

- clinical experience with a battery of skin-test reagents. *J ALLERGY CLIN IMMUNOL* 1982;69:238-44.
23. Galant S, Posner L, Lee N, Hicks D, Wilkinson B, Ellis M. Immediate hypersensitivity to semisynthetic penicillins in cystic fibrosis [Abstract]. *J ALLERGY CLIN IMMUNOL* 1987;79:199.
 24. Blanca M, Fernandez J, Miranda A, et al. Cross-reactivity between penicillins and cephalosporins: clinical and immunologic studies. *J ALLERGY CLIN IMMUNOL* 1989;83:381-5.
 25. Moss RB, McClelland E, Rubio T, Hillman B, Williams R, Adkinson NF. In vivo and in vitro evaluation of immunologic cross-reactivity of aztreonam in cystic fibrosis patients allergic to penicillin and/or cephalosporin antibiotics [in press]. *Rev Infect Dis*.
 26. Adkinson NF. Relative immunogenicity of β -lactam antibiotics. In: Pilcher WJ, Stadler BM, Dahinden C, et al., eds. *Progress in allergy and clinical immunology*. Toronto: Hogrefe & Huber, 1989, pp. 439-41.
 27. Brown LA, Goldberg ND, Shearer WT. Long-term ticarcillin desensitization by the continuous oral administration of penicillin. *J ALLERGY CLIN IMMUNOL* 1982;69:51-4.
 28. Hilman BC, Rao K. Desensitization with anti-pseudomonal penicillins after allergic reactions to ureidopenicillins in cystic fibrosis [Abstract]. *J ALLERGY CLIN IMMUNOL* 1986;77:167.
 29. Earl HS, Sullivan TJ. Acute desensitization of a patient with cystic fibrosis allergic to both beta-lactam and aminoglycoside antibiotics. *J ALLERGY CLIN IMMUNOL* 1987;79:477-83.
 30. Van Dellen RG. Skin testing for penicillin allergy. *J ALLERGY CLIN IMMUNOL* 1981;68:169-70.

Neuropeptide Y, a putative cotransmitter in noradrenergic neurons, induces mast cell degranulation but not prostaglandin D_2 release

Carmen Arzubiaga, MD, Jason Morrow, MD, L. Jackson Roberts II, MD, and Italo Biaggioni, MD Nashville, Tenn.

*Recent evidence suggests that neural transmitters, including neuropeptides, may modulate the release of mast cell mediators. Because neuropeptide Y (NPY) has recently been recognized as a putative cotransmitter in noradrenergic neurons, we studied the effect of NPY on purified rat peritoneal mast cells. NPY induced mast cell degranulation, as assessed by a dose-dependent increase in net release of β -hexosaminidase. The concentration that produced 50% of the maximal effect, approximately 10 $\mu\text{mol/L}$, evoked a $40\% \pm 3\%$ release. As previously reported for other neuropeptides, release was fast with maximal release already achieved at 60 seconds. Release was at 4°C . In contrast to its effects on mast cell degranulation, NPY had no effect on the generation of prostaglandin D_2 , the major mast cell cyclooxygenase product. By comparison, the calcium ionophore A23187, at doses (4 $\mu\text{mol/L}$) that evoked comparable release of β -hexosaminidase, stimulated a net release of 37 ± 9 ng of PGD_2 per 10^6 mast cells. These results raise the possibility that NPY may act as a modulator between the autonomic nervous system and mast cells. The results also imply that with neuropeptide stimulation, the release of preformed and newly formed mast cell mediators are mediated through independent pathways. (*J ALLERGY CLIN IMMUNOL* 1991;87:88-93.)*

From the Divisions of Clinical Pharmacology and Dermatology, Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tenn.

Supported by American Heart Association Grant-in-aid BRSG 2S07-RR05424-27 and GM15431.

Presented in abstract form, *Clin Res* 1989;37:341A.

Received for publication July 11, 1989.

Revised July 24, 1990.

Accepted for publication July 26, 1990.

Reprint requests: Italo Biaggioni, MD, AA-3228 MCN, Department of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Dr. Arzubiaga was an American Heart Association (Tennessee affiliate) Research Fellow.

1/1/24311

An interrelation between mast cells and nerve structures has long been recognized. Histologically, "mucosal" mast cells are in close proximity to nerve endings and blood vessels.¹ Nerve stimulation has been demonstrated to cause degranulation of mast cells in the skin² that contain mostly "serosal" mast cells.³ Nerve stimulation also results in the release of histamine from guinea pig atrium and rat ileum, in which there is no evidence for histaminergic neurones.⁴ It has been postulated therefore that mast cells are involved in neurogenic inflammation.

There are also pathologic conditions characterized by mast cell activation in the absence of obvious al-

Abbreviations used

NPY:	Neuropeptide Y
PGD ₂ :	Prostaglandin D ₂
Con A:	Concanavalin A
DNAse:	Deoxyribonuclease

lergen exposure, such as chronic idiopathic urticaria, heat-induced urticaria, cold-induced urticaria, or cholinergic urticaria. It has therefore been suggested that these forms of urticaria are neurogenic in nature.⁵ Likewise, episodes of asthma and mast cell activation in patients with systemic mastocytosis can be triggered by exercise and/or stress in the absence of known allergen exposure. Since such events are associated with activation of the autonomic nervous system, a link between such activation and mast cell degranulation appears possible.

It has therefore been suggested that neurotransmitters may modulate the release of mast cell mediators. In particular, neuropeptides have been implicated in mast cell activation,⁵ and neuropeptides, most notably substance P, appear to play a role in the axon reflex.⁶ It has been postulated that activation of sensory receptors results in orthodromic transmission of impulses through afferent nerve fibers to the dorsal horn of the spinal cord and in antidromic transmission of impulses to terminal arborizations of the same fibers. This antidromic stimulation would then cause the release of neuropeptides, for example, substance P, which then stimulate mast cells causing the release of histamine and a flare reaction.⁶ Moreover, substances that deplete neuropeptides, such as capsaicin, inhibit the flare reaction.⁷

NPY has recently been recognized as a putative neurotransmitter/neuromodulator⁸ and has been found to be costored with norepinephrine in noradrenergic neurons.⁹ Given the possible interaction between the autonomic nervous system and mast cell activation, our aim was to determine if NPY, like other neuropeptides, could produce mast cell activation.

MATERIAL AND METHODS

Isolation of mast cells

Sprague-Dawley male rats (400 to 500 gm, retired breeders) were purchased from Charles Rivers (Raleigh, N.C.). Purified peritoneal mast cells were obtained as previously described.¹⁰ Briefly, rats were killed by rapid decapitation and were exsanguinated. Tyrode's buffer (15 ml) lacking calcium and magnesium but containing gelatin, 1 gm/L, and heparin, 50 mg/L, was injected into the peritoneal cavity through a small midline abdominal incision. The abdomen was gently massaged for approximately 1 minute, and the contents were then exposed through a midline in-

cision. Peritoneal fluid was aspirated by gentle suction through a plastic catheter into a plastic syringe and centrifuged at 100 g for 5 minutes at 25° C. Cells from each rat were resuspended in calcium- and magnesium-free Tyrode's buffer (2 ml) containing gelatin and DNAse, 30 mg/L, and placed over 22.5% metrizamide in 2.5 ml of the same buffer. Mast cells were separated by centrifugation at 400 g for 15 minutes at 25° C. The mononuclear cell layer was discarded, and the mast cell pellet was washed twice in 2 ml of the same buffer at 100 g for 5 minutes at 25° C. Cells from different rats (6 to 8 per experiment) were then pooled and kept at room temperature before study. Cells were resuspended to a concentration of $5 \pm 0.7 \times 10^6$ cells per milliliter in Tyrode's buffer containing DNAse and gelatin, 1 mmol/L of MgCl₂, and 1.6 mmol/L of CaCl₂, unless otherwise noted. Cells were counted with a hemocytometer chamber.

Release of mediators

Cells were prewarmed at 37° C for 5 to 10 minutes. Incubations were started by adding cells ($0.7 \pm 0.1 \times 10^6$ per tube) into plastic tubes containing the secretagogues or buffer alone, at a final reaction volume of 250 μ l. Each set of experiments included a tube without secretagogue to determine unstimulated mediator release and a tube containing calcium ionophore A23187 as a positive control. Phosphatidylserine was not added unless otherwise noted.

Release reactions were stopped by dilution with 0.75 ml of ice-cold, calcium- and magnesium-free Tyrode's buffer, and the cells were immediately sedimented at 100 g for 5 minutes at 4° C. The supernatant fractions were decanted, and the cell pellets were resuspended to 1 ml with calcium- and magnesium-free buffer. The resuspended cell pellets were disrupted by sonication (35% power with microtip, for 1 minute, on ice) (Fisher Sonic Dismembrator, model 300, Fisher Scientific, Pittsburgh, Pa.). It was demonstrated in ancillary studies that this procedure did not affect β -hexosaminidase activity. The net percentage of release of β -hexosaminidase and histamine was calculated by the formula

$$\text{Net \% release} = \frac{\text{Sup (S)} - \text{Sup (U)}}{\text{Sup (S)} + \text{Pellet (S)} - \text{Sup (U)}}$$

where Sup = supernatant, S = stimulated, and U = unstimulated.

Net release of PGD₂ was calculated as the difference between the concentration of PGD₂ in the supernatants of the stimulated minus the unstimulated tubes. Results are presented as mean $\pm$ SEM.

Measurements of mediators

β -Hexosaminidase activity was quantified by release of *p*-nitrophenol from *p*-nitrophenol- β -D-2-acetamido-2-deoxyglucopyranoside in reaction mixtures containing a final substrate concentration of 4 mmol/L and either 50 μ l of cell pellet or 100 μ l of supernatant fractions. Incubations were done in citrate buffer, pH 4.2, containing 0.5% Triton X-100 for 2 hours at 37° C. Reactions were stopped by addition of 1.5 ml of 0.2 mol/L of glycine, pH 10.7. Chrom-

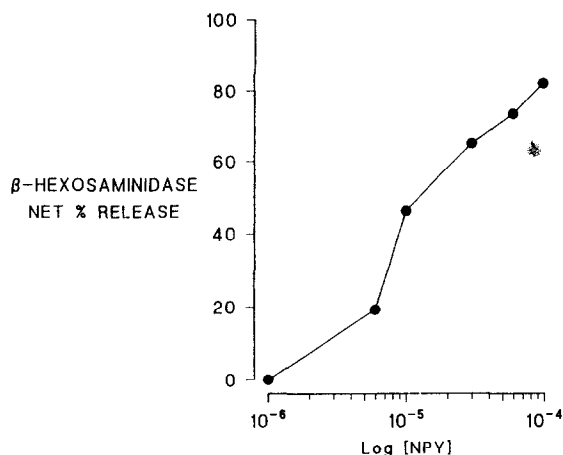


FIG. 1. Dose-response curve for the release of β -hexosaminidase induced by the human NPY in rat peritoneal mast cells. Results illustrated are from a single experiment.

ogen release was quantified by absorbance at 410 nm and compared to a standard curve run with each experiment.

Histamine was quantitated with either 200 μ l of the supernatant fractions or the cell pellets resuspended in 200 μ l of methanol/water (1:20). Histamine [$\alpha, \alpha, \beta, \beta$ - $^2\text{H}_4$]-dihydrochloride, 10 ng (MSD Isotopes, Montreal, Canada), was then added to each sample, which was then purified and derivatized as previously described.¹¹ After derivatization and evaporation, the residue was dissolved in 500 μ l of toluene to which 500 μ l of saturated Tris base was added and vortexed. After brief centrifugation, the toluene layer was transferred to a silica Sep-Pak preparative column (Waters Associates, Milford, Mass.) previously washed with 5 ml of methylene chloride. The column was then washed with 2 ml of methylene chloride and eluted with 5 ml of methanol/ethyl acetate (1:9), evaporated to dryness, and reconstituted for analysis in 20 μ l of ethyl acetate. Quantification was accomplished by negative ion chemical ionization-gas chromatography-mass spectrometry, with selected ion monitoring, and with a 0.25 micron DB-1 fused silica capillary column (J & W Scientific, Inc., Folsom, Calif.). Ions monitored were $m/z = 430$ for the endogenous compound, and $m/z = 434$ for [$^2\text{H}_4$]-histamine. Quantitation of endogenous histamine was based on the ratio of m/z 430 to m/z 434.

PGD₂ was quantitated in 200 μ l of the supernatant fractions. Briefly, 3 ng of [$^2\text{H}_7$]-PGD₂¹⁰ was added to each sample. The mixture was then acidified to pH 3, extracted with Sep-Pak C₁₈ preparative columns, and purified as previously described.¹² The final pentafluorobenzyl ester, trimethylsilyl ether derivative was quantified with negative ion chemical ionization-gas chromatography-mass spectrometry with selected ion monitoring. PGD₂ was quantified by determining the ration of m/z 524 ([$^2\text{H}_6$]-PGD₂) to the sum of m/z 530, 531, 532 ([$^2\text{H}_6$], [$^2\text{H}_7$], [$^2\text{H}_8$]-PGD₂), as previously described.¹⁰

Material and sources

Reagents were obtained from the following sources: human NPY, Bachem Co. (Torrance, Calif.) and Peninsula Laboratories (Belmont, Calif.); DNase, 85,000 U/mg from bovine pancreas and calcium ionophore A23187, Calbiochem (San Diego, Calif.); and metrizamide, Nycomed (Oslo, Norway). All other reagents were purchased from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

The mean β -hexosaminidase release from unstimulated cells was $5\% \pm 0.9\%$. NPY produced a dose-dependent net release of β -hexosaminidase (Fig. 1). The concentration that produced 50% of the maximal effect was approximately 10 μ mol/L. This concentration evoked a $40\% \pm 3\%$ net release of β -hexosaminidase ($n = 7$) and was used in subsequent studies. By comparison, the calcium ionophore A23187, which activates mast cells by causing an influx of calcium ions, evoked a $58\% \pm 3\%$ net release of β -hexosaminidase ($n = 8$) (Fig. 2, upper panel). Con A, in the presence of phosphatidylserine, which activates mast cells through cross-linkage of IgE, evoked a $16\% \pm 0.2\%$ net release of β -hexosaminidase release ($n = 4$). Although β -hexosaminidase release has been demonstrated to be linearly correlated to histamine release in the same cell preparation,¹³ it appeared important to us to validate mast cell degranulation with histamine measurements. Indeed, NPY, 10 μ mol/L, also evoked a $37\% \pm 2\%$ release of histamine ($n = 3$).

The time course of NPY-induced degranulation is illustrated in Fig. 3. Degranulation was fast with maximal release already apparent at 60 seconds. To determine if mast cell degranulation induced by NPY was the result of an energy-dependent process, the effect of temperature was determined. NPY-induced degranulation was submaximal at 4° C compared to 37° C (Fig. 3), suggesting that it is mediated through an energy-dependent and not a cytotoxic process. Furthermore, after exposure to NPY, mast cells excluded trypan blue, an estimate of viability.¹⁴

To determine the effect of extracellular calcium on NPY-induced mediator release, cells were isolated as described and divided in half before the last washing. One half was resuspended in calcium-containing Tyrode's buffer and the other half in calcium-free buffer. Release experiments were then carried out in parallel as previously described. NPY, 20 μ mol/L, produced $48\% \pm 2\%$ release of β -hexosaminidase in the presence of extracellular calcium, but only $7\% \pm 0.4\%$ release in cells incubated in a calcium-free buffer ($n = 3$).

In contrast to its effects on preformed mediator

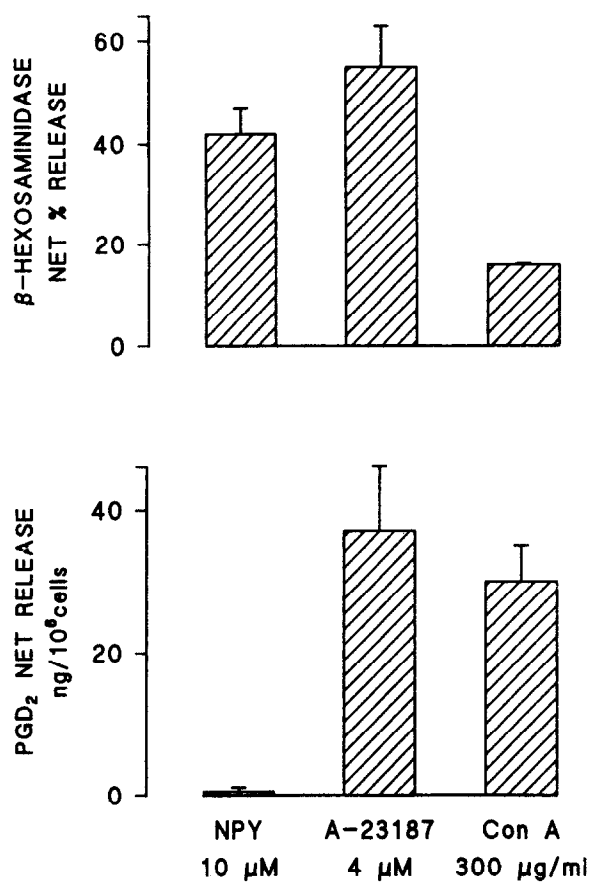


FIG. 2. Upper panel, Net percent release of β -hexosaminidase produced by NPY, 10 μ M/L, ($n = 7$), the calcium ionophore A23187, 4 μ M/L, ($n = 8$), and Con A, 300 μ g/ml ($n = 4$). Lower panel, Net release of PGD₂ produced by NPY ($n = 5$), the calcium ionophore A23187, ($n = 6$), and Con A ($n = 4$).

release, NPY had no significant effect on the net release of PGD₂ ($+ 0.5 \pm 0.6$ ng of PGD₂ per 10⁶ cells; $n = 5$), a newly formed mediator and the major mast cell cyclooxygenase product. By comparison, calcium ionophore A23187, at doses that produced comparable increases in β -hexosaminidase release as NPY, produced a net release of 37 ± 9 ng of PGD₂ per 10⁶ cells ($n = 6$; Fig. 2, lower panel). Con A produced a net release of 30 ± 5 ng of PGD₂ per 10⁶ cells ($n = 4$).

DISCUSSION

NPY is a 36 amino acid polypeptide present in the brain, in the spinal cord, and in peripheral neurons. It is closely associated with, or costored in, noradrenergic neurons.⁹ NPY is synthesized in the neuronal cell body and reaches nerve terminals via axonal transport.¹⁵ Subcellular fractionation studies have suggested that norepinephrine occurs mainly in small ves-

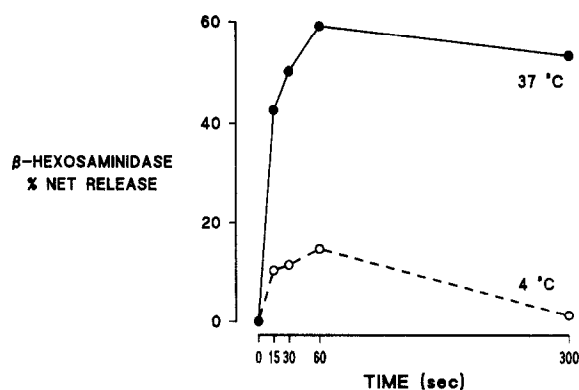


FIG. 3. Time course of β -hexosaminidase release produced by NPY in rat peritoneal mast cells at 37°C (—) and at 4°C (---). Results illustrated are from a single experiment.

icles (diameter, 40 to 60 nm), whereas NPY is contained together with norepinephrine in the less numerous large vesicles (diameter, 70 to 100 nm).¹⁶ These two distinct subcellular stores permit a differential release of norepinephrine or NPY, with NPY being preferentially released at relatively high-frequency stimulation (20 Hz).¹⁷ It has been demonstrated in vivo that situations characterized by sympathetic activation are associated with the release of NPY.¹⁸ In addition to coexisting in catecholamine-containing neurons, NPY may also coexist in neurons containing somatostatin.¹⁹

Because of the potential importance of NPY as a neurotransmitter/neuromodulator and given the precedent of other neuropeptides producing mast cell activation, we studied the effects of NPY on mast cell activation. The results presented here indicate that NPY produces mast cell degranulation. The concentration required to produce half-maximal degranulation was in the low micromolar range. Although the concentrations present in the vicinity of nerve terminals are unknown, the concentrations found here are higher than concentrations required for vascular actions.²⁰ However, other peptides that activate rat peritoneal mast cells do so also at low micromolar concentrations, including substance P,¹⁴ somatostatin,²¹ and β -endorphins.²² Likewise, the concentration of substance P required for rat peritoneal mast cell degranulation are 1000-fold higher than concentrations that stimulate smooth muscle.¹⁴ Although the importance of these neuropeptides on mast cell function in vivo is not clearly established, it has been suggested that substance P produces mast cell activation in vivo.⁶

Mast cell degranulation induced by NPY was fast, and maximal release was apparent at 60 seconds. This

appears to be a general feature of peptide-induced mast cell activation as compared to immunologic stimulation that reaches a peak 3 minutes after challenge.²³ NPY-induced release was significantly lower in the absence of extracellular calcium. This differs from substance P-induced release, which is greater in calcium-free media.²⁴ However, this phenomena is not universal to all types of mast cells or peptides studied. For example, neurotensin-induced mast cell degranulation is not significantly affected by changes in extracellular calcium concentration.²² Similar findings are found in human skin mast cells activated by vasoactive intestinal peptides and somatostatin. Furthermore, histamine release produced by substance P in these cells is actually decreased in the absence of extracellular calcium.²⁵ In contrast, it is universally accepted that immunologically mediated histamine release is critically dependent on extracellular calcium.

NPY induced mast cell degranulation but had no effect on PGD₂ generation. This finding is in sharp contrast to the effect of calcium ionophore and the reported effects of immunologic stimulation of mast cells, which induce both degranulation with release of preformed mediators, as well as generation of newly formed mediators. These results imply that, at least with neuropeptide stimulation, the release of preformed and newly formed mast cell mediators are mediated through independent pathways. Furthermore, NPY could be used as a probe to examine the intracellular mechanisms involved in preformed versus newly formed mediator release. In agreement with the hypothesis that there is differential release of preformed and newly formed mediators, it has been described that substance P, vasointestinal peptide, and somatostatin release histamine from human skin mast cells but have virtually no effect on PGD₂ or leukotriene C₄ generation.⁵ Likewise, dog mastocytoma cells release comparable amounts of histamine when cells are stimulated by either antigen or compound 48/80 but generate approximately tenfold less leukotriene C₄ after compound 48/80 challenge.²⁶ Finally, it has recently been reported that substance P, bradykinin, and neurotensin trigger histamine release from rat peritoneal mast cells but produce only minor generation of PGD.²⁷

From our studies, we, of course, cannot draw conclusions on the physiologic role of NPY on mast cell function. Nonetheless, these results raise the possibility that NPY may act as a modulator between the autonomic nervous system and mast cells. This finding may be of relevance in pathologic situations, which, even though they are characterized by mast cell activation, are triggered by mechanisms other than

allergen exposure, such as exertion or stress. However, the physiologic importance of mast cells³ and neuropeptides⁵ in these forms of neurogenic inflammation or allergy remains to be determined.

REFERENCES

1. Neuson B, Dahlstrom A, Enerback L, Ahlman H. Suggestive evidence for a direct innervation of mucosal mast cells. *Neuroscience* 1983;10:565-70.
2. Kiernan JA. The involvement of mast cells in vasodilatation due to axon reflex in injured skin. *Q J Exp Physiol* 1972;57:311-7.
3. Irani AMA, Schwartz LB. Mast cell heterogeneity. *Clin Exp Allergy* 1989;19:143-55.
4. Bani-Sachi T, Barattini M, Bianchi S, et al. The release of histamine by parasympathetic stimulation in guinea pig auricle and rat ileum. *J Physiol* 1986;371:29-43.
5. Church MK, Lowman MA, Robinson C, Holgate S, Benyon RC. Interaction of neuropeptides with human mast cells. *Int Arch Allergy Appl Immunol* 1989;88:70-8.
6. Foreman JC. Substance P and calcitonin gene-related peptide: effects on mast cells and in human skin. *Int Arch Allergy Appl Immunol* 1987;82:366-71.
7. Bernstein JE, Swift RM, Soltari K, Llorincz A. Inhibition of axon reflex vasodilatation by topically applied capsaicin. *J Invest Dermatol* 1981;76:394-5.
8. Tseng CJ, Mosqueda-Garcia R, Appalsamy M, Robertson D. Cardiovascular effects of neuropeptide Y in rat brainstem nuclei. *Circ Res* 1988;64:55-61.
9. Allen YS, Adrian TE, Allen JM, Takemoto K, Crow TJ. Neuropeptide Y distribution in the rat brain. *Science* 1983;221:877-9.
10. Roberts LJ II, Lewis RA, Oates JA, Austen KF. Prostaglandin, thromboxane, and 12-hydroxy-5,8,10,14-eicosatetraenoic acid production by ionophore-stimulated rat serosal mast cells. *Biochim Biophys Acta* 1979;575:185-92.
11. Roberts LJ II, Oates JA. Accurate and efficient method for quantification of urinary histamine by gas chromatography negative ion chemical ionization mass spectrometry. *Anal Biochem* 1984;136:258-63.
12. Parsons WG III, Roberts LJ II. Transformation of prostaglandin D₂ to isomeric prostaglandin F₂ compounds by human eosinophils. *J Immunol* 1988;141:2413-9.
13. Schwartz LB, Austen KF, Wasserman SI. Immunologic release of β -hexosaminidase and β -glucuronidase from peritoneal rat serosal mast cells. *J Immunol* 1979;330:393-411.
14. Fewtrell CMJ, Foreman JC, Jordan CC, Oehme P, Renner H, Steward JM. The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. *J Physiol* 1982;330:393-411.
15. Fried G, Lundberg J, Theodorsson-Norheim E. Subcellular storage and axonal transport of neuropeptide Y (NPY) in relation to catecholamines in the cat. *Acta Physiol Scand* 1985;125:145-52.
16. Frif G, Terenius L, Hokfelt T, Goldstein M. Evidence for differential localization of noradrenaline and neuropeptide Y in neuronal storage vesicles isolated from rat vas deferens. *J Neuroscience* 1985;5:450-8.
17. Lundberg JM, Rudchild A, Sollen A, Theodorsson-Norheim A, Hamberger B. Frequency and reserpine-dependent chemical coding of sympathetic transmission: differential release of noradrenaline and neuropeptide Y from pig spleen transmission. *Neurosci Lett* 1986;63:96-100.

18. Lundberg JM, Torssell L, Sollevi A, et al. Neuropeptide Y and sympathetic vascular control in man. *Regul Pept* 1985; 13:41-52.
19. Chronwall BM, Chase TN, O'Donohue TL. Coexistence of neuropeptide Y and somatostatin in rat and human cortical and hypothalamic neurons. *Neurosci Lett* 1984;52:213-7.
20. Tseng CJ, Robertson D, Light RT, Atkinson JR, Robertson RM. Neuropeptide Y is a vasoconstrictor of human coronary arteries. *Am J Med Sci* 1988;296:11-6.
21. Kassessinoff TA, Pearce FL. Histamine secretion from mast cells stimulated with somatostatin. *Agents Actions* 1988;23: 211-3.
22. Sydbom A. Mast cells and neuropeptides: effects of β -endorphin on rat mast cells. *Agents Actions* 1988;23:204-6.
23. Ishisaka T, Hirota F, Ishisaka K, Axelrod J. Stimulation of phospholipid methylation, Ca^{2+} influx, and histamine release by bridging of IgE receptors on rat mast cells. *Proc Natl Acad Sci USA* 1980;77:1903-6.
24. Goetzl EJ, Phillips MJ, Gold W. Stimulus specificity of the generation of leukotrienes by dog mastocytoma cells. *J Exp Med* 1983;158:731-7.
25. Lowman MA, Benyon RC, Church MK. Characterization of neuropeptide-induced histamine release from human dispersed skin mast cells. *Br J Pharmacol* 1988;95:121-30.
26. Kowalski ML, Kalinen MA. Neurogenic inflammation, vascular permeability, and mast cells. *J Immunol* 1988;140:3905-11.
27. Levi-Schaffer F, Shalit M. Differential release of histamine and prostaglandin D_2 in rat peritoneal mast cells activated with peptides. *Int Arch Allergy Appl Immunol* 1989;90:352-7.

The effect of the oral leukotriene antagonist, ICI 204,219, on leukotriene D_4 and histamine-induced cutaneous vascular reactions in man

Jonathan A. Bernstein, MD, Paul A. Greenberger, MD, Roy Patterson, MD, Mitchell Glass, MD,* Robert Krell, PhD,* and Per T. Thyrum, MD, PhD*
Chicago, Ill., and Wilmington, Del.

Eighteen normal male subjects were recruited for a double-blind, placebo-controlled, randomized, two-period crossover study to determine the effects of a new, potent, highly selective, oral peptide leukotriene antagonist (ICI 204,219) on cutaneous vasculature. Skin testing for leukotriene D_4 (LTD_4) and histamine was performed at three different time intervals after receiving ICI 204,219 or placebo. The LTD_4 threshold dose responses were recorded in duplicate to verify reproducibility. These data confirm known remarkable dose-response variation of cutaneous vascular reactions to LTD_4 since some subjects reacted to a dose as low as 0.001 fmol. Twelve subjects (67%) demonstrated a more than one-half log increase in their threshold response after receiving ICI 204,219. Five of these 12 subjects (28%) had >1 log-dose response after ICI 204,219. Two of these subjects (11.1%) had a 5 or 10 log increase in their threshold response after receiving ICI 204,219. Given the role of leukotrienes as potentially important mediators in immediate-type reactions, our results raise the possibility for additional investigation of the antagonistic effect of ICI 204,219 in nonallergic and allergic cutaneous diseases. (J ALLERGY CLIN IMMUNOL 1991;87:93-8.)

From the Sections of Allergy-Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Ill., and *ICI Pharmaceuticals Group, Wilmington, Del.

Supported by United States Public Health Services Grant AI 11403 and the Ernest S. Bazley Grant.

Received for publication Jan. 23, 1990.

Revised Aug. 6, 1990.

Accepted for publication Aug. 14, 1990.

Reprint requests: Paul A. Greenberger, MD, Northwestern University Medical School, 303 E. Chicago, Ave., Chicago, IL 60611.

1/1/24691

Leukotrienes comprise a group of bioactive mediators that are formed from the oxidative metabolism of arachidonic acid through the 5'-lipoxygenase pathway. Previously referred to as slow-reacting substances of anaphylaxis, the peptides LTC_4 , LTD_4 , and LTE_4 are known to be potent bronchoconstrictors in addition to stimulating mucous secretion and increasing capillary permeability in postcapillary venules.¹ Leukotrienes are believed to play an important role in immediate hypersensitivity reactions, notably