Enhanced responsiveness of ovalbumin-sensitized guinea-pig alveolar macrophages to tachykinins

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1 We have evaluated the ability of substance P (SP), neurokinin A (NKA) and the selective NK₂ receptor agonist [β -Ala⁸]-NKA(4–10) to induce superoxide anion (O₂^{•-}) production and prostanoid (prostaglandin E₂, thromboxane B₂) release from alveolar macrophages (AMs) isolated from control or actively sensitized guinea-pigs.

2 The dose-response curves for NKA and SP were shifted to the left (three orders and one order of magnitude, respectively) in AMs isolated from sensitized animals, with no variation in maximal effects.

3 By evaluating the effects of [β -Ala⁸]-NKA(4–10), we observed that not only was the concentration-response curve shifted to the left in both the functional parameters examined, but also maximal effects were significantly enhanced in AMs isolated from sensitized guinea-pigs.

4 This varied responsiveness seems to be specific for tachykinins, as it was not reproduced by another AM stimulant, the bacterial peptide N-formylmethionyl-leucyl-phenylalanine (fMLP).

5 Only small amounts of β -glucuronidase were released following tachykinin or ovalbumin stimulation both in control and sensitized AMs.

6 These results indicate that AMs isolated from sensitized guinea-pigs show an increased responsiveness to NK₂ receptor stimulation and further stress the role played by AMs in allergic lung diseases.

Keywords: Tachykinin receptors; alveolar macrophages, ovalbumin sensitization

Introduction

Alveolar macrophages (AMs), which play a key role in the local inflammatory process associated with bronchial asthma (Rankin, 1989), can be activated by different agonists *in vitro*. Upon stimulation, AMs undergo a respiratory burst, secrete regulatory cytokines (especially, interleukin-1 (IL-1) and tumour necrosis factor α (TNF- α)) and lysosomal enzymes, release biologically active lipids, e.g., PAF (platelet activating factor), cyclo-oxygenase and lipoxygenase metabolites (Sibille & Reynolds, 1990).

We have previously shown that mammalian tachykinins dose-dependently evoke superoxide anion (O₂^{•-}) generation from guinea-pig AMs: a comparative evaluation of the effects of natural tachykinins and selective synthetic peptide analogues indicated that macrophage activation is mainly mediated by NK₂ receptors (Brunelleschi *et al.*, 1990).

Neurogenic inflammation, which involves the release of neuropeptides, including the tachykinins, substance P (SP) and neurokinin A (NKA), from capsaicin-sensitive primary neurones is known to contribute to airway disease, namely asthma (Barnes *et al.*, 1991; Frossard & Advenier, 1991). Saria *et al.* (1983) and Matsuse *et al.* (1991) reported that capsaicin pretreatment provides significant protection against antigen-induced bronchoconstriction and airway hyperresponsiveness in sensitized guinea-pigs. Dusser *et al.* (1989) demonstrated that the bronchoconstrictor responses to SP and capsaicin, but not to acetylcholine, are potentiated during viral respiratory infections in the guinea-pig, thus suggesting that airway smooth muscle responsiveness to sensory neuropeptides could be impaired in pathophysiological conditions.

Binding sites for IgG and IgE have been demonstrated on both rabbit and human AMs (Arendt & Mannick, 1973; Spiegelberg *et al.*, 1983; Rossman *et al.*, 1986; Kindt *et al.*, 1991) as well as an impaired responsiveness to β -adrenoceptor stimulation in AMs obtained from sensitized guinea-pigs (Bachelet *et al.*, 1989; Beusenberg *et al.*, 1989; 1991).

Furthermore, IgE-stimulated canine AMs enhance the neurally-mediated contraction of bronchial smooth muscle *in vitro*: this effect appears to be associated with release of mediators (namely thromboxane B₂ (TXB₂)) from immunologically stimulated AMs (Tamaoki *et al.*, 1991).

The present study was undertaken to assess the effects of natural (SP and NKA) and synthetic ([β -Ala⁸]-NKA(4–10); a selective NK₂ receptor agonist) tachykinins in AMs obtained from control or ovalbumin-sensitized guinea-pigs and to compare these effects with those elicited, in the same preparations, by the bacterial peptide N-formylmethionyl-leucyl-phenylalanine (fMLP).

Methods

Sensitization procedure

Male guinea-pigs (380–500 g) were actively sensitized by two s.c. injections at a 2-week interval of 0.5 ml of saline containing 10 μ g ovalbumin dispersed in 1 mg Al(OH)₃ according to Pretolani *et al.* (1989). Animals were used for the experiments 7–10 days after the second injection. Control animals were injected with the same volume of saline and Al(OH)₃.

Sensitization of the animals was verified by measuring ovalbumin-induced contractile responses in guinea-pig tracheal spirals obtained from the same guinea-pigs, by use of techniques described by Brunelleschi *et al.* (1987).

Harvesting and purification of guinea-pig alveolar macrophages

AMs were harvested from control or ovalbumin-sensitized guinea-pigs as previously described (Brunelleschi *et al.*, 1990). Briefly, guinea-pigs were anaesthetized with pentobarbitone sodium (50 mg kg⁻¹, i.p.) and the trachea was cannulated. Twelve successive bronchoalveolar lavages were performed with 5 ml aliquots of phosphate buffered saline (pH 7.4, 37°C) injected with a plastic syringe through a polyethylene

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cannula inserted into the trachea. Fluid of the two first lavages was discarded, as it was found to contain a large quantity of cells other than macrophages (especially, epithelial cells and eosinophils). After centrifugation and lysis of erythrocytes by hypotonic shock, the bronchoalveolar lavage pellet was resuspended in MEM (Minimum Essential Medium with Earle's salts) supplemented with 5% foetal calf serum, 20 mM HEPES, 2 mM glutamine, 50 $\mu\text{g ml}^{-1}$ streptomycin and 5 u ml^{-1} penicillin and microscopically evaluated (May-Grunwald/Giemsa). Cell viability, as assessed by trypan blue exclusion, was $>95\%$.

Aliquots of cell suspension ($0.8-1 \times 10^6$ cells) were plated in 6-well tissue culture plates (35 mm diameter, Costar, Cambridge) and allowed to adhere for 2 h at 37°C in a humidified atmosphere containing 5% CO_2 . Non-adherent cells were removed by washing monolayers three times with MEM.

Activation of guinea-pig alveolar macrophages

Adherent AMs, obtained from both control or sensitized animals, were incubated with fMLP, ovalbumin, SP, NKA or the synthetic NK_2 receptor agonist, $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ at 37°C in a CO_2 incubator for different times, according to the functional parameter evaluated (see below). Challenge with the different stimuli used was terminated by placing the culture plates on ice.

Determination of superoxide anion (O_2^-) production from guinea-pig alveolar macrophages

In these experiments, adherent AMs were incubated (final volume 2 ml) with the stimuli for 30 min at 37°C in a humidified atmosphere containing 5% CO_2 in the presence of cytochrome C (1 mg ml^{-1}), as previously described (Brunelleschi *et al.*, 1990).

Production of O_2^- was measured by the superoxide dismutase-inhibitable cytochrome C reduction, the absorbance changes being recorded at 550 nm in a Perkin Elmer 552S spectrophotometer. Release of O_2^- was expressed as nmol cytochrome C reduced per 10^6 AMs per 30 min, using an extinction coefficient of $2.1 \times 10^{-4} \text{ M min}^{-1}$.

To avoid interference with spectrophotometric recordings of O_2^- production, monolayers were incubated in MEM (supplemented by HEPES, glutamine and antibiotics; see above) without phenol red.

Determinations of prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) production in guinea-pig alveolar macrophages

The concentrations of TXB_2 and PGE_2 in the cell supernatant were determined by specific enzyme-immunoassay (EIA) after suitable dilutions (1:2 to 1:100) in EIA buffer without prior extraction or purification, according to the manufacturer's instructions. The threshold sensitivities of these assays were: 9 pg ml^{-1} for PGE_2 and 20 pg ml^{-1} for TXB_2 .

In these experiments adherent AMs were challenged with the stimuli in a CO_2 incubator at 37°C , in the presence of MEM without phenol red, supplemented by HEPES, glutamine and antibiotics. The incubation time was 18 h, according to Hartung & Tokya (1983), and was confirmed by preliminary time-course studies (see Results). Supernatants were collected, immediately frozen and lyophilized before being evaluated for their prostanoid content. Results are expressed as ng TXB_2 per 10^6 AMs or pg PGE_2 per 10^6 AMs.

Determination of β -glucuronidase release from guinea-pig alveolar macrophages

Adherent AMs ($1-1.2 \times 10^6$) were incubated for 10 min with cytochalasin B ($5 \mu\text{g ml}^{-1}$) at 37°C in a CO_2 incubator and then challenged for 30 min with the stimuli, according to

Hartung *et al.* (1986). The reaction was stopped by placing monolayers on ice and the supernatants were collected. Pellets were treated with 0.1% Triton X-100 to lyse the cells.

β -Glucuronidase (EC 3.2.1.31) activity was chosen as a marker of lysosomal enzymes according to Arnoux *et al.* (1987) and was measured spectrophotometrically after 16 h incubation at 37°C , with phenolphthalein glucuronide used as a substrate (Fishman, 1974). Total macrophage enzyme activity was calculated by summation of the activity in the supernatant with that in the pellet; enzyme release was expressed as the percentage of the total enzyme activity.

Data analysis

Experiments were performed in duplicate or triplicate and basal values (e.g., values obtained in the absence of any treatment) were subtracted from all determinations. All results are expressed as mean $\pm$ s.e.mean; statistical significance was evaluated by Student's *t* test.

Chemicals

The compounds used and their sources were: SP and NKA (Peninsula: St. Helens, Merseyside); fMLP and Triton X-100 (Serva: Heidelberg, Germany); HEPES (2-(4-(2-hydroxyethyl)-1-piperazino-ethan-sulfonic acid) and cytochalasin B (Aldrich Chemical Co: Milwaukee, WI, U.S.A.); superoxide dismutase, cytochrome C type III, phenolphthalein glucuronide and ovalbumin (Sigma: St. Louis, MO, U.S.A.); MEM with or without phenol red, glutamine, heat-inactivated foetal calf serum, penicillin and streptomycin (Gibco: Paisley). $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ was synthesized at Menarini Laboratories, Firenze (Italy); $[\text{Pro}^9]\text{SP}$ sulphone was kindly supplied by Prof. D. Regoli, University of Sherbrooke (Canada). EIA kits for PGE_2 and TXB_2 measurements were purchased from Cayman Chemical Company (Ann Arbor, MI, U.S.A.).

Results

Effect of ovalbumin sensitization in the bronchoalveolar population

No significant differences in body weight or the number of cells in bronchoalveolar lavages were observed between control or sensitized animals. Control and sensitized animals provided a mean of $27.6 \pm 2.2 \times 10^6$ and $29.8 \pm 4 \times 10^6$ cells per guinea-pig, respectively ($n = 6$). The different cell populations present in lavage fluid from control and sensitized animals are listed in Table 1: a significant increase in the number of eosinophils and lymphocytes was induced by the sensitization procedure. In contrast, the percentage of AMs present in bronchoalveolar lavage from sensitized guinea-pigs was lower ($P < 0.05$) than in control animals, but the absolute number of macrophages did not differ significantly ($24.8 \pm 3 \times 10^6$ in controls, $23 \pm 3.5 \times 10^6$ in sensitized animals).

Table 1 Cell populations in the bronchoalveolar lavage from control or ovalbumin-sensitized guinea-pigs

	% Composition	
	Control	Sensitized
Macrophages	88.7 ± 0.1	$78 \pm 2^*$
Lymphocytes	10.2 ± 0.5	$17.2 \pm 2^*$
Eosinophils	0.7 ± 0.5	$5.3 \pm 1^{**}$
Neutrophils	0.6 ± 0.4	0.7 ± 0.5

Data are mean $\pm$ s.e.mean for 6 animals.

* $P < 0.05$ vs control animals; ** $P < 0.01$ vs control animals (Student's unpaired *t* test).

Superoxide anion generation in alveolar macrophages obtained from control or sensitized guinea-pigs

Basal O_2^- production (e.g., O_2^- production in the absence of any treatment) was not different in AMs obtained from either experimental group (1.2 ± 0.2 vs 1.35 ± 0.3 nmol cytochrome C reduced per 10^6 AMs; $n = 8$) and was subtracted from all values.

The challenge of AMs isolated from sensitized guinea-pigs with ovalbumin ($100 \mu\text{g ml}^{-1}$) resulted in O_2^- generation (3 ± 0.5 nmol cytochrome C reduced per 10^6 AMs; $n = 8$); the antigen was devoid of activity in AMs obtained from control animals (data not shown).

We previously found that NKA and SP dose-dependently evoked O_2^- production from guinea-pig AMs (Brunelleschi *et al.*, 1990): these effects were not significantly varied in the presence of $10 \mu\text{M}$ thiorphan or a cocktail of inhibitors (thiorphan, captopril, bestatin, each $1 \mu\text{M}$, data not shown). When AMs isolated from sensitized animals were challenged with SP, a leftward displacement of the dose-response curve by approximately one order was observed, ED_{50} values being 0.56 nM in control and 0.04 nM in sensitized cells (Figure 1a). The amount of maximal activation was similar in both experimental groups (2.33 ± 0.3 vs 2.37 ± 0.7 nmol cytochrome C reduced per 10^6 AMs; $n = 6$) and was achieved at 10 nM in sensitized and 100 nM in control cells (Figure 1a). When AMs were challenged with NKA, the leftward shift in the concentration-response curve observed with cells from sensitized animals was more than two orders of magnitude and ED_{50} values were 0.14 pM and 0.09 nM in sensitized and control cells respectively (Figure 1b). Maximal O_2^- production, which did not differ significantly between the two groups, was observed at 0.1 nM NKA in sensitized and $0.1 \mu\text{M}$ NKA in control animals (Figure 1b). The synthetic peptide analogue, $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$, which is a selective NK_2 receptor agonist (Rovero *et al.*, 1989), dose-dependently (0.01 nM– $0.1 \mu\text{M}$) evoked O_2^- production in AMs from control guinea-pigs. The dose-response curve for this NK_2 receptor agonist was shifted to the left in experiments performed on AMs from sensitized animals: ED_{50} values were 3 pM and 0.9 nM in sensitized and control cells respectively (Figure 1c). Unlike results with natural tachykinins, the extent of O_2^- production induced by $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ was higher in AMs isolated from sensitized animals and maximal activation (4.08 ± 0.8 nmol cytochrome C reduced per 10^6 AMs; $n = 5$) was achieved at 1 nM (Figure 1c). In contrast, no significant variation in the dose-response curves for $[\text{Pro}^9]\text{-SP}$ sulphone, a selective NK_1 agonist, was observed between control and sensitized AMs (data not shown). As previously reported (Brunelleschi *et al.*, 1990), $[\text{Pro}^9]\text{-SP}$ sulphone acted at high concentrations ($0.1 \mu\text{M}$ – $1 \mu\text{M}$) only.

The bacterial peptide fMLP, which interacts with specific receptors in AMs (Daniele *et al.*, 1982), evoked O_2^- production in both control and ovalbumin-sensitized guinea-pig AMs. Neither maximal effects (18.4 ± 2.1 vs 18 ± 1.7 nmol cytochrome C reduced per 10^6 AMs; $n = 5$) nor ED_{50} values (10.9 vs 11.6 nM) were significantly different in control and sensitized cells (Figure 2).

Prostanoid release in alveolar macrophages obtained from control or sensitized guinea-pigs

Prostanoid release from AMs of both experimental groups was evaluated after 18 h incubation with the stimuli. Preliminary time-course studies revealed that basal prostanoid release (which was subtracted from all determinations) increased with time (data not shown). PGE_2 release evoked by a fixed concentration of $0.1 \mu\text{M}$ NKA amounted to 60, 90, 125, 220 and 230 pg per 10^6 AMs, after 1, 6, 12, 18 and 24 h, respectively (means of two determinations in triplicate). The release of PGE_2 by $0.1 \mu\text{M}$ fMLP was 380–400 pg per 10^6 AMs, reached a plateau after 6 h of incubation and remained

Figure 1 Tachykinin-evoked superoxide anion generation in alveolar macrophages isolated from control (○) or ovalbumin-sensitized (●) guinea-pigs. (a) Dose-response curve to substance P (SP) ($n = 6$). (b) Dose-response curve to neurokinin A (NKA) ($n = 8$). (c) Dose-response curve to $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ ($n = 5$). Cells were challenged for 30 min with tachykinins. Data show mean values ($\pm$ s.e.mean, vertical bars). *Denotes $P < 0.01$ (Student's t test for paired samples).

stable until 18 h. Therefore, the 18 h incubation period was selected in these experiments.

Basal TXB_2 release differed significantly ($P < 0.01$) between the two groups: 13.9 ± 1.9 and 27.2 ± 5 ng per 10^6 AMs ($n = 3$), in control and sensitized AMs, respectively. These values were subtracted from all determinations with stimuli. Ovalbumin $100 \mu\text{g ml}^{-1}$ released 14.43 ± 8.7 ng per 10^6 AMs ($n = 3$) from sensitized AMs, with no effect on control cells. Moreover, TXB_2 release evoked by $0.1 \mu\text{M}$ fMLP was not modified in either group: 28.5 ± 4 and 21 ± 5 ng per 10^6 AMs ($n = 3$), in sensitized and control cells, respectively.

By measuring tachykinin-evoked TXB_2 release, we confirmed the pattern of results seen when O_2^- generation

Figure 2 Superoxide anion generation evoked by N-formylmethionyl-leucyl-phenylalanine (fMLP) in alveolar macrophages (AMs) isolated from control (○) or ovalbumin-sensitized (●) guinea-pigs. Data are mean ($\pm$ s.e.mean, vertical bars) of 5 experiments.

was measured. As depicted in Figure 3a, SP dose-dependently evoked TXB_2 release from control or sensitized AMs, a greater than one order of magnitude leftward shift in its dose-response curve being observed in sensitized animals. ED_{50} values were 70 pM in control and 1 pM in sensitized AMs. Maximal release, which was similar in both experimental groups, was obtained at 10 nM SP (Figure 3a). When AMs were stimulated by NKA, the leftward shift in the dose-response curve observed with cells harvested from sensitized animals was about three orders of magnitude and ED_{50} values were 0.2 pM and 0.4 nM in sensitized and control AMs, respectively (Figure 3b). Maximal TXB_2 release, which was similar in both experimental groups (8.2 ± 0.4 and 6.2 ± 2.5 ng per 10^6 AMs; $n = 3$), was measured at 0.1 nM NKA in sensitized and 0.1 μM NKA in control AMs (Figure 3b). Moreover, the results obtained by evaluating, in both groups, the effect of the NK_2 receptor agonist $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ matched those observed by measuring O_2^- production (see above): ED_{50} values were 1 nM in control and 10 pM in sensitized AMs and the evoked TXB_2 release was significantly higher in AMs from sensitized animals (Figure 3c).

Also, by evaluating tachykinin-evoked PGE_2 release from AMs of both groups, we observed that dose-response curves in sensitized cells were shifted leftwards (data not shown). Maximal PGE_2 release induced by both SP and NKA was similar and did not differ significantly between the two experimental groups (SP = 207 ± 41 and 264 ± 30 pg per 10^6 AMs in control or sensitized cells; NKA = 216 ± 37 and 293 ± 63 pg per 10^6 AMs in control or sensitized cells; $n = 4$). ED_{50} values were as follows: SP = 0.48 nM in control and 8 pM in sensitized AMs; NKA = 0.2 nM in control and 1 pM in sensitized AMs. Basal PGE_2 release, which was subtracted from all determinations, was 630 ± 76 pg per 10^6 AMs in control and 472 ± 72 pg per 10^6 AMs in sensitized cells ($n = 4$). A

Figure 3 Tachykinin-evoked thromboxane B_2 release in alveolar macrophages (AMs) isolated from control (○) or ovalbumin-sensitized guinea-pigs (●). (a) Dose-response curve to substance P (SP). (b) Dose-response curve to neurokinin A (NKA). (c) Dose-response curve to $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$. Data show mean ($\pm$ s.e.mean, vertical bars) of three experiments in duplicate. *Denotes $P < 0.01$.

total of 369 ± 68 pg PGE_2 per 10^6 AMs in sensitized animals and 311 ± 93 pg PGE_2 per 10^6 AMs in control animals was released by 0.1 μM fMLP ($n = 4$). Ovalbumin-evoked PGE_2 release was detected only in sensitized AMs and amounted to 138 ± 48 pg per 10^6 AMs ($n = 4$).

β -Glucuronidase release in alveolar macrophages obtained from control or sensitized guinea-pigs

Basal enzyme release did not differ significantly between the two experimental groups ($6.4 \pm 0.8\%$ and $7.2 \pm 1\%$ in control and sensitized AMs, respectively; $n = 6$). Basal values were subtracted from all determinations.

SP, NKA and the NK_2 receptor agonist $[\beta\text{-Ala}^8]\text{-NKA}(4-10)$ caused a net enzyme release at micromolar concentrations only: the extent of β -glucuronidase release was very small in both experimental groups (SP 100 μM = $5.3 \pm 1.3\%$ vs $6 \pm 1.5\%$; SP 10 μM = $2 \pm 0.2\%$ vs $2.5 \pm 0.5\%$; NKA

10 μM = $3.5 \pm 0.4\%$ vs $3.1 \pm 0.5\%$; NKA 1 μM = $2.5 \pm 0.5\%$ vs $2.8 \pm 0.6\%$; [β -Ala⁸]-NKA(4–10) 10 μM = 2.8 ± 0.3 vs $3.5 \pm 0.6\%$ in control and sensitized AMs, respectively; $n = 6$).

Ovalbumin (100 $\mu\text{g ml}^{-1}$) released $3.5 \pm 0.7\%$ of total β -glucuronidase content in sensitized cells, but had no effect on control cells.

The peptide fMLP (0.1 μM), which is more potent than tachykinins in evoking O_2^- production, released $6.4 \pm 0.7\%$ and $7.5 \pm 0.4\%$ of the total β -glucuronidase activity in control and sensitized cells, respectively ($n = 6$) and the calcium ionophore A23187 1 μM released $20 \pm 1.7\%$ and $23 \pm 1.8\%$ in control and sensitized AMs, respectively ($n = 6$).

Discussion

Our study indicates that AMs isolated from ovalbumin-sensitized guinea-pigs exhibit an increased responsiveness to tachykinins. By assessing O_2^- production and prostanoid release, concentration-response curves for NKA and SP are shifted leftwards, with no variation in maximal response: the left shift is far more pronounced for NKA (about three orders of magnitude) than for SP (only one order of magnitude). The involvement of NK_2 receptors in this event is further confirmed by the results obtained with a selective NK_2 receptor agonist: the concentration-effect curve for [β -Ala⁸]-NKA(4–10) is not only shifted leftwards, but also maximal activation is strongly enhanced in AMs isolated from sensitized animals. In contrast, no variation is observed between AMs obtained from control or sensitized guinea-pigs by evaluating the effects of the selective NK_1 agonist [Pro⁹]-SP sulphone or those of the bacterial peptide fMLP. However, when we evaluated the ability of mammalian tachykinins to induce lysosomal enzyme release from AMs, only minimal amounts of β -glucuronidase could be detected and tachykinins acted at concentrations higher than those needed to induce O_2^- generation or prostanoid release. This fact warrants some explanations. Firstly, lysosomal enzyme release is not a very sensitive parameter of AM activation compared to O_2^- generation. According to Brieland *et al.* (1987), rat AMs secrete lysozyme and N-acetyl- β -D-glucosaminidase either spontaneously or following zymosan stimulation. However, when data are expressed as net enzyme release, only 1.5% of the total lysozyme activity is observed after challenge with opsonized zymosan (5 $\mu\text{g ml}^{-1}$; Brieland *et al.*, 1987). Moreover, Joseph *et al.* (1983) observed that the release of the lysosomal acid hydrolase, β -glucuronidase, from passively sensitized human AMs or AMs obtained from asthmatic patients is not significantly different and amounts to 4–8% after a 30 min contact time with the specific antigen, *in vitro*. Similar results were also reported by Arnoux *et al.* (1987).

The fact that micromolar concentrations of tachykinins are needed to induce lysosomal enzyme secretion from guinea-pig AMs whereas nanomolar concentrations evoke O_2^- generation and prostanoid release suggests that different pattern(s) of cell activation exhibit a varied coupling to second messengers for the release of the selected mediator. In many cell types, the interaction of agonists with specific membrane receptors stimulates phospholipase C, yielding inositol 1,4,5-trisphosphate (IP_3), which in turn mobilizes intracellular calcium and 1,2-diacylglycerol (which directly activates protein kinase C, PKC) (Nishizuka, 1984; Berridge & Irvine, 1984). Enzyme exocytosis is primarily a calcium-dependent response, while the respiratory burst may also occur in the absence of added calcium in the medium.

Sakata *et al.* (1987) suggested that in guinea-pig peritoneal macrophages, arachidonic acid release plays an essential role in the activation of the O_2^- generating system, with PKC having a significant but not an obligatory role, in the respiratory burst. In guinea-pig AMs, the stimulation of arachidonic acid release by fMLP involves several mechanisms and

is regulated by both protein kinase C and A, the latter exerting a negative modulation (Kadiri *et al.*, 1990).

Most of the physiological effects of tachykinins depend on their terminal sequence, involving NK_1 , NK_2 and NK_3 receptors. Activation of NK_2 receptors is coupled to a guanine nucleotide-binding protein (G-protein) and stimulates phosphatidylinositol hydrolysis in different tissues (Frossard & Advenier, 1991). However, little information is available concerning the signal transduction mechanisms afforded by tachykinins in inflammatory cells, and only SP-induced effects have been investigated. In human neutrophils, activation of the respiratory burst by SP is insensitive to 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7, a PKC inhibitor), indicating that PKC is not obligatory for the activation of NADPH-oxidase, and is only partially inhibited by pertussis toxin (Serra *et al.*, 1988). SP also induces a cytochalasin B-dependent secretion of lysosomal enzymes from human neutrophils (Serra *et al.*, 1988).

In this study, the varied responsiveness of AMs to tachykinins *in vitro* follows a sensitization procedure which, according to Garcez Do Carmo *et al.* (1986), Pretolani *et al.* (1989) and Desquand *et al.* (1991), causes serum levels of both IgG and IgE to be significantly enhanced. AMs obtained from actively sensitized guinea-pigs have been shown to possess an impaired adenylate cyclase activity, conflicting results being reported (Bachelet *et al.*, 1989; Beusenberg *et al.*, 1989; 1991). While Bachelet *et al.* (1989) noted that PGE_2 and β -adrenoceptor agonists are less effective in increasing the intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) content of AMs from ovalbumin-sensitized compared to control guinea-pigs, Beusenberg *et al.* (1989, 1991) demonstrated that both agonists stimulate adenylate cyclase activity more effectively in AMs obtained from sensitized animals. These authors also reported that sensitization and antigen challenge do not affect β -adrenoceptor binding and suggested that the enhanced adenylate cyclase responsiveness results from alterations in the α_s -subunit of a G-protein (Beusenberg *et al.*, 1991).

In our experiments, AMs isolated from sensitized guinea-pigs demonstrate an enhanced responsiveness to tachykinins (and, especially to NKA and NK_2 receptor stimulation), but not to the bacterial peptide fMLP, thus showing selectivity. We can, therefore, suggest a role for NK_2 receptor stimulation in immunomodulation, since O_2^- generation and prostanoid release evoked by the peptide analogue [β -Ala⁸]-NKA(4–10) are significantly enhanced in AMs obtained from sensitized guinea-pigs. The involvement of NK_2 receptors in the modulation of primary antibody response has been recently discussed by Eglezos *et al.* (1991). In their experiments, rats treated neonatally with capsaicin to destroy primary afferent nerves presented a diminished antibody response: NKA was twelve times more potent than SP in restoring the immune response (Eglezos *et al.*, 1991). Furthermore, in keeping with the observations by Nohr & Weihe (1991), who demonstrated the presence of tachykinin- and calcitonin gene-related peptide-fibres in alveolar walls of different species, including the guinea-pig, and suggested that tachykinins released from alveolar fibres might influence epithelial and inflammatory cells, we think it possible to hypothesize that the binding of immunoglobulins to guinea-pig AM membrane may be the trigger for increased responsiveness to tachykinins.

In conclusion, these results indicate that AMs isolated from actively sensitized guinea-pigs show enhanced responsiveness to tachykinins and point to AMs as important cells for the development of allergic lung disease.

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