} M indomethacin showed no effect on PACAP-stimulated tracer-release (PACAP(1–27) control: 48.2 ± 3.2, PACAP(1–27) + indomethacin: 38.1 ± 3.8).

4. Discussion

In the present investigation we have confirmed a recent observation by Mori et al. (1994) that some N-terminally truncated PACAP-fragments (PACAP(6–27) or PACAP(16–38)) stimulate secretion in rat peritoneal mast cells with higher potency and a similar efficacy than the naturally occurring PACAP-peptides (PACAP(1–27) and PACAP(1–38)). This finding indicates a high-affinity PACAP receptor-independent mechanism of action. Similar atypical high affinity receptor-independent effects have been observed in rat peritoneal mast cells with a number of other basic peptide hormones, neuropeptides and basic random peptides (Lagunoff and Martin, 1983; Repke et al., 1987; Mousli et al., 1995; Cross et al., 1995). Basic peptides, as well as some basic non-peptide molecules (e.g., compound 48/80), seem to directly activate al least one ptx-sensitive heterotrimeric G-protein of the G_i-type, which is coupled to the phosphatidylinositol-specific phospholipase C signaling cascade (Higashijima et al., 1990; Bueb et al., 1990; Fischer et al., 1993). G_i-proteins have been demonstrated to be specifically inhibited by a number of different alkylamines like benzalkonium chloride (Read and Kiefer, 1979; Higashijima et al., 1990; Fischer et al., 1993). In line with these observations we demonstrated that the PACAP-induced secretory response of rat peritoneal mast cells could be inhibited by preincubation with ptx and BAC. If the main effects of ptx and BAC on the peptidergic pathway of mast cell activation are due to the inhibition of a phospholipase C-coupled G_i protein or a phospholipase C-independent $G\alpha_{i3}$ which is localized more downstream in the signaling cascade is currently a matter of controversy (Lindau and Nüße, 1987; Fischer et al., 1993; Aridor et al., 1993).

The impact of the phosphatidylinositol-specific phospholipase C and the phosphatidylcholine-specific phospholipases C and D in the exocytotic response of intact rat peritoneal mast cells, when challenged by basic peptides, is yet not fully understood. Therefore, we assessed the influence of different cell-permeant inhibitors of these enzymes (U73122, ET-18-OCH_3 and D609) on PACAP(1–27)-induced serotonin-release in rat peritoneal mast cells.

4.1. U73122, U73343 and ET-18-OCH_3

It was demonstrated in two preliminary reports (Smith et al., 1990; Bleasdale et al., 1990) that the aminosteroid U73122 acts as a cell-permeant, rapidly acting (within seconds) and selective inhibitor of phosphatidylinositol-specific phospholipase C in polymorphenuclear neutrophils, while its close derivative U73343 was ineffective, and therefore recommended as a negative control. In a number of studies, the inhibitory effect of U73122 on the phosphatidylinositol-specific phospholipase C in different cell types was confirmed showing most often a maximum inhibition (IC_{100}) at 1–10 μM, while the IC_{50} values were in a range between 0.06–5 μM. Furthermore, a number of phospholipase C-independent effects were observed for U73122 and also for U73343 (Mogami et al., 1997; Muto et al., 1997).

The ether lipid ET-18-OCH_3 has been shown to effectively inhibit the phosphatidylinositol-specific phospholipase C and to exert only a weak inhibitory effect on the phosphatidylcholine-specific phospholipases C and D. Unfortunately, a large number of other phospholipase C-independent effects have also been reported for this compound (Heesbeen et al., 1991). In this study we observed a dose-dependent inhibition of PACAP(1–27) induced [^{3}H]serotonin release by U73122 showing a maximum effect at 10 μM. The IC_{50} value of 4 μM was in the same range as recently described for the inhibition on platelet aggregation (1–5 μM) by U73122 (Bleasdale et al., 1990). U73343 also inhibited the response of PACAP(1–27) but with a 5-fold lower potency. At concentrations above 10 μM both inhibitors themselves increased the extracellular concentration of [^{3}H]serotonin. A similar dual response pattern was observed for ET-18-OCH_3, which reduced the PACAP-response only at concentrations below 30 μM. The IC_{50}-value (17.7 μM) obtained in this study for inhibition of PACAP(1–27)-induced [^{3}H]serotonin release by ET-18-OCH_3 was in a comparable range as previously described (Stella et al., 1997) for phospholipase C-inhibition in cortical neurons (IC_{50}: 5.0 μM). The fact that U73122 and ET-18-OCH_3 reduced the PACAP-induced secretion, as well as the finding that U73122 showed a relative higher potency than its inactive analogue U73343 can be taken as indirect evidence, that the phosphatidylinositol-specific phospholipase C associated signaling cascade is involved in the stimulus secretion coupling of basic peptides in rat peritoneal mast cells. The mechanism that leads to an increased extracellular concentration of serotonin when higher concentrations of all three phospholipase inhibitors were used was not further investigated in this study; a possible explanation for this phenomenon may be an inhibition of amine-uptake transporters (Purcell et al., 1989).

4.2. D609

Tricyclodecan-9-ylxanthogenate (D609) has been shown to directly inhibit the enzymatic activity of the phosphatidylcholine-specific phospholipase C without affecting the enzymatic activity of other phospholipases like phospholipase A2, phospholipase D or the phosphatidylinositol-specific phospholipase C (Schütze et al., 1992; Amtmann, 1996). Recently, Kiss and Tomono (1995) reported that D609 exerted an inhibitory influence on phospholipase D-activity in fibroblasts; but in this study indirect effects due to activity of the phosphatidylcholine-specific phospholipase C have not been excluded. In a further study Powis et al. (1991) demonstrated that D609 exerts an inhibitory influence on the activity of purified phosphatidylinositol-specific phospholipase C only at concentrations higher than 10^{-4} M. Thus D609, in concentrations below 100 μM, can be considered as an selective inhibitor of phosphatidylcholine-specific phospholipases.

It has been demonstrated in previous studies that the phospholipase D-signaling pathway plays an important role in the immunoglobulin E (IgE)-induced exocytotic response of rat peritoneal mast cells (Gruchalla et al., 1990; Yamada et al., 1991; Ishimoto et al., 1996); in contrast the phosphatidylcholine-specific phospholipase C does not seem to be involved in this signaling cascade (Dinh and Kennerly, 1991). In the present study preincubation of rat peritoneal mast cells with D609 dose-dependently reduced the PACAP(1–27)-induced release of [^{3}H]serotonin with an IC_{50} of 4.1×10^{-5} M (10.1 μg/ml). A maximal inhibition was observed at 10^{-4} M ($\approx$ 27 μg/ml). These data are in good agreement with the recently reported inhibitory effects of D609 on TNFα-induced activation of the phosphatidylcholine-specific phospholipase C in jurkat cells ($IC_{100} \approx 30$ μg/ml) and on phospholipase D-activity ($IC_{100} = 50$ μg/ml) in fibroblasts (Schütze et al., 1992; Kiss and Tomono, 1995). At concentrations above 10^{-4} M D609 itself induced a small insignificant, but discernible release of [^{3}H]serotonin. We conclude, that an activation of phospholipase D and/or phosphatidylcholine-specific phospholipase C may contribute to PACAP-induced signaling-processes in rat peritoneal mast cells.

4.3. Staurosporine

Diacylglyceroles are intracellular messenger molecules, known to act via modulation of different important signaling enzymes (e.g., protein kinase C, acidic sphinogomyelinase and others) (Quest et al., 1996). Despite their generation by the phosphatidylinositol-specific phospholipase C, diacylglycerols are also directly or indirectly produced by the phosphatidylcholine-specific phospholipases C and D (Exton, 1997). This tempted us to investigate the effect of protein kinase C-inhibitor on PACAP(1–27) induced secretion in rat peritoneal mast cells. Therefore, staurosporine a protein kinase inhibitor with some specificity for protein kinase C, which has previously been shown to inhibit protein kinase C-effects in rat peritoneal mast cells (Koopmann and Jackson, 1990) was tested. Sixty-minute preincubation of rat peritoneal mast cells with staurosporine resulted in a dose-dependent reduction of PACAP-induced [^{3}H]serotonin release (IC_{50}: 600 nM). White and Zembryki (1989) have recently demonstrated that the IgE-induced histamine-release in rat peritoneal mast cells was inhibited by preincubation with staurosporine with an IC_{50} value of 110 nM, while no effect on compound 48/80-induced secretion was observed.

Based on these data we cannot exclude that the effects of staurosporine on PACAP(1–27)-induced serotonin-release are due to protein kinase C-independent actions of the drug. It was shown recently that staurosporine interferes with purified protein kinase C with K_i values of 3 nM (Hidaka and Kobayashi, 1992). Due to diffusional barriers and degradation, the inhibition constants of stau-

rosporine for inhibition of protein kinase C in intact cells may probably be higher. The almost complete inhibition of the PACAP-response by staurosporine in this study fits well into the concept that within the phosphatidylinositol-specific phospholipase C signaling cascade the protein kinase C-pathway may be of greater importance than the Ca^{2+}-signal, which alone is neither sufficient nor necessary for the induction of exocytotic responses in mast cells (Neher, 1988).

4.4. Nordihydroguaiaretic acid

By means of the specific phospholipase A2-inhibitor *para*-bromophenacylbromide it has been shown that this enzyme is functionally involved in the immunoglobulin E (IgE)- and compound 48/80-induced exocytotic response of rat peritoneal mast cells (Bronner et al., 1990; Kuno et al., 1993). Free arachidonic acid, the product of phospholipase A2 serves as substrate for two further enzymes, namely lipoxygenase and cyclooxygenase. Recently, Kuno et al. (1993) demonstrated that an inhibition of the lipoxygenase (by nordihydroguaiaretic acid) but not of the cyclooxygenase (by indomethacin) prevents compound 48/80-induced histamine-release in rat peritoneal mast cells (Kuno et al., 1993). Therefore, the effect of NGDA on PACAP(1–27) induced serotonin-release was also assessed in the present study. NGDA inhibited PACAP(1–27) induced secretion with the same potency that was recently described for compound 48/80 stimulated secretion ($IC_{50} \approx 20\ \mu M$). This finding indicates a functional involvement of the lipoxygenase within the peptidergic pathway of mast cell secretion. The mechanism by which an increased lipoxygenase-activity promotes secretion in peritoneal mast cells is so far not understood. Extracellular applied leukotrienes have been shown not to influence the exocytotic responses of rat peritoneal mast cells and rat basophilic leukaemia cells (Conti et al., 1992). Alternatively, some lipoxygenase-products seem to act as intracellular messenger molecules (Campbell and Halushka, 1995). The finding that an inhibition of either of the signaling enzymes investigated (lipoxygenase, phosphatidylcholine-specific phospholipase C, phospolipase C and D) resulted in a nearly complete inhibition of PACAP(1–27) induced exocytosis, may allow to conclude that these pathways are activated in a sequential manner.

In summary, we have demonstrated in the present investigation that PACAP(1–27) stimulates secretion of [^{3}H]serotonin in rat peritoneal mast cells in a high-affinity PACAP receptor-independent manner, probably by direct interaction with at least one G-protein of the G_i-type. Indirect evidence was presented, that this direct G-protein-activation of PACAP may consecutively induce at least three different signaling enzymes (phospatidylinositol-specific phospholipase C, phosphatidylcholine-specific phospholipase C and/or D, and the lipoxygenase) in a sequential manner. The messenger molecules derived from each of these pathways may converge at the level of one or more staurosporine-sensitive protein kinase(s).

Acknowledgements

We would like to thank W. Gloyna (Dept. of Pharmacology) for expert technical assistance. This study was supported by grants from the Deutsche Forschungsgemeinschaft (Schm 805/4-2; Schm 1223/4-1).

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