

## Slow reacting substances (leukotrienes): Enzymes involved in their biosynthesis

(glutathione transferase/ $\gamma$ -glutamyltransferase/guinea pig lung/RBL-1 cells)

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**ABSTRACT** Slow reacting substances (leukotrienes C<sub>4</sub>, D<sub>4</sub>, E<sub>4</sub>) are synthesized *in vivo* by a combination of two previously unrelated pathways: lipoxygenase oxygenation of arachidonic acid and the glutathione detoxification pathway. Enzymes involved in the latter pathway (glutathione transferase [RX: glutathione R-transferase, EC 2.5.1.18];  $\gamma$ -glutamyltransferase [(5-glutamyl)-peptide: amino acid 5-glutamyltransferase, EC 2.3.2.2]) have been investigated in guinea pig lung and rat basophilic leukemia (RBL-1) cells. We report data on levels of enzymic activity both before and during the release of slow reacting substances. Both glutathione transferase and  $\gamma$ -glutamyltransferase are present in significant quantities in guinea pig lung and RBL-1 cells. A model for the changes in  $\gamma$ -glutamyltransferase during leukotriene release is proposed for the cell line, and differences from the guinea pig lung system are reported. Leukotriene C<sub>4</sub> is converted to the more potent leukotriene D<sub>4</sub> by the action of  $\gamma$ -glutamyltransferase on guinea pig ileum during bioassay.  $\gamma$ -Glutamyltransferase may represent a control feature in the biosynthesis of leukotriene D<sub>4</sub>, and thus be involved in leukotriene-induced bronchoconstriction in the lung.

For more than 40 years since their discovery (1), the structures of a group of closely related materials, known collectively as the slow reacting substances, remained unknown. Perhaps the most interesting of these substances, which are released from various tissues by a number of different stimuli, was the immunologically generated material slow reacting substances of anaphylaxis (SRS-A) because of its implied role in hypersensitivity reactions such as asthma.

It is only recently that the structures of these substances have been determined as a novel class of peptidolipids—the leukotrienes. The first covalent structure reported was that of leukotriene C<sub>4</sub> (LTC<sub>4</sub>) determined as the S-glutathionyl conjugate of an arachidonic acid metabolite 5-(S)-hydroxy-6-(R)- $\gamma$ -glutamylcysteinylglycyl-7,9,11,14-icosatetraenoic acid (2, 3). Concomitantly, the major biologically active species of SRS-A was defined as the related structure 5-(S)-hydroxy-6-(R)-cysteinylglycyl-7,9,11,14-icosatetraenoic acid (4–6), later termed “leukotriene D<sub>4</sub>” (LTD<sub>4</sub>) (7).

Examination of the structure of LTC<sub>4</sub> and LTD<sub>4</sub> clearly indicated that they were both formed by the same biosynthetic route: a combination of oxygenation of arachidonic acid by 5-lipoxygenase (8–10) and further metabolism by the glutathione detoxification pathway (11), involving the enzymes glutathione transferase (GSHTase; RX: glutathione R-transferase, EC 2.5.1.18) and  $\gamma$ -glutamyltransferase [GluTase; (5-glutamyl)-peptide: amino acid 5-glutamyltransferase, EC 2.3.2.2]. Fur-

ther steps on this pathway would be expected to result in the formation of a cysteinyl and an N-acetylcysteinyl leukotriene; the former has now been reported and termed “leukotriene E<sub>4</sub>” (LTE<sub>4</sub>) (12).

The action of a 5-lipoxygenase in the biosynthesis of SRSs (LTC<sub>4</sub>, -D<sub>4</sub>, and -E<sub>4</sub>) has been studied (10, 13). To determine the role of two of the enzymes in the glutathione detoxification pathway (GSHTase and GluTase), with the view to their possible control *in vivo*, we have investigated their levels in both rat basophilic leukemia cells (RBL-1 cells) and guinea pig lung systems during the release of SRSs.

High basal levels of both enzymes are present in lung and the cultured cells. However, only GluTase increases concomitantly with SRS release but this increase does not represent a simple leakage of cell contents. We also report the conversion (and inhibition of it) of LTC<sub>4</sub> to the more potent LTD<sub>4</sub> by GluTase in guinea pig ileum during bioassay.

### MATERIALS AND METHODS

**Bioassay.** Samples were assayed on strips of guinea pig ileum smooth muscle that were superfused in series in the presence of mepyramine, hyoscine, and indomethacin (14). FPL 55712 (Fisons, Loughborough, England) was superfused over at least one strip so that any SRS-like contractile activity could be tested for antagonism by the SRS-A antagonist.

**LTC<sub>4</sub> Activation by Guinea Pig Ileum.** LTC<sub>4</sub> and LTD<sub>4</sub> (gifts from J. Rokach, Merck-Frosst Laboratories, Pointe-Claire, Quebec, Canada) were assayed in a cascade of six strips of longitudinal smooth muscle from guinea pig ileum superfused in two banks of three in series. The time required for these leukotrienes to pass from the first tissue to the last tissue was 1 min.

**Enzyme Assays.** GSHTases were measured by the method of Habig *et al.* (15). One unit of enzyme causes conjugation of 1  $\mu$ mol of 1-chloro-2,4-dinitrobenzene with glutathione per minute at 30°C and pH 6.5. The epoxide substrate 1,2-oxido-3-(3-nitrophenoxy) propane was used in some assays.

GluTase was measured by the method of Szasz (16). One unit of enzyme releases 1  $\mu$ mol of *p*-nitroaniline per min at 37°C and pH 8.6. Glycylglycine was present as the  $\gamma$ -glutamyl acceptor. Glucose-6-phosphate dehydrogenase was measured by the method of Kornberg and Horecker (17). One unit of enzyme activity converts 1  $\mu$ mol of substrate per min at 30°C and pH 8.

Reagents for the enzyme assays, and partially purified

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Abbreviations: LTA<sub>4</sub>, -C<sub>4</sub>, -D<sub>4</sub>, -E<sub>4</sub>, leukotrienes A<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub>, E<sub>4</sub>; SRS (-A), slow reacting substances (of anaphylaxis); GSHTase, glutathione transferase; GluTase,  $\gamma$ -glutamyltransferase; RBL-1 cells, rat basophilic leukemia cells; U, unit.

GluTase as a standard, were obtained from Sigma. Changes in the UV/visible absorption were monitored on a Cary 210 spectrophotometer.

**GluTase Inhibitors.** Serine/borate ( $K_i = 1.45$  mM) (18) was prepared by using Analar grade chemicals (BDH), azaserine ( $K_i = 5$  mM) (19) was obtained from Sigma, and  $\delta$ -D-glutamyl-(*O*-carboxy)phenylhydrazine ( $K_i = 22.5$   $\mu$ M) (18) was prepared by J. Rokach. Inhibition studies were carried out with these inhibitors over a range of concentrations from 0.1 to 10  $K_i$ .

**RBL-1 Cells.** These were grown in spinner culture in Eagle's minimal essential medium supplemented with a mixture of penicillin and streptomycin, 10% fetal calf serum, and 10% newborn calf serum (GIBCO) in an atmosphere of 5% CO<sub>2</sub> in air. Cells were harvested and incubated ( $10^7$  cells per ml) as reported (4). LTD<sub>4</sub> was generated in the presence of indomethacin (1  $\mu$ g/ml), arachidonic acid (25  $\mu$ g/ml), and L-cysteine (1.2 mg/ml) by stimulating the cells with calcium ionophore A 23187 (10  $\mu$ g/ml); additions were at 0, 15, 30, and 32.5 min, respectively (final concentrations given in parentheses). Indomethacin and arachidonic acid were prepared as ethanolic solutions, L-cysteine was added as a solid, and ionophore was a solution in dimethyl sulfoxide; the final concentrations of ethanol and dimethyl sulfoxide did not exceed 0.2%. Indomethacin was supplied by Merck Sharp and Dohme, the ionophore was from Calbiochem, and the other reagents were obtained from Sigma.

Two aliquots (1 ml) were removed at various stages of the incubation and centrifuged immediately ( $10,000 \times g$ , 1 min). One set of supernatants was assayed on guinea pig ileum smooth muscle strips for biological activity; the other set was assayed for GSHTase, GluTase, or glucose-6-phosphate dehydrogenase. The cells removed by centrifugation were lysed in water ( $10^7$  cells per ml), centrifuged ( $11,500 \times g$ , 10 min), and assayed as above. Separate aliquots (cells plus supernatant) were treated with Triton X-100 (1% in the incubation buffer), centrifuged ( $10,000 \times g$ , 1 min) and assayed as described above.

Control incubations were carried out at the same time as normal incubations but in the absence of (i) indomethacin, (ii) arachidonic acid, (iii) L-cysteine, or (iv) ionophore; ethanol or dimethyl sulfoxide was added as appropriate. 5,8,11,14,17-icosapentaenoic acid (NU Chek Prep, Elysian, MN) was used in place of arachidonic acid in two incubations.

**Guinea Pig Lung.** Perfused lungs from guinea pigs previously sensitized to ovalbumin were challenged with antigen as reported (14) and the perfusate was collected. During the perfusion, lung pieces (100 mg) were removed and homogenized at 0°C in Tyrode solution (with and without Triton X-100) and centrifuged ( $5,000 \times g$ , 15 min). The supernatant was assayed for enzyme activity and biological activity on muscle strips. The experiments were repeated with perfused lung from unsensitized guinea pigs.

## RESULTS

### GSHTase

**RBL-1 Cells.** Low basal levels of GSHTase were observed in the cell supernatant (2.5 mU/ml) and increased approximately 2-fold during the incubation, although maximal increase of GSHTase did not always correspond with ionophore-induced leukotriene release. Lack of indomethacin in the incubation medium did not appear to affect the enzyme level. Somewhat higher levels of GSHTase were observed in the centrifuged and lysed cells (5 mU/ml) and they increased approximately 2.5 times on addition of ionophore. In both cases, a wide spread of data points was observed at this level, which was at the minimal level of detection of the enzyme.

**Guinea Pig Lung.** Prior to challenge, higher basal levels of GSHTase were observed in homogenized lung pieces from unsensitized animals (26.5 U/g) compared with samples from sensitized animals (21.5 U/g). On challenge, a slight increase (after a 5-min lag) was observed in the sensitized lung pieces (Fig. 1). Very low levels of GSHTase were observed in the perfusate of the sensitized lung (<1 U/ml); no observable increase occurred on challenge. The earliest release of SRS-A from the sensitized lung occurred 30 sec after challenge; and a maximum was reached after 2.5 min, after which there was a slow decrease in output over the next 15 min. No GSHTase activity was observed in guinea pig lung (in the amount of tissue described) with the epoxide substrate, 1,2-oxido-3-(3-nitrophenoxy)propane.

### GluTase

**RBL-1 Cells.** High basal levels of GluTase were observed in both the supernatant (12 mU/ml) and the lysed cells (60 mU/ml). A general increasing trend in GluTase levels in the cell supernatant was observed on addition of indomethacin, arachidonic acid, and L-cysteine. However, on addition of ionophore, a rapid decrease to almost basal level was observed, followed by an increasing release of GluTase into the incubation medium (to approximately 40 mU/ml at 52.5 min) (Fig. 2a). A small increase (<10%) of the GluTase level in the supernatant occurred between 52.5 and 62.5 min (not shown in Fig. 2a). In each incubation the characteristic dip-followed-by-rapid-rise pattern in GluTase levels was observed.

SRS release, assayed as LTD<sub>4</sub>, was observed after addition of arachidonic acid (<20%) and reached a maximum (100%) which coincided with the maximum increase in GluTase ( $t = 52.5$  min); the level of SRS remained constant for a further 20 min. In the absence of indomethacin, L-cysteine, or arachidonic acid, the final GluTase level was decreased—namely, 85%, 80%, and 60% of normal levels, respectively, at  $t = 52.5$  min.

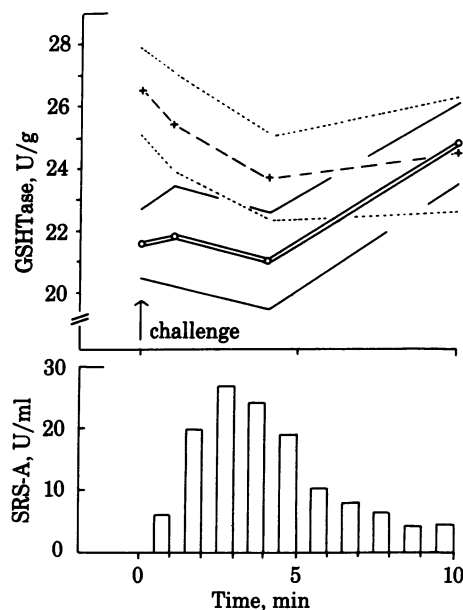


FIG. 1. Total GSHTases in guinea pig lung pieces (1-chloro-2,4-dinitrobenzene as substrate in the assay). SRS-A, assayed as LTD<sub>4</sub>, was released only from the sensitized lung on challenge; release occurred into the perfusate after 30 sec and reached a maximum at 2.5 min. No direct correlation may be drawn between total GSHTase levels and SRS-A (LTD<sub>4</sub>) release; however, differences between the sensitized (○—○) and unsensitized (+---+) lungs are apparent. Mean ( $\pm$ SEM) values of five sets of experiments are plotted. GSHTase was observed in the lung perfusate but at minimal levels of detection.

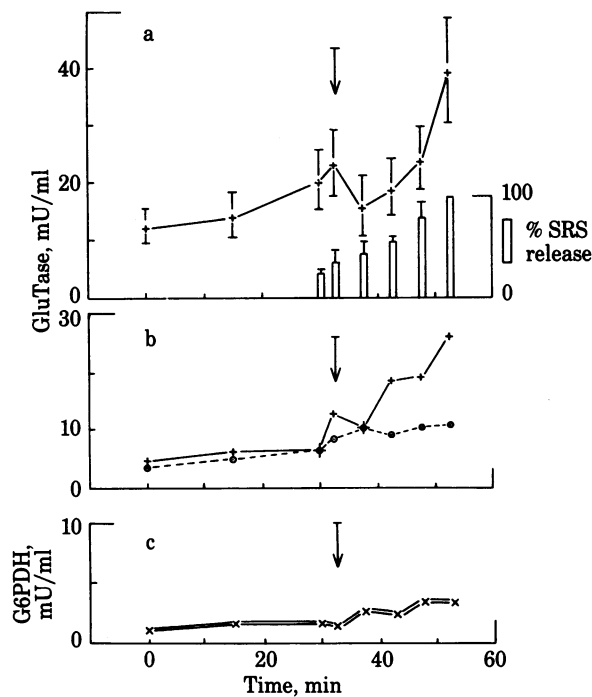


FIG. 2. (a) GluTase levels (+) in RBL-1 cell supernatant showing the characteristic dip-followed-by-rise pattern on addition of ionophore at  $t = 32.5$  min (arrow). The change in GluTase levels between 52.5 and 62.5 min was  $<10\%$  (not shown). SRS (vertical bars), assayed as LTD<sub>4</sub>, was released on addition of ionophore and reached a maximum concomitantly with GluTase release (52.5 min); no further change occurred over the following 20 min. Mean ( $\pm$ SEM) values of 13 experiments (9 at  $t = 37.5, 42.5,$  and  $47.5$  min) are plotted. (b) Control incubations. When dimethylsulfoxide alone was added ( $\circ$ ) at  $t = 32.5$  min (without the ionophore), the final GluTase levels were greatly reduced. The dip-followed-by-rise pattern was not present. Means ( $\pm$ SEM) of six paired experiments are plotted. During the course of this study the basal level of GluTase released into the supernatant decreased. (c) Release of the cytosolic enzyme glucose-6-phosphate dehydrogenase (G6PDH) into the supernatant did not mirror GluTase release, suggesting that simple cell leakage was not the primary cause of increased GluTase levels.

When 5,8,11,14,17-icosapentaenoic acid was used in place of arachidonic acid the final level of GluTase was again decreased (75%). In a control experiment using only the ionophore solvent dimethyl sulfoxide, the final GluTase level ( $t = 52.5$  min) was  $<40\%$  of the normal value (Fig. 2b); also, the dip-followed-by-rapid-rise pattern shown in Fig. 2a was not observed.

A decrease in the basal level of GluTase in the cell supernatant was seen during the course of this study (16 months); however, on ionophore treatment the characteristic pattern of GluTase release was always observed. The basal level of glucose-6-phosphate dehydrogenase (1 mU/ml) increased 3-fold during incubation but the change was approximately linear and did not follow the variations in GluTase activity (Fig. 2c).

The GluTase level in the lysed cells did not change significantly after ionophore treatment although a small upward trend in activity was observed during the incubation.

To determine whether reagents used in SRS (leukotriene) generation caused activation of total cell GluTase, the enzyme solubilized by Triton X-100 was measured in samples containing both cells and supernatant during the incubation. In the presence of Triton X-100, 10-fold higher GluTase levels (650 mU/ml) were observed, compared with enzyme from lysed cells (without Triton X-100) plus the supernatant measured separately. No significant change in GluTase level occurred during the incubation in the presence of Triton X-100. No activation/

inhibition of commercially available GluTase occurred during control assays with the incubation reagents (indomethacin, arachidonic acid, L-cysteine, and ionophore).

**Guinea Pig Lung.** High basal levels of GluTase were observed in homogenates of lung tissue from both sensitized (219 mU/g) and unsensitized (272 mU/g) guinea pigs. On antigen challenge only sensitized lung responded immediately with an increase in the enzyme (21%). A small increase ( $<10\%$ ) was observed for the unsensitized lung but only after a definite time lag (Fig. 3a). When the lung pieces were homogenized in the presence of Triton X-100, an increase in GluTase was again observed in the sensitized lung on challenge (14% increase from the higher basal level of 237 mU/g); however, GluTase levels in samples from unsensitized lung remained constant (Fig. 3b). Very little GluTase was released into the lung perfusate, but a small increase was observed on antigen challenge.

SRS-A (LTD<sub>4</sub>) was released into the perfusate only by the sensitized lung; release began 30 sec after challenge and reached a maximum after 2.5 min.

#### LTC<sub>4</sub> activation

LTC<sub>4</sub> and -D<sub>4</sub> were assayed on six strips of smooth muscle suspended in series in two banks of three tissues. When 40 pmol of LTC<sub>4</sub> was administered, a contraction equal in height to that due to 4 pmol of LTD<sub>4</sub> was obtained in the first tissue (Fig. 4). This LTC<sub>4</sub>-induced contraction increased progressively and, 60 sec later, in the last tissue, the height of the response to LTC<sub>4</sub> was greater than that caused by 4 pmol of LTD<sub>4</sub>. Furthermore, the shape of the LTC<sub>4</sub>-induced contraction had changed to shorter duration and more LTD<sub>4</sub>-like on the last tissue.

In the presence of 0.25 mM  $\delta$ -D-glutamyl-(*O*-carboxy)-phenylhydrazine, a change in the shape and relative height

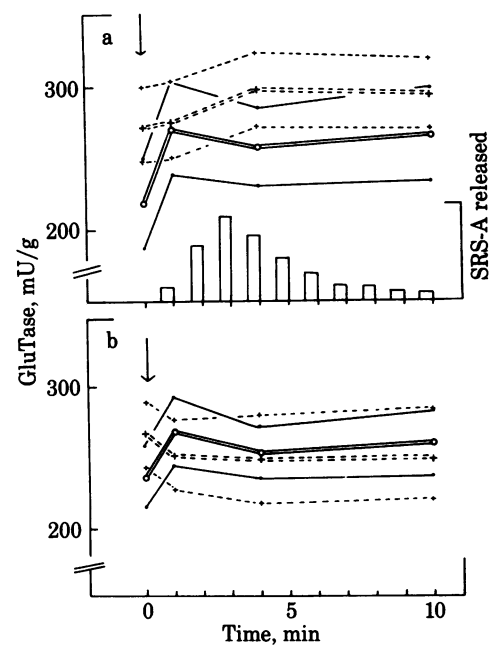


FIG. 3. (a) GluTase levels in homogenized guinea pig lung pieces. Only the sensitized lung ( $\circ$ ) responded immediately on challenge (arrow) with an increased GluTase level. +---+, unsensitized levels. SRS-A (LTD<sub>4</sub>) (vertical bars) was released into the perfusate only from the sensitized lung after 0.5 min; maximal release occurred at 2.5 min. Mean ( $\pm$ SEM) results of nine experiments are plotted. (b) GluTase levels in lung pieces homogenized in the presence of Triton X-100. An increase in GluTase on challenge was again observed only for the sensitized lung (from an increased basal level). GluTase was released into the perfusate, but at just detectable levels.

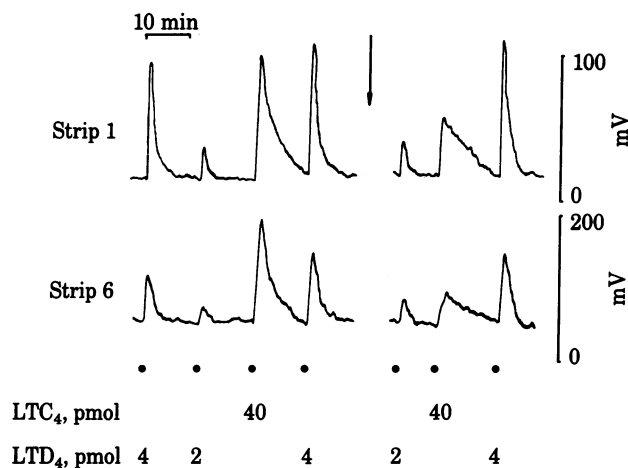


FIG. 4. Activation of LTC<sub>4</sub> during superfusion over smooth muscle strip from guinea-pig ileum. LTC<sub>4</sub> and LTD<sub>4</sub> were superfused over six strips; results with the first and sixth strips are shown, the time lag between them being 60 sec. (Left) In the first strip 40 pmol of LTC<sub>4</sub> caused contraction equivalent in height to that produced by 4 pmol of LTD<sub>4</sub>. In the last strip this had increased significantly. (Right) In the presence of 0.25 mM  $\delta$ -D-glutamyl-(O-carboxy)phenylhydrazine (added at arrow), the contraction elicited by 40 pmol of LTC<sub>4</sub> was reduced to the equivalent of that elicited by approximately 2 pmol of LTD<sub>4</sub>, and the duration of the response increased. The presence of GluTase on the strip surface was confirmed by the method of Szasz (14).

of contraction elicited by LTC<sub>4</sub> was observed. The LTC<sub>4</sub>-induced contraction (40 pmol) became slower and its relative height decreased from the first to the sixth tissue; the contraction due to LTD<sub>4</sub> remained unaffected.

## DISCUSSION

The possible role of the slow reacting substances [in particular, SRS-A (LTD<sub>4</sub>)] in allergic bronchospasm (asthma) in man lends direct pathophysiological relevance to a study of the biosynthetic enzymes involved in their generation. The action of 5-lipoxygenase on arachidonic acid, producing the active oxido intermediate leukotriene A<sub>4</sub> (LTA<sub>4</sub>), has been well studied (20) but to date little is known about the role of either GSHTase or GluTase during leukotriene biosynthesis. Some work on inhibition of GluTase (and LTD<sub>4</sub> production) in RBL-1 cells has been reported (21); however, most information as to the role of these enzymes has come from the extensive literature on carcinogen detoxification (11).

In this study GSHTase levels in homogenates from sensitized lung tissue showed an increase after antigen challenge. The increase occurred 5 min after challenge. No increase in GSHTase activity occurred in unsensitized tissue; in fact, a decrease in enzyme levels normally was seen. It is interesting to note that the basal level of GSHTase was higher in unsensitized than in sensitized lung tissue which is the opposite of what one might have expected. It would appear, therefore, that the release of slow reacting substances into the perfusate upon challenge cannot be related directly with the level of total GSHTase.

Although an increase in GSHTase was observed for RBL-1 cells during the incubation, there was a wide spread of data points, and the levels of enzyme found were around the minimum detectable in our assay. This reduced the significance of the change, and no firm conclusions may be drawn from these data.

The results described show that no direct correlation between total GSHTase and leukotrienes in either lung tissue or RBL-1 cells can be seen. It should be noted that, with 1-chloro-

2,4-dinitrobenzene as substrate, total GSHTase levels have been assayed. However, at least six forms of this enzyme have been reported (11, 22), each with a different substrate specificity. It is possible that one enzyme, specific for LTA<sub>4</sub>, may in fact be produced or activated during SRS-A formation but that an increased level may be undetectable in the overall GSHTase change. Assays using the epoxide substrate 1,2-oxido-3-(3-nitrophenoxy)propane failed to detect any "epoxide-specific" enzyme in the guinea pig lung pieces before or during the challenge. At this stage it is not possible to tell whether such enzymes are truly absent from lung or are present but at levels below the level of detection in the 100-mg lung pieces assayed. Further studies using larger quantities of lung or cells and involving purification of the enzymes present will be required to elucidate the role of GSHTase in leukotriene biosynthesis.

In the study of the levels of GluTase the picture is somewhat clearer. An increase in GluTase in sensitized lung homogenates immediately preceded release of LTD<sub>4</sub> and low levels of the enzyme into the perfusate. The same effect was observed when these lung pieces were homogenized in the presence of Triton X-100 to solubilize membrane-bound GluTase, suggesting that sensitization had resulted in an activation or synthesis of the enzyme during leukotriene synthesis rather than a release of enzyme from a membrane-bound store. These changes, which do not occur in unsensitized lung, indicate that GluTase may play some controlling role in the formation of LTD<sub>4</sub> in the guinea pig lung. Because antigen challenge of guinea pig lung is a model for allergic bronchospasm, a clearer understanding of this system should aid studies into the control of leukotriene-induced bronchoconstriction *in vivo*.

It is interesting that, as in the case of total GSHTase levels, GluTase is higher in unsensitized lung compared with sensitized lung. It is not known whether this effect occurs with all lung enzymes or only with those of the glutathione detoxification pathway.

In the RBL-1 cells, the release of GluTase into the cell supernatant on addition of indomethacin, arachidonic acid, and L-cysteine probably arises from a general cell leakage (a phenomenon observed throughout the incubation with the cytosolic enzyme glucose-6-phosphate dehydrogenase). On addition of ionophore, however, the characteristic dip-followed-by-rapid-rise pattern (Fig. 2a) was observed, with maximal increase of LTD<sub>4</sub> and GluTase occurring at the same time. This release is not simply cell leakage as evidenced by the dimethyl sulfoxide control incubations and the glucose-6-phosphate dehydrogenase levels (Fig. 2). The results described suggest that there are a number of steps in the generation and release of LTD<sub>4</sub> from RBL-1 cells and lung tissue. On addition of ionophore, leukotriene biosynthesis is fully activated with probable production of LTC<sub>4</sub>. Available GluTase is then used to convert this initial burst of LTC<sub>4</sub> to LTD<sub>4</sub>, and thus enzyme leakage into the supernatant decreases markedly. The ionophore then mobilizes more GluTase from the membrane store (which is itself constant) over a 20-min period as more LTC<sub>4</sub> is biosynthesized; excess GluTase then passes into the supernatant, by partial exocytosis, together with LTD<sub>4</sub>.

The somewhat lower final GluTase levels observed when indomethacin, arachidonic acid, or L-cysteine was omitted from the incubation medium may be related to a reduction in the quantity of LTC<sub>4</sub> formed prior to metabolism by GluTase. For example, in the absence of indomethacin, arachidonic acid will be partially converted to prostaglandins, thus reducing the eventual requirement for GluTase. Similarly, 5,8,11,14,17-icosapentaenoic acid, which is a poorer substrate for lung SRS-A production (10), results in decreased GluTase output.

The cell membrane acts as a relatively large constant store

of GluTase; the enzyme is available from this store when required without material depletion occurring (Triton X-100 data). The cell line is thus utilizing GluTase differently from the manner in which it is used by guinea pig lung (where some activation/synthesis of the enzyme probably occurs). Therefore it should not be assumed that the cell line is similar to the guinea pig lung for studies relevant to the genesis and control of asthma, although RBL-1 cells provide a good system for the generation of leukotrienes.

The high levels of GluTase both in lung and in RBL-1 cell supernatant are sufficient to convert tens of nanomoles of LTC<sub>4</sub> to LTD<sub>4</sub> per minute; under such circumstances, most, if not all, LTC<sub>4</sub> released would be expected to be converted to LTD<sub>4</sub> by the end of the incubation. In our original work on the structure of RBL-1 slow reacting substances and guinea-pig lung SRS-A, we found no evidence of the presence of LTC<sub>4</sub>. Other workers, however, have found LTC<sub>4</sub> in mouse mastocytoma cells (2), human and rat SRS-A (23), and human polymorphonuclear leukocytes (24). Each of these methods of leukotriene production differs in many respects from those used in our laboratories and may account for differences in the LTC/LTD ratios. Possible differences could include decreased GluTase levels (or its complete absence), decreased time of contact with GluTase, or, perhaps, increased glutathione levels which could force the equilibrium, in the presence of GluTase, in favor of LTC<sub>4</sub>.

The presence of GluTase must also be considered in other systems—for example, in the guinea pig ileum. Direct bioassay of LTC<sub>4</sub> on ileum shows a prolonged tissue response (different from that of “classical” SRS-A/LTD<sub>4</sub>). LTC<sub>4</sub> is approximately 1/10th as potent as SRS-A (LTD<sub>4</sub>) on guinea pig ileum (25). In the presence of GluTase inhibitors at concentrations at 1–10 K<sub>i</sub> [i.e., K<sub>i</sub> = 22.5 μM for δ-D-glutamyl-(O-carboxy)phenylhydrazine] the LTC<sub>4</sub> response is diminished, suggesting that at least part of the “biological activity” of LTC<sub>4</sub> arises from its conversion to LTD<sub>4</sub>. Conversion of LTC<sub>4</sub> to LTD<sub>4</sub> on the smooth muscle strip was readily observed in the dual-bank superfusion system in which the time between contact with the first and last tissue is 1 min. Here the response to LTC<sub>4</sub> both increased in height and shortened in duration on the second bank of tissues as GluTase on the ileum surface metabolized LTC<sub>4</sub>. As expected, the activity was decreased in the presence of GluTase inhibitors serine, borate, and δ-D-glutamyl-(O-carboxy)phenylhydrazine. Therefore, care must be taken in attributing biological activity to LTC<sub>4</sub> on the basis of bioassay procedures, because we have clearly demonstrated its conversion on the bioassay tissue to other active products.

In summary, our data indicate that GluTase may be important in the control of leukotriene biosynthesis *in vivo* (and thus bronchoconstriction in the lung). Further studies are required, however, to elucidate the role of GSHTase, the putative enzyme controlling an earlier stage in the biosynthesis of the peptidolipid leukotrienes.

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