

Increased contraction and inositol phosphate formation of tracheal smooth muscle from hyperresponsive guinea pigs

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Tracheal smooth muscle from guinea pigs with documented airway hyperresponsiveness in vivo after multiple antigen challenges produced 30% to 50% greater force than tracheas from control guinea pigs, when stimulated with carbachol, histamine, or leukotriene D₄. When cultured smooth muscle cells were incubated with myo[2-³H]inositol, basal uptake of [³H]inositol was similar in cells from normal and hyperresponsive guinea pigs, but when these cells were stimulated with contractile agonists, there was increased uptake of inositol in hyperresponsive cells. Analysis of inositol phosphates by column chromatography and high-performance liquid chromatography revealed the presence of inositol-1,4,5-triphosphate, inositol-1,3,4-trisphosphate, inositol-1,4-bisphosphate, and inositol-1-monophosphate. The release of inositol-1,4,5-triphosphate, inositol-1,4-bisphosphate, and inositol-1-monophosphate by smooth muscle cells stimulated with carbachol, leukotriene D₄, or histamine was 20% to 40% greater in cells derived from hyperresponsive animals than cells from normal animals. These data demonstrate that the increased muscle contraction of hyperresponsive guinea pig tracheas is associated with increased inositol phosphate metabolism in these cells. Delineating the mechanisms of airway smooth muscle contraction should provide new pharmacologic targets for the inhibition of bronchoconstriction in asthma. (J ALLERGY CLIN IMMUNOL 1992;90:918-26.)

Key words: Smooth muscle, inositol phosphate, carbachol, histamine, LTD₄, airway hyperresponsiveness

Nonspecific airway hyperresponsiveness is a hallmark of human asthma. The factor(s) responsible for this exaggerated airway narrowing in asthmatic patients remain to be clarified. In atopic asthma, inhalation of allergen induces an immediate bronchoconstriction and increases airway responsiveness to various stimuli such as histamine or methacholine.¹ In addition, studies have demonstrated increased responsiveness of airways from sensitised animals after antigen challenge.²⁻⁶

Airway tissues from human asthmatic subjects have

Abbreviations used

LTD ₄ :	Leukotriene D ₄
PIP ₂ :	Phosphatidylinositol-4,5-bisphosphate
PIP:	Phosphatidylinositol-4-monophosphate
IP ₂ :	Inositol-1,4-bisphosphate
IP ₃ :	Inositol-1,4,5-triphosphate
IP ₄ :	Inositol-1,3,4,5-phosphate
IP ₁ :	Inositol-1-monophosphate
HBSS:	Hanks' balanced salt solution
DMEM:	Dulbecco's modified Eagle's medium
FBS:	Fetal bovine serum
HPLC:	High-performance liquid chromatography
TLC:	Thin layer chromatography
PLC:	Phospholipase C
BSA:	Bovine serum albumin
PCA:	Provocative concentration of antigen

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demonstrated increased isometric force generation versus that from nonasthmatic subjects.⁷⁻⁹ Similar observations have also been made with animal models of hyperresponsiveness. It has been reported that

airway smooth muscle from dogs with allergic bronchospasm shorten more than those from control animals.^{10, 11}

Ishida et al.¹² showed increased isovolumetric force generation and isobaric shortening of the isolated trachea from hyperresponsive guinea pigs versus control animals. Other investigators have failed to demonstrate such differences in muscle mechanics between normal and hyperresponsive tissues.^{13, 14} Whether airway hyperresponsiveness is due to increased force generation and shortening of the muscle cells from sensitized animals is still a matter of controversy.

After binding of contractile agonists to the cell membrane, a cascade of biochemical processes are activated leading to the hydrolysis of membrane-bound phosphoinositides and generation of second messengers such as inositol trisphosphate (IP₃) and diacylglycerol. IP₃ binds to intracellular stores causing calcium release.¹⁵ Increased intracellular calcium leads to the contraction of smooth muscle cells.¹⁶ In this study we report that in tracheal smooth muscle from hyperresponsive guinea pigs agonists caused greater contraction and released more inositol phosphates.

MATERIAL AND METHODS

Materials

Fetal bovine serum (FBS) tissue culture media, and antibiotics were purchased from Gibco Laboratories (Grand Island, NY). Leukotriene D₄ (LTD₄), trypsin, collagenase, elastase (type IV), carbachol, histamine, phosphatidylinositol, lyso-phosphatidylinositol, phosphatidylinositol-4,5-bisphosphate (PIP₂), phosphatidylinositol-4-monophosphate (PIP), and ovalbumin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Smooth muscle-specific monoclonal antibodies to α -actin were purchased from BioGenex Laboratories (San Ramon, Calif.). Myo-[2-³H]inositol (10 to 20 Ci/mmol), and phosphatidylinositol-4,5-bisphosphate ([myo-inositol-2-³H{N}] [1 to 5 Ci/mmol]) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, Mo.). Dowex-1 resin (anion exchanger) was purchased from Bio-Rad Laboratories (Richmond, Calif.).

Sensitization procedures

Cam-Hartley male guinea pigs were sensitized to ovalbumin by either injection of 10 mg ovalbumin adsorbed to 1 μ g of aluminum hydroxide intraperitoneally or by inhalation of an aerosolized solution of 1% ovalbumin with 4% *Bordetella pertussis* vaccine. To induce hyperresponsiveness, animals were injected intraperitoneally with 10 μ g of ovalbumin-Al(OH)₃ twice per month for a total period of 6 months, during which all the animals demonstrated a Provocative concentration of antigen (PCA) titer of approximately 1 in 3000 for IgG.¹⁷ Control guinea pigs received saline injection or inhalation by means of the same protocol.

Tracheal contraction in vitro

Spirals of approximately 0.5 cm long were placed in oxygenated Krebs'-Henseleit solution in a 5 ml jacketed organ bath under 1 gm resting tension. Isometric force was measured with a Grass FT.03 (Grass Instrument Co., Quincy, Mass.) transducer and recorded on a polygraph. Before the addition of drugs, the tissues were allowed to equilibrate for 30 minutes to obtain a stable baseline. All drugs were added in 50 μ l aliquots. Cumulative dose-response curves were obtained, with the maximal contractile activity to carbachol (1 mmol/L) assessed at the end of each experiment.

Cell culture

Airway smooth muscle cells from guinea pig trachea were isolated by the method of Devore-Carter et al.¹⁸ Guinea pigs (150 to 300 gm) were killed by saturating dose of pentobarbital sodium (500 mg/kg). Tracheas were excised aseptically, combined, and rinsed twice in ice cold Hanks' balanced salt solution (HBSS), supplemented with penicillin (100 units/ml), streptomycin (100 units/ml), and nystatin (10 units/ml). The connective tissues were carefully stripped off by blunt dissection with frequent rinsing of the tissue in HBSS to prevent desiccation. The tracheas were cut longitudinally on both sides of the muscle strip (through the rings opposite the muscle). The tracheas were incubated with an enzyme solution containing 0.2% collagenase and 0.05% elastase at 37° C for 30 minutes. The dissociated cells that were predominantly epithelial cells and other loosely attached non-smooth muscle cells were discarded. The tissue fragments were washed in HBSS once to remove all the dissociated non-smooth muscle cells. The remaining tissue was put in a solution of 0.2% collagenase and 0.05% elastase and digested for 1 to 1.5 hours in an atmosphere of 5% CO₂ at 37° C. The enzymatically digested trachea were then passed through a nylon mesh to remove the large debris and fragments. The cells were formed into pellets by spinning in a centrifuge at 250 g for 5 minutes. The supernatant was removed by aspiration, and the cells were resuspended in 1 to 5 ml of modified Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1; vol/vol) with 1% FBS without growth factors for 24 hours to eliminate the non-smooth muscle cells. After 24 hours the cells were washed with culture medium, and fresh medium containing 10% FBS was added. When cells were seeded at 1×10^4 /ml, they became confluent, usually after 10 to 14 days. To confirm that primary cultures were smooth muscle cells and not fibroblasts, several parameters of smooth muscle structure and function were analyzed. These included response to histamine and positive immunocytochemical staining techniques with use of monoclonal antibodies to smooth muscle α -actin. Smooth muscle cells in culture under the phase-contrast optics were spindle shaped, and when histamine (1 mmol/L) was added they became round/oval in shape. To carry out immunocytochemistry, cells were cultured on 1 cm glass coverslips and fixed in formalin. The fixed cells were incubated with anti- α -actin as described by BioGenex. With use of monoclonal antibodies, cells stained

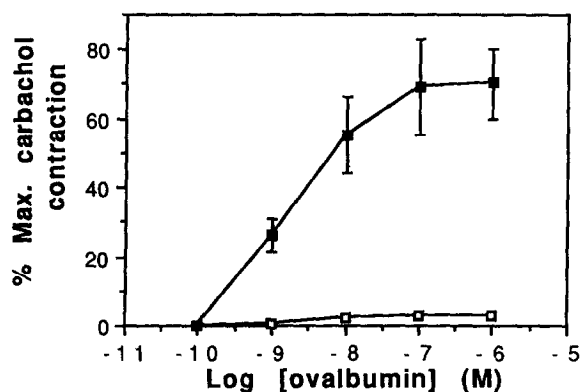


FIG. 1. Isometric responses of isolated guinea pig tracheal spirals from normal animals (□) and hyperresponsive animals challenged with ovalbumin (■) stimulated with increasing concentrations of ovalbumin. Mean $\pm$ SD, $n = 30$.

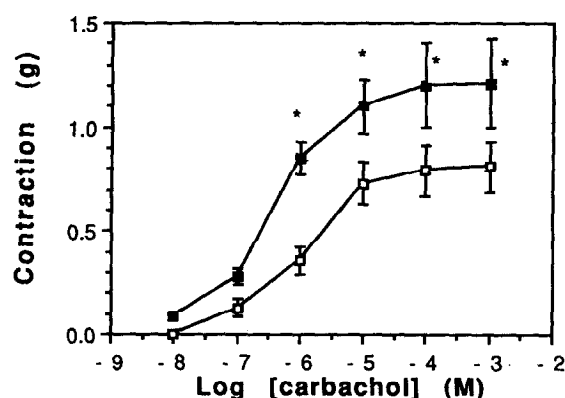


FIG. 2. Dose-response curve for carbachol-induced contraction of tracheal smooth muscles from normal (□) and hyperresponsive (■) guinea pigs. Mean $\pm$ SD, $n = 20$, $*p < 0.05$.

positive for α -actin. Cells were cultured in 60 mm Petri dishes and directly challenged with the agonists.

Polyphosphoinositide hydrolysis

Unless indicated cells were labeled with 2.5 μ Ci/ml myo[2- ^{3} H]inositol, total volume 2 ml, for 24 hours in an inositol-free DMEM medium (Gibco) and supplemented with 1% FBS. After 24 hours the unincorporated [^{3} H]inositol was removed by washing. Cells were then incubated in 2 ml of HBSS containing 2 mmol/L Ca^{2+} , 0.1% bovine serum albumin and 10 mmol/L lithium fluoride for 5 minutes. The cells were then challenged with the various contractile agonists (histamine, LTD $_4$, or carbachol) for various time periods. The reaction was stopped by the addition of 50 μ l concentrated HCl. To the reaction mixture 4.8 ml of methanol followed by 2.4 ml of chloroform and an additional 2.4 ml of water was added. The mixture was whirled and spun in a centrifuge at 1000 g for 10 minutes. From the supernatant 1.5 ml was removed and analyzed for the

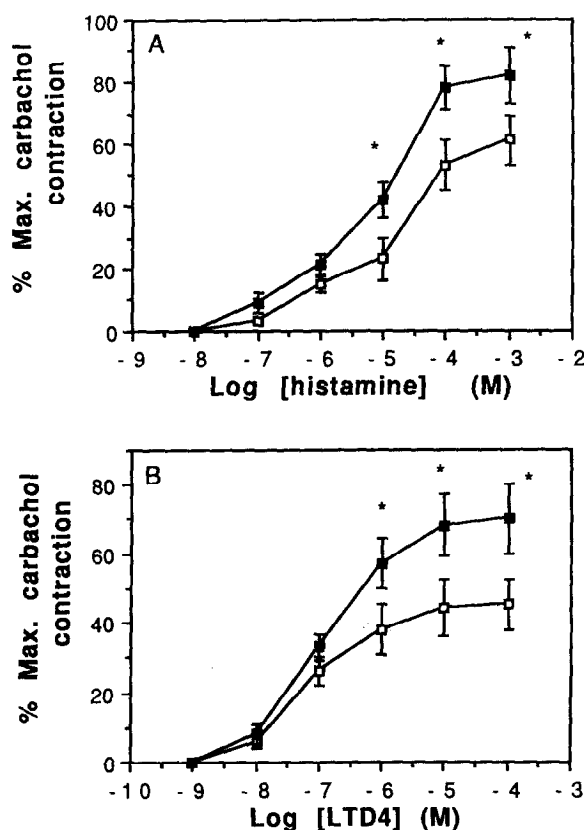


FIG. 3. Isometric responses of isolated guinea pig tracheal spirals from normal (□) and hyperresponsive animals (■) stimulated with increasing concentrations of histamine (A) and LTD $_4$ (B). Results are mean $\pm$ SE of eight experiments, $*p < 0.05$.

presence of inositol phosphates as described by Watson et al.¹⁹ For confirmation of inositol phosphate metabolites, the fractions were analyzed by high-performance liquid chromatography (HPLC) as described by Batty et al.²⁰

Statistical analysis. Data were expressed as means $\pm$ SE. Tracheal contractile responses to cumulative drug dosages were compared by use of a repeated measurements analysis of variance (ANOVA). Cellular inositol phosphate measurements were compared by use of Student's t test with correction for multiple comparisons.

RESULTS

Tracheal contraction

Tracheal smooth muscle from guinea pigs challenged with and sensitized to ovalbumin demonstrated dose-dependent contractile responses to addition of ovalbumin, whereas tracheas from unsensitized controls challenged with saline showed no response (Fig. 1).

Active force generation in grams of tension after addition of carbachol was greater in tracheas from antigen-challenged hyperresponsive guinea pigs than from controls challenged with saline (Fig. 2). The

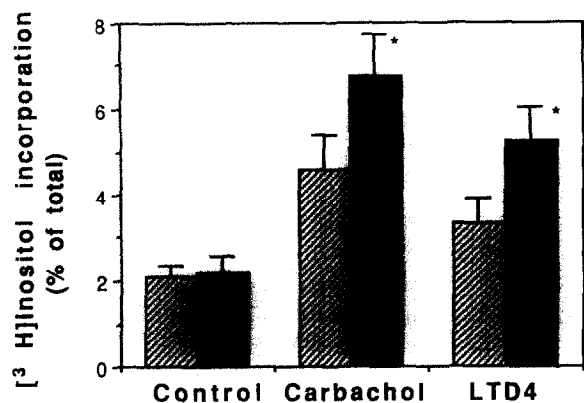


FIG. 4. Effect of labeling smooth muscle cells (1×10^7) from normal (▨) and hyperresponsive guinea pigs (■) in the presence of carbachol, LTD₄, or buffer alone. Results are mean $\pm$ SE of six experiments, * $p < 0.05$. In this experiment smooth muscles were incubated with 20 μ Ci/ml of myo[2-³H]inositol for 2 hours in the presence or absence of agonists.

maximal contractile response to 10^{-3} mol/L carbachol was significantly greater for tracheas from hyperresponsive animals compared with those from controls (1.13 ± 0.18 vs 0.81 ± 0.12 gm, for hyperresponsive and normal animals, respectively; $n = 20$, $p < 0.05$). The isometric response of guinea pig tracheas to cumulative doses of histamine and LTD₄ are shown in Figs. 3 A and B, respectively. As noted for carbachol, the response of tracheas from hyperresponsive guinea pigs was 30% to 50% greater than the response of tracheas from normal guinea pigs.

Inositol phosphate formation

Initial studies investigated the rate of myo[2-³H]inositol incorporation into smooth muscle cells from normal and hyperresponsive guinea pigs over a 2-hour period in the presence or absence of contractile agonists (carbachol and LTD₄). As can be seen from Fig. 4, stimulation by carbachol or LTD₄ caused cells of hyperresponsive animals to incorporate greater amounts of [³H]inositol than cells of normal animals. However, under basal conditions (i.e., absence of agonist) the rate of myo[2-³H]inositol incorporation was similar in both cell sources.

The dose-response curves for agonists causing inositol phosphate formation in smooth muscle cells from normal guinea pigs are shown in Fig. 5. When [³H]inositol-labeled cells were stimulated with carbachol, LTD₄, or histamine, appreciable amounts of inositol phosphates (IP₁, IP₂, and IP₃) were generated. Carbachol was the strongest stimulus and histamine the weakest (Fig. 5). Carbachol and LTD₄ induced inositol phosphate formation at doses similar to those

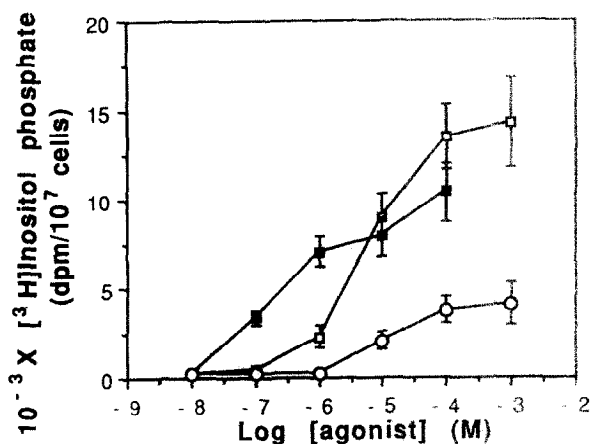


FIG. 5. Dose-response effects of carbachol (□), histamine (○), and LTD₄ (■) on the hydrolysis of PIP₂ in airway smooth muscles (1×10^7 cells) in culture from normal guinea pigs. Values are the total radioactivity associated with inositol phosphates (IP₁, IP₂, and IP₃) together, released by smooth muscle cells from normal guinea pigs during 30 minutes of stimulation. Mean $\pm$ SE of six experiments

TABLE I. Approximate IC₅₀ values for contraction and inositol phosphate formation in airway smooth muscle cells from normal guinea pigs after stimulation with various agonists

Agonist	Contraction IC ₅₀ (μmol/L)	Inositol phosphate formation IC ₅₀ (μmol/L)
Carbachol	1	10
LTD ₄	0.1	0.7
Histamine	10	50

that caused contraction of tracheal muscles. For all agonists the threshold contractile responses occurred at doses approximately 1 log less than those required to detect changes in inositol phosphate formation (Table I). The doses required to obtain maximal inositol phosphate formation for carbachol, LTD₄, and histamine were 100 μmol/L, 1 μmol/L and 1 mmol/L, respectively. The above concentrations of agonists were therefore used throughout the present study.

Guinea pig smooth muscles activated with carbachol, LTD₄, and histamine generated IP₃ as the predominant product, but an appreciable amount of inositol-1,3,4-trisphosphate was also generated, particularly at times greater than 5 minutes (percentage of IP₃ in the form of inositol-1,4,5-P₃ vs inositol-1,3,4-P₃ after 5, 30, 60, 120, 300, and 420 seconds of stimulation with carbachol were 100 vs 0, 100 vs 0,

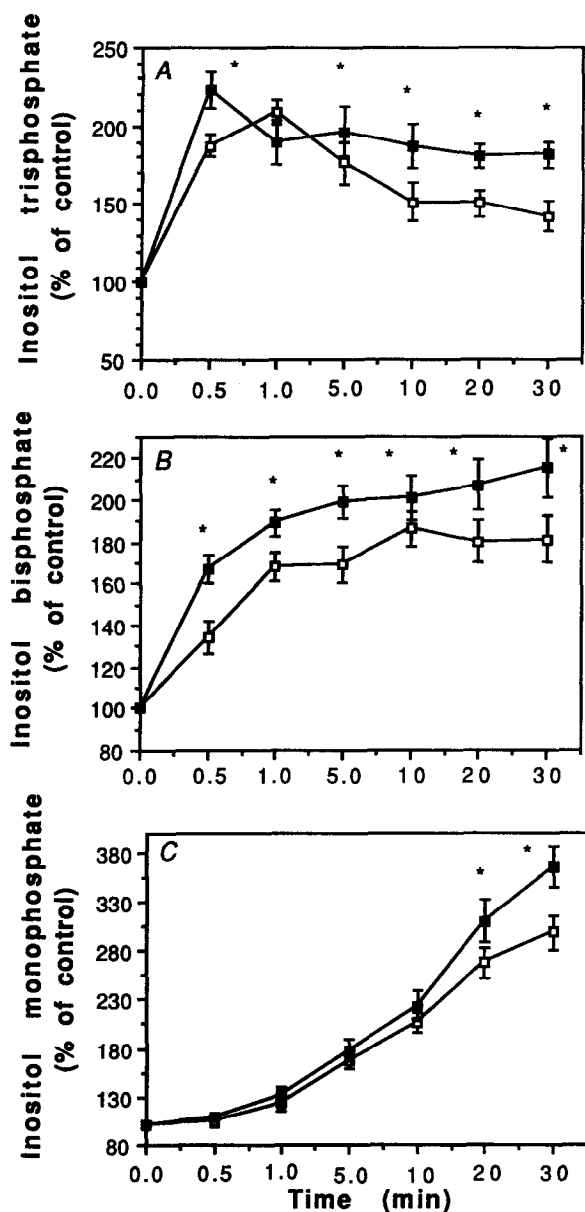


FIG. 6. Formation of inositol phosphates, IP₃ (A), IP₂ (B), IP₁ (C), in smooth muscle cells (5×10^6 cells) from normal (□) and hyperresponsive guinea pigs (■) after activation with 100 $\mu\text{mol/L}$ carbachol. Results are mean $\pm$ SE of eight experiments, * $p < 0.05$.

90 vs 10, 80 vs 20, 60 vs 40, and 60 vs 40, respectively), and no appreciable amount of IP₄ was detected with the HPLC system. For that the data presented for IP₃ in the present study are the combined IP₃ isotypes.

When [³H]inositol-labeled smooth muscle cells from normal and hyperresponsive guinea pigs were challenged with 100 $\mu\text{mol/L}$ carbachol, a rapid metabolism of polyphosphoinositides was observed. Fig. 6, A to C show the kinetics of [³H]IP₁, [³H]IP₂, and [³H]IP₃ formation in the normal and hyperresponsive

smooth muscle cells stimulated with 100 $\mu\text{mol/L}$ carbachol. The level of IP₃ increased rapidly in both smooth muscle cell populations, reaching a maximum at 0.5 to 1 minute. In normal cells the level of IP₃ decreased after 1 minute to approximately 40% above the unstimulated level, whereas in hyperresponsive cells the IP₃ level remained at 80% above the baseline. As can be seen from Fig. 6, A, the IP₃ generated by hyperresponsive cells was approximately 30% to 50% higher than in normal cells. The maximal release of IP₂ was observed after 5 minutes (Fig. 6, B). The level of IP₂ in cells from hyperresponsive animals was approximately 20% greater than the level in normal smooth muscle cells. The formation of IP₁ in both cell sources was slow, and a plateau of response was not obtained (Fig. 6, C). In both cell sources the formation of IP₁ began after 1 minute and continued to increase for 30 minutes. At 30 minutes the level of IP₁ was three times above baseline and 20% greater in hyperresponsive cells (Fig. 6, C).

Fig. 7, A to C shows the generation of [³H]IP₁, [³H]IP₂, and [³H]IP₃ from normal and hyperresponsive guinea pigs stimulated with 1 $\mu\text{mol/L}$ LTD₄ for various lengths of time. As seen in Fig. 7, A, LTD₄ caused a rapid hydrolysis of PIP₂ leading to the formation of IP₃. The maximal formation of IP₃ in both cell sources was seen at 1 minute. After 1 minute the level of IP₃ in normal animals declined, whereas it remained steady for 20 minutes in cells from hyperresponsive animals. Smooth muscle cells from hyperresponsive guinea pigs generated 30% more IP₃ than those from normal animals. The levels of IP₂ and IP₁ were also greater in cells from hyperresponsive animals than cells from normal animals (Fig. 7, B and C). The kinetics of IP₂ and IP₁ formation in both cell sources stimulated with LTD₄ were similar to those stimulated with carbachol; however, the magnitude of response was different (i.e., cells stimulated with carbachol released more IP₂ and IP₁ than the cells stimulated with LTD₄).

With use of 1 mmol/L histamine as stimulus, cells of hyperresponsive animals released approximately 20% more inositol phosphates than cells of normal animals. Since histamine was found to be a weak agonist of PIP₂ hydrolysis the major difference was seen at the IP₁ level. The level of IP₃ and IP₂ after 30 minutes of histamine stimulation in both cell sources were low, but the level of IP₁ was approximately two-fold above the baseline.

To investigate the lipid source of inositol phosphates in smooth muscle cells the total phospholipid analyses were performed with use of thin layer chromatography (TLC) technique.²¹ Cells were incubated with 1 $\mu\text{Ci/ml}$ of [³H]phosphatidylinositol, [³H]PIP, or [³H]PIP₂ for 1 hour before stimulation with carbachol.

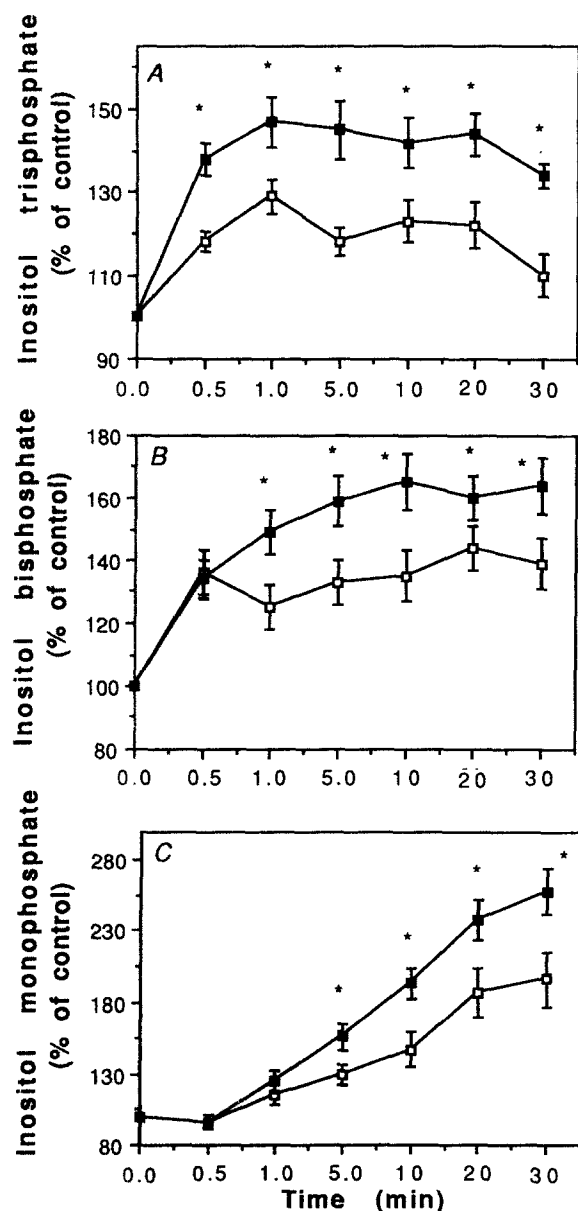


FIG. 7. Formation of inositol phosphates, IP_3 (A), IP_2 (B), IP_1 (C), in smooth muscle cells from normal ($\square$) and hyperresponsive guinea pigs ($\blacksquare$) after activation with $1 \mu\text{mol/L}$ LTD_4 . Results are mean of nine experiments $\pm$ SE, $*p < 0.05$.

The degradation of radiolabeled phospholipids was monitored in cells of normal and hyperresponsive animals. As can be seen in Fig. 8, $[\text{H}]\text{PIP}_2$ was the only phospholipid whose level dropped after carbachol stimulation. Approximately 20% more degradation occurred of $[\text{H}]\text{PIP}_2$ in cells of sensitized guinea pigs.

DISCUSSION

Our results show that increased smooth muscle contractile activity of tracheal preparations from guinea

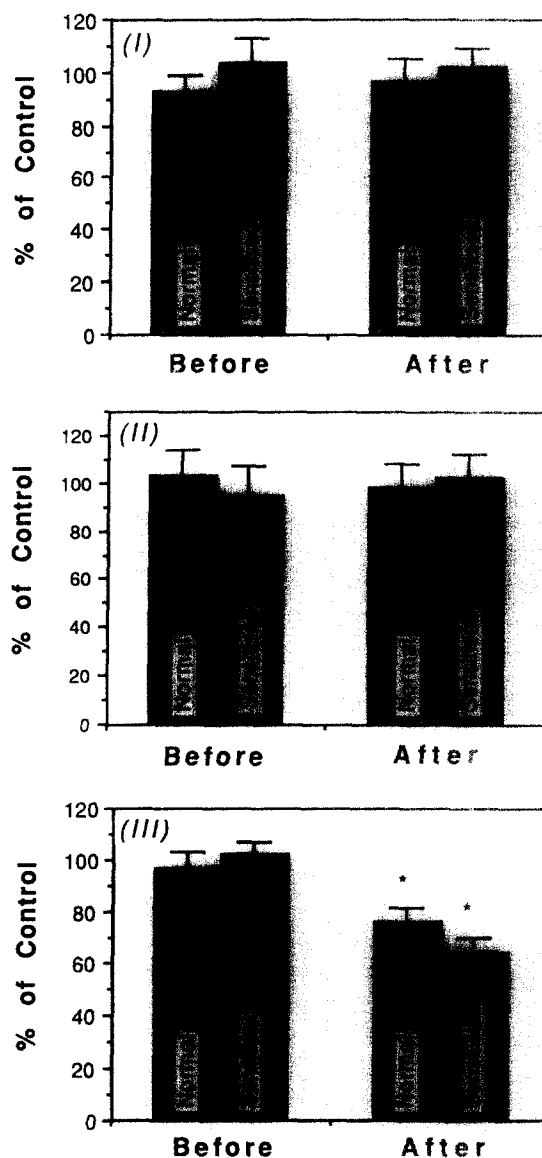


FIG. 8. Polyphosphoinositide hydrolysis in airway smooth muscles from normal and hyperresponsive guinea pigs before and after stimulation. Cells (10×10^7) from either animal sources were incubated with $1 \mu\text{Ci}$ of either $[\text{H}]\text{PI}$, $[\text{H}]\text{PIP}$, or $[\text{H}]\text{PIP}_2$ for 1 hour before stimulation with $100 \mu\text{mol/L}$ carbachol. The membrane lipids were analyzed by TLC, and the radioactivity associated with each lipid bands were counted. The degradation of $[\text{H}]\text{phosphatidylinositol}$ (I), $[\text{H}]\text{PIP}$ (II), and $[\text{H}]\text{PIP}_2$ (III) are shown. Results are the mean of four experiments in triplicate $\pm$ SE, $*p < 0.05$.

pigs demonstrating hyperresponsiveness in vitro is associated with increased polyphosphoinositide hydrolysis. The increased tracheal contractile responses confirm the work of Ishida et al.¹² and Antonissen et al.¹¹ in which they also observed increased contractility of tracheal smooth muscle from hyperresponsive animals. However, our finding of enhanced polyphosphoinositide hydrolysis in smooth muscle cells

of hyperresponsive animals has not been previously reported.

Earlier studies with tracheal smooth muscle from hyperresponsive dogs have suggested that there are alterations in resting membrane potentials,^{22, 23} myofibrillar adenosine triphosphatase activity.²⁴⁻²⁶ It is difficult to define a common mechanism by which these three changes may be brought about, or to relate them to alterations in inositol phosphates.

Our findings that the smooth muscle cells of hyperresponsive guinea pigs have enhanced inositol uptake while undergoing stimulation suggest that the changes must be associated with the agonist-stimulated cellular events, since no significant changes were seen with the two cell populations under resting conditions. The increased myo[2-³H]inositol uptake by hyperresponsive cells during stimulation indicates a rapid turnover of polyphosphoinositides during metabolism of inositol phosphates. Since cells of hyperresponsive animals generate greater amount of inositol phosphates, it is likely that these cells have enhanced phospholipid synthesis. Otherwise the persistent activation of phospholipase C (PLC) would deplete phosphoinositide pools. The extent of agonist-induced [³H]inositol labeling in tracheal smooth muscle is considerable. In the present study we found an almost threefold increase in [³H]inositol labeling, which is comparable to that shown for α_1 -adrenoceptor.²⁷ The increase in [³H]inositol labeling by cells of hyperresponsive animals suggests enhanced PLC activity, which causes the breakdown of phospholipid pools.²⁸⁻³⁰ Both muscarinic and histaminic receptors are coupled to PIP₂-specific PLC. Hydrolysis of PIP₂ leads to the generation of IP₃ that undergoes dephosphorylation to IP₂ and subsequent dephosphorylation to IP₁.^{31, 32} Although it has been reported that bovine tracheal smooth muscles generate greater amount of inositol-1,3,4-trisphosphate than inositol-1,4,5-trisphosphate,³¹ we found in guinea pig smooth muscles IP₃ to be predominant, with no appreciable accumulation of IP₄. It is not known whether the differences in metabolism of inositol phospholipids in cells from bovine and guinea pigs are due to their species differences or are due to differences in culture versus whole tissue slices.

The mechanism(s) for increased phosphatidylinositol hydrolysis in hyperresponsive cells is not known, but several hypotheses might explain these changes. These hypotheses are derived from the studies dealing with the mechanisms of exaggerated contraction of smooth muscles in hypertensive animals. In rabbits and rats the level of inositol phosphate formation in vascular tissues was found to be significantly higher in hypertensive animals than in normotensive

ones.³³⁻³⁶ The increased inositol phosphate formation was found to be due to the enhancement in PLC activity in these cells, because the level of inositol phosphates before stimulation was similar in both cell sources. In our study we did not observe significant differences in the basal level of inositol phosphate formation between cells from the two animal groups, either when expressed per number of cells or milligram of protein. This suggests that the increased inositol phosphate formation was not caused by an increased uptake of myo[2-³H]inositol within one cell population. Thus in hyperresponsive animals an alteration occurs in phosphatidylinositol metabolism when these cells undergo repeated activation during the period of sensitization. One possible mechanism for enhanced phosphatidylinositol hydrolysis in hyperresponsive guinea pig airway smooth muscles could be an enhancement of PLC activity. The other potential mechanism for enhanced inositol phosphate formation is an increase in receptor number or receptor affinity.^{37, 38} It is possible that airway smooth muscles from hyperresponsive guinea pigs have developed alterations at the receptor level for various contractile agonists. We think this is unlikely because of the large number of receptor types that would need to be affected.

In rat brain cortical slices the potentiation of inositol phosphate formation was seen with cells undergoing depolarization.³⁹ Since the sensitized smooth muscles have also been found to be partially depolarized,^{22, 23} the possibility that increased inositol phosphate formation is due to this factor should be taken into consideration. The enhanced phosphatidylinositol hydrolysis can also be due to alteration in receptor-coupled G-proteins and G-protein distribution. Asano et al.^{40, 41} found that the arterial relaxant responses to β -adrenoceptor agonists were decreased in spontaneously hypertensive rats when compared with normotensive rats, and this was due to an impairment in the function of stimulatory G-protein. Reduction in the activity of the stimulatory G-protein in the myocardium of spontaneously hypertensive rats has also been demonstrated by Murakami et al.,⁴² suggesting that abnormal signal transduction is operating in the hypertensive rats. Whether similar mechanisms operate in airway smooth muscles of hyperresponsive guinea pigs is not known. The marked increase in inositol labeling between normal and hyperresponsive cells may suggest that there are important differences in receptor occupation and response relationships in these tissues that relate to their function in pharmacomechanical coupling.

In conclusion, our results demonstrate a significant difference in the formation of inositol phosphates

within airway smooth muscle cells from normal and hyperresponsive guinea pigs. Further studies are required to determine whether an abnormal activity of PLC or other components of second messenger systems account for airway hyperresponsiveness.

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