Tachykinins activate guinea-pig alveolar macrophages: involvement of NK₂ and NK₁ receptors

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1 The effects of substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) were evaluated on superoxide anion (O₂⁻) production by guinea-pig alveolar macrophages (AM).

2 SP dose-dependently (ED₅₀ = 0.7 nM) evoked O₂⁻ production from guinea-pig AM; the N-terminal heptapeptide, SP(1–7), was ineffective. In the presence of thiorphan (10⁻⁵ M), an enkephalinase inhibitor, the stimulating effects of SP were not significantly modified. NKA and NKB were both able to induce O₂⁻ production from guinea-pig AM, ED₅₀ values being 0.1 and 1.3 nM, respectively. Therefore, the rank order of activity of natural tachykinins was NKA > SP > NKB. Tachykinin-evoked effects were quantitatively similar to those elicited by the autacoid mediator PAF-acether and less than those induced by the synthetic peptide N-formylmethionyl-leucyl-phenylalanine (FMLP).

3 The NK₂ receptor agonist [β -Ala⁸]-NKA (4–10) dose-dependently evoked O₂⁻ production from guinea-pig AM; the NK₁ receptor agonist [Pro⁹]-SP sulphone acted only at high concentrations, while the NK₃ receptor agonist [Me,Phe⁷]-NKB was ineffective.

4 These findings indicate that guinea-pig AM possess NK₂ and possibly some NK₁ tachykinin receptors and further suggest tachykinin involvement in lung pathophysiology.

Introduction

Several studies indicate that natural tachykinins may play a role in lung pathophysiology (Barnes, 1987; Lundberg & Saria, 1987; Barnes *et al.*, 1988). Among mammalian tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are the most likely candidates for a neurotransmitter(s) role. SP, NKA and NKB cause, with different potencies, airway smooth muscle contraction in several animal species, increase plasma exudation in guinea-pig airways and have been implicated in lung diseases, namely asthma (Barnes, 1987; Lundberg & Saria, 1987; Sekizawa *et al.*, 1987; Rogers *et al.*, 1988; Frossard *et al.*, 1989).

The undecapeptide SP is recognized as a chemical mediator of neurogenic inflammation, since it participates in vasodilatation, increases vascular permeability and exerts modulatory effects on cells involved in inflammatory and immune processes (Lembeck & Holzer, 1979; Foreman & Jordan, 1984; Payan *et al.*, 1984).

In addition, SP degranulates rat mast cells, stimulates DNA and protein synthesis from human T lymphocytes, evokes the release of inflammatory cytokines from human blood monocytes, enhances the phagocytic activity of human neutrophils and rat peritoneal macrophages, promotes lysosomal enzyme release and oxy-radical production from human neutrophils (Bar-Shavit *et al.*, 1980; Fewtrell *et al.*, 1982; Payan *et al.*, 1983; Lotz *et al.*, 1988; Serra *et al.*, 1988).

In elicited guinea-pig peritoneal macrophages, SP induces oxy-radical production and arachidonic acid metabolite release by interacting with specific receptor sites (Hartung & Toyka, 1983; Hartung *et al.*, 1986), but no attempts were made to characterize the receptor subtype(s) mediating these effects.

We evaluated the effects of natural tachykinins on guinea-pig alveolar macrophages (AM), a cell type which plays an important role in the local inflammatory process associated with bronchial hyperreactivity in asthma (Hunninghake *et al.*, 1979).

Previous studies have demonstrated that guinea-pig AM possess specific receptors for chemotactic formylpeptides (e.g., N-formylmethionyl-leucyl-phenylalanine; FMLP) and PAF-acether, and are capable, when challenged by these agents, to undergo a respiratory burst, which in turn leads to the production of reactive oxygen metabolites known to promote tissue damage (Daniele *et al.*, 1982; Desquand *et al.*, 1986; Henson & Johnston, 1987).

Therefore, we decided to ascertain whether natural tachykinins (SP, NKA, NKB) evoke superoxide anion (O₂⁻) production from guinea-pig AM. By using synthetic peptide analogues that act as selective stimulants at tachykinin receptors (Drapeau *et al.*, 1987; Rovero *et al.*, 1989), we tried to identify which tachykinin receptor(s) mediate the activation of guinea-pig AM.

Methods

Harvest of broncho-alveolar cells and macrophage culture

Alveolar macrophages (AM) were harvested from male guinea-pigs (250–300 g), according to the method of Myrvik *et al.* (1961), with minor modifications. Briefly, the animals were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.p.) and the trachea was cannulated. Sixty ml of phosphate buffered saline (pH 7.4, 37°C) was infused into the lungs in 5 ml aliquots and reaspirated after gentle massage. Fluid of the two first lavages was discarded, as it was found to contain a large quantity of cells other than macrophages on microscopic examination. The lavage fluid was then centrifuged (1700 r.p.m., room temperature, 10 min) and cells were resuspended in Minimum Essential Medium with Earle's salts (MEM).

After lysis of red blood cells by hypotonic shock, cellular populations were diluted in MEM and microscopically evaluated. The preparation thus obtained consisted of more than 85% macrophages, other cells being lymphocytes and eosinophils (May-Grunwald/Giemsa staining). Cell viability was

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determined by trypan blue exclusion and consistently exceeded 95%.

Aliquots of cell suspension ($0.7-1 \times 10^6$ cells) were plated in 6-well tissue culture plates (35 mm diameter, Costar, Cambridge, U.K.) containing 2 ml of MEM supplemented with 20 mM HEPES, 2 mM glutamine and 5% foetal calf serum and were allowed to adhere for 2 h at 37°C in 5% CO₂. Thereafter, non-adherent cells were removed by washing monolayers three times with Hank's balanced salt solution. The monolayers consisted of at least 98% macrophages (May-Grunwald/Giemsa staining); cell viability (trypan blue exclusion) was approximately 95%.

Activation of guinea-pig alveolar macrophages and superoxide anion production

Adherent macrophages were incubated with SP, NKA, NKB and drugs reported to be selective NK-receptor subtype agonists: [Pro⁹]-SP sulphone (NK₁ agonist), [β -Ala⁸]-NKA (4-10) (NK₂ agonist) and [Me,Phe⁷]-NKB (NK₃ agonist) (Drapeau *et al.*, 1987; Rovero *et al.*, 1989) up to 60 min at 37°C in a humidified atmosphere containing 5% CO₂ in the presence of cytochrome C (1 mg ml^{-1}). In some experiments, adherent AM were preincubated 30 min with thiorphan (10^{-5} M) or vehicle and then exposed, in the presence of cytochrome C, to increasing concentrations of SP for further 60 min. The stimulating effects of neuropeptides were terminated by placing the wells on ice. O₂⁻ production was measured by the superoxide dismutase-inhibitable cytochrome C reduction, the absorbance changes being recorded at 550 nm in a Perkin Elmer 552S spectrophotometer. O₂⁻ production was expressed as nmol cytochrome C reduced/ 10^6 cells, using an extinction coefficient of 21.1 mM (Massey, 1959).

To avoid interference with spectrophotometrical recordings of O₂⁻ production, monolayers were incubated in MEM without phenol red.

Data analysis

Experiments were performed in triplicate and control values (e.g., O₂⁻ production in the absence of any treatment) were subtracted from all determinations. All results are expressed as means $\pm$ s.e.mean. Statistical analysis was carried out by ANOVA.

Chemicals

The compounds used and their sources were: SP, SP(1-7), NKA and NKB (Peninsula); HEPES (2-(4-(2-hydroxyethyl)-1-piperazino-ethansulfonic acid: Aldrich); thiorphan, superoxide dismutase, cytochrome C type VI (Sigma); FMLP (Serva); PAF-acether (Bachem); MEM with or without phenol red, glutamine and foetal calf serum (Gibco).

[β -Ala⁸]-NKA(4-10) was synthesized at Menarini Laboratories, Florence (Italy); [Me,Phe⁷]-NKB and [Pro⁹]-SP sulphone were kindly supplied by Prof. D. Regoli, University of Sherbrooke (Canada).

Results

Substance P-induced superoxide anion generation from guinea-pig alveolar macrophages

In the concentration range 10^{-11} – 10^{-6} M , SP dose-dependently evoked O₂⁻ production, maximal activation ($2.18 \pm 0.4 \text{ nmol cytochrome C reduced}/10^6 \text{ cells}$; $n = 8$) being achieved at 10^{-7} M SP, the ED₅₀ value being 0.7 nM (Figure 1a). The N-terminal heptapeptide, SP(1-7), was ineffective up to 10^{-6} M (Figure 1a).

Figure 1 Superoxide anion production by guinea-pig alveolar macrophages incubated with substance P (SP). (a) Dose-response curve to SP (●; $n = 8$) and SP(1-7) (○; $n = 4$). Cells were incubated 60 min with SP or SP(1-7). Means with s.e.mean shown by vertical bars. (b) Effect of thiorphan 10^{-5} M on superoxide anion production evoked by SP; open column, substance P; solid columns, thiorphan 10^{-5} M + SP. Means with s.e.mean. (vertical bars) of three experiments. $P > 0.5$.

Activation of guinea-pig AM by two other stimuli, the autacoid mediator PAF-acether and the chemotactic peptide N-formylmethionyl-leucyl-phenylalanine (FMLP), also resulted in O₂⁻ production. However, while O₂⁻ production evoked by 10^{-6} M PAF-acether ($2.05 \pm 0.5 \text{ nmol cytochrome C reduced}/10^6 \text{ cells}$; $n = 6$) was similar to that measured with SP, 10^{-7} M FMLP evoked a greater response ($15.8 \pm 1.2 \text{ nmol cytochrome C reduced}/10^6 \text{ cells}$; $n = 8$) than SP or PAF-acether (data not shown).

Time-course experiments demonstrated that O₂⁻ generation evoked by a given concentration of SP (10^{-7} M SP) was 0.6 ± 0.4 , 0.66 ± 0.29 , 0.79 ± 0.5 , 1.58 ± 0.2 and $1.73 \pm 0.1 \text{ nmol cytochrome C reduced}/10^6 \text{ cells}$ ($n = 4$), after 5, 10, 15, 30 and 60 min, respectively. Therefore, since no significant differences were observed between the 30 and 60 min challenge period ($P > 0.05$), the 60 min period was selected for all subsequent experiments.

The enkephalinase inhibitor, thiorphan has been shown to enhance the effector responses to exogenous and endogenous tachykinins in different muscle tissues (Devillier *et al.*, 1988; Maggi *et al.*, 1989).

In the presence of thiorphan, O₂⁻ generation evoked by increasing concentrations of SP was not significantly modified as compared to controls (Figure 1b).

Neurokinin A and B-induced superoxide anion production from guinea-pig alveolar macrophages

The two natural tachykinins NKA and NKB stimulated guinea-pig AM (Figure 2). In the concentration range 10^{-12} –

10^{-6} M, NKA evoked O_2^- production from guinea-pig AM: maximal activation was observed at 10^{-7} M (1.89 ± 0.5 nmol cytochrome C reduced/ 10^6 cells; $n = 8$), the ED_{50} value being 0.1 nM (Figure 2).

NKB evoked O_2^- generation from guinea-pig AM in a dose-dependent manner: maximal activation (2.42 ± 0.7 nmol cytochrome C reduced/ 10^6 cells; $n = 5$) was obtained at 10^{-6} M, with an ED_{50} value of 1.3 nM (Figure 2). However, the maximum effects produced by NKA and NKB, as well as those evoked by SP, were not significantly different.

Effects of selective tachykinin-receptor agonists on guinea-pig alveolar macrophages

In the concentration range 10^{-10} – 10^{-6} M, [β -Ala⁸]-NKA(4–10), a selective NK₂ receptor agonist (Rovero *et al.*, 1989), evoked O_2^- production from guinea-pig AM, with maximal effects (2.52 ± 0.9 nmol cytochrome C reduced/ 10^6 cells; $n = 7$) at 10^{-7} M (Figure 3).

In the same concentration-range, the NK₁ receptor agonist [Me,Phe⁷]-NKB (Drapeau *et al.*, 1987) was almost ineffective,

Figure 2 Superoxide anion production by guinea-pig alveolar macrophages incubated with neurokinin A (NKA) or NKB. Cells were incubated 60 min with NKA (●; $n = 8$) or NKB (○; $n = 5$). Means are shown with s.e.mean indicated by vertical bars.

Figure 3 Superoxide anion production by guinea-pig alveolar macrophages incubated with selective tachykinin receptor agonists. Cells were incubated 60 min with [Pro⁹]-SP sulphone (○; selective NK₁ agonist); [β -Ala⁸]-NKA(4–10) (●; selective NK₂ agonist) or [Me,Phe⁷]-NKB (△; selective NK₃ agonist). Means $\pm$ s.e.mean $n = 7$.

while the NK₁ receptor agonist [Pro⁹]-SP sulphone (Drapeau *et al.*, 1987) displayed some stimulant action at high concentrations (10^{-7} – 10^{-6} M) (Figure 3).

Discussion

These results indicate that SP, NKA and NKB are able to activate guinea-pig AM, as monitored by O_2^- production. Tachykinin-induced effects on guinea-pig AM are quantitatively similar to those evoked by the autacoid mediator PAF-acether, but less than those induced by the chemotactic peptide FMLP. The ability of FMLP to evoke a greater response in comparison with PAF-acether is similar to the situation with human neutrophils, where we have previously demonstrated that the FMLP-induced maximal response is 3–4 fold higher than that elicited by PAF-acether (Brunelleschi *et al.*, 1987). The *in vitro* concentrations at which SP activates cellular populations involved in inflammatory and immune processes greatly vary, depending on the cell type and the functional parameter evaluated. In human monocytes and guinea-pig peritoneal macrophages, the stimulant action of SP was found in the nM range (Hartung & Toyka, 1983; Lotz *et al.*, 1988), whereas mast cell degranulation occurs at μ M concentrations (Shibata *et al.*, 1985). Repke & Bienert (1987) have also shown that SP degranulates rat mast cells through a receptor-independent mechanism and Bar-Shavit *et al.* (1980) have reported that enhancement of human neutrophil phagocytosis by SP resides in the N-terminal tetrapeptide, SP(1–4), which resembles tuftsin in its structure. In this study, SP stimulates O_2^- generation from guinea-pig AM at concentrations as low as 10^{-10} M ($ED_{50} = 0.7$ nM), thus suggesting the activation of a receptor-mediated mechanism. Furthermore, the N-terminal heptapeptide, SP(1–7), lacks the ability to induce O_2^- generation, thus indicating that SP-evoked effects in this cell population are mediated by the C-terminal region of the peptide.

Enkephalinase (endopeptidase 24.11), which hydrolyzes a variety of neuropeptides, exists in many peripheral tissues including lungs of different animal species (Sekizawa *et al.*, 1987); inhibitors of this metalloendopeptidase have been shown to enhance significantly tachykinin-induced effects, for example in guinea-pig trachea (Devillier *et al.*, 1988; Frossard *et al.*, 1989). However, in the guinea-pig AM, the stimulant effects of SP were not significantly modified in the presence of thiorphan, suggesting that, *in vitro*, the undecapeptide did not undergo degradation by enkephalinase.

Actually, three distinct tachykinin receptors have been recognized, each one of them having a preferential affinity for one of the three natural tachykinins (Buck & Burcher, 1986; Lee *et al.*, 1986; Regoli *et al.*, 1989).

In our experiments, NKA and NKB shared the ability of SP to induce O_2^- generation from guinea-pig AM, the former being more active ($ED_{50} = 0.1$ nM) than the latter ($ED_{50} = 1.3$ nM). Therefore, the rank order of activity of natural tachykinins (NKA > SP > NKB) might suggest that the NK₂ receptors mediate O_2^- generation in guinea-pig AM. However, the finding that SP was almost as active as NKA, does not fit with this hypothesis, because SP has been shown to have much lower affinity than NKA for NK₂ receptors in mono-receptorial assays (Buck & Burcher, 1986; Lee *et al.*, 1986; Regoli *et al.*, 1989).

Natural tachykinins at high concentrations stimulate all three receptors indiscriminately; for this reason, synthetic receptor agonists with improved or absolute selectivity for one receptor have been developed (Drapeau *et al.*, 1987; Regoli *et al.*, 1989; Rovero *et al.*, 1989).

In this study, the NK₂ receptor agonist [β -Ala⁸]-NKA(4–10) dose-dependently evoked O_2^- production from guinea-pig AM, with maximal effects at 10^{-7} M; the NK₁ receptor agonist [Pro⁹]-SP sulphone displayed some activity at high concentrations only, whereas the NK₃ receptor agonist [Me,Phe⁷]-NKB was ineffective.

We conclude that the NK₂ receptors are the main mediators of guinea-pig AM response to natural tachykinins. The weak activity of the NK₁-selective agonist suggests the presence of a minor population of NK₁ sites on this cell population. The possibility that the effect of [Pro⁹]-SP sulphone might involve activation of NK₂ or NK₃ receptors seems unlikely, because this peptide is ineffective at these sites, even at high concentrations (Drapeau *et al.*, 1987).

References

- BARNES, P.J. (1987). Airway neuropeptides and asthma. *Trends Pharmacol. Sci.*, **8**, 24–27.
- BARNES, P.J., CHUNG, K.F. & PAGE, C.P. (1988). Inflammatory mediators and asthma. *Pharmacol. Rev.*, **40**, 49–84.
- BAR-SHAVIT, Z., GOLDMAN, R., STABINSKY, Y., GOTTLIEB, P., FRIDKIN, M., TEICHBERG, V.I. & BLUMBERG, S. (1980). Enhancement of phagocytosis—A newly found activity of substance P residing in its N-terminal tetrapeptide sequence. *Biochem. Biophys. Res. Commun.*, **94**, 1445–1451.
- BRUNELLESCHI, S., RUBINO, A., TARLI, S., MIAN, M., LEDDA, F., GIOTTI, A. & FANTOZZI, R. (1987). Modulation of oxygen radical production from human neutrophils by calcium entry blockers. *Adv. Biosci.*, **66**, 139–146.
- BUCK, S.H. & BURCHER, E. (1986). The tachykinins: a family of peptides with a brood of receptors. *Trends Pharmacol. Sci.*, **7**, 65–68.
- DANIELE, R.P., DIAMOND, M.S. & HOLIAN, A. (1982). Demonstration of a formylpeptide receptor on lung macrophages. Correlation of binding properties with chemotaxis and release of superoxide anion. *Am. Rev. Respir. Dis.*, **126**, 274–280.
- DESQUAND, S., TOUVAY, C., RANDON, J., LAGENTE, V., VILAIN, B., MARIDONNEAU-PARINI, I., ETIENNE, A., LEFORT, J., BRAQUET, P. & VARGAFTIG, B.B. (1986). Interference of the BN52021 (ginkgolide B) with the bronchopulmonary effects of PAF-acether in the guinea-pig. *Eur. J. Pharmacol.*, **127**, 83–95.
- DEVILLIER, P., ADVENIER, C., DRAPEAU, G., MARSAC, J. & REGOLI, D. (1988). Comparison of the effects of epithelium removal and of an enkephalinase inhibitor on the neurokinin-induced contractions of guinea-pig trachea. *Br. J. Pharmacol.*, **94**, 675–684.
- DRAPEAU, G., D'ORLEANS-JUSTE, P., DION, S., RHALEB, N.E., ROUISSI, N.E. & REGOLI, D. (1987). Selective agonists for substance P and neurokinin receptors. *Neuropeptides*, **10**, 43–54.
- FEWTRELL, C.M.S., FOREMAN, J.C., JORDAN, C.C., OEHME, P., RENNERT, H. & STEWART, J.M. (1982). The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. *J. Physiol.*, **330**, 393–411.
- FOREMAN, J.C. & JORDAN, C.C. (1984). Neurogenic inflammation. *Trends Pharmacol. Sci.*, **5**, 116–119.
- FROSSARD, N., RHODEN, K.J. & BARNES, P.J. (1989). Influence of epithelium on guinea-pig airway responses to tachykinins: role of endopeptidase and cyclooxygenase. *J. Pharmacol. Exp. Ther.*, **248**, 292–298.
- HARTUNG, H.P. & TOYKA, K.V. (1983). Activation of macrophages by substance P: induction of oxidative burst and thromboxane release. *Eur. J. Pharmacol.*, **89**, 301–305.
- HARTUNG, H.P., WOLTERS, K. & TOYKA, K.V. (1986). Substance P: binding properties and studies on cellular responses in guinea-pig macrophages. *J. Immunol.*, **136**, 3856–3863.
- HENSON, P.M. & JOHNSTON, R.B. (1987). Tissue injury in inflammation. Oxidants, proteinases and cationic proteins. *J. Clin. Invest.*, **79**, 669–674.
- HUNNINGHAKE, G.W., GADEK, J.E., KAWANAMI, O., FERRANS, W.J. & CRYSTAL, R.G. (1979). Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am. J. Pathol.*, **97**, 149–206.
- LEE, C.M., CAMPBELL, N.J., WILLIAMS, B.J. & IVERSEN, L.L. (1986). Multiple tachykinin binding sites in peripheral tissue and brain. *Eur. J. Pharmacol.*, **130**, 209–217.
- LEMBECK, F. & HOLZER, P. (1979). Substance P as a neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **310**, 175–183.
- LOTZ, M., VAUGHAN, J.H. & CARSON, D.A. (1988). Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science*, **241**, 1218–1221.
- LUNDBERG, J.M. & SARIA, A. (1987). Polypeptide-containing neurones in airway smooth muscle. *Ann. Rev. Physiol.*, **49**, 557–572.
- MAGGI, C.A., PATACCHINI, R., RENZI, D., SANTICIOLI, P., REGOLI, D., ROVERO, P., DRAPEAU, G., SURRENTI, C. & MELI, A. (1989). Effect of thiorphan on the response of the guinea-pig gallbladder to tachykinins. *Eur. J. Pharmacol.*, **165**, 51–61.
- MASSEY, V. (1959). The microestimation of succinate and the extinction coefficient of cytochrome c. *Biochem. Biophys. Acta*, **34**, 255–256.
- MYRVIK, Q.N., LEAKE, E.S. & FARISS, B. (1961). Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a state of purity. *J. Immunol.*, **86**, 128–132.
- PAYAN, D.G., BREWSTER, D.R. & GOETZL, E.J. (1983). Specific stimulation of human T lymphocytes by substance P. *J. Immunol.*, **131**, 1613–1615.
- PAYAN, D.G., LEVINE, J.D. & GOETZL, E.J. (1984). Modulation of immunity and hypersensitivity by sensory neuropeptides. *J. Immunol.*, **132**, 1601–1604.
- REGOLI, D., DRAPEAU, G., DION, S. & D'ORLEANS-JUSTE, P. (1989). Receptors for substance P and related neurokinins. *Pharmacology*, **38**, 1–15.
- REPKE, H. & BIENERT, M. (1987). Mast cell activation—a receptor-independent mode of substance P action? *FEBS Lett.*, **221**, 236–240.
- ROGERS, D.F., BELVISI, M.G., AURSUDKIJ, B., EVANS, T.W. & BARNES, P.J. (1988). Effects and interactions of sensory neuropeptides on airway microvascular leakage in guinea-pigs. *Br. J. Pharmacol.*, **95**, 1109–1116.
- ROVERO, P., PESTELLINI, V., RHALEB, N.E., DION, S., ROUISSI, N., TOUSIGNANT, C., TELEMAQUE, S., DRAPEAU, G. & REGOLI, D. (1989). Structure-activity studies of neurokinin A. *Neuropeptides*, **13**, 263–270.
- SEKIZAWA, K., TAMAOKI, J., GRAF, P.D., BASBAUM, C.B., BORSON, D.B. & NADEL, J.A. (1987). Enkephalinase inhibitor potentiates mammalian tachykinin-induced contraction in ferret trachea. *J. Pharmacol. Exp. Ther.*, **243**, 1211–1217.
- SERRA, M.C., BAZZONI, F., DELLA BIANCA, V., GRESKOWIAK, M. & ROSSI, F. (1988). Activation of human neutrophils by substance P. Effect on oxidative metabolism, exocytosis, cytosolic Ca²⁺ concentration and inositol phosphate formation. *J. Immunol.*, **141**, 2118–2124.
- SHIBATA, H., MIO, M. & TASAKA, K. (1985). Analysis of the mechanism of histamine release induced by substance P. *Biochim. Biophys. Acta*, **846**, 1–7.

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