Biochemical Pharmacology, Vol. 38, No. 15, pp. 2521–2526, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Maxwell Pergamon Macmillan plc

EFFECTS OF GLYCYRRHIZIN AND GLYCYRRHETINIC ACID ON DEXAMETHASONE-INDUCED CHANGES IN HISTAMINE SYNTHESIS OF MOUSE MASTOCYTOMA P-815 CELLS AND IN HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

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(*Received* 18 *July* 1988; *accepted* 16 *December* 1988)

Abstract—The effects of glycyrrhizin and its aglycone, glycyrrhetinic acid, on dexamethasone-induced changes in the histamine synthesis of mastocytoma P-815 cells and in the histamine release from antigen-stimulated rat peritoneal mast cells were investigated. Glycyrrhetinic acid but not glycyrrhizin, at concentrations from 20 to 35 μM, almost completely inhibited the dexamethasone-induced increases in both the histamine content and histidine decarboxylase activity of cultured mastocytoma P-815 cells. Glycyrrhetinic acid, however, showed practically no inhibition of [^{3}H]dexamethasone binding to the cytoplasmic receptor. On the other hand, glycyrrhetinic acid but not glycyrrhizin markedly inhibited the release of histamine from antigen-stimulated rat mast cells, and intensified the inhibitory activity induced by dexamethasone. Glycyrrhetinic acid inhibited the antigen-induced release and incorporation of [^{3}H]arachidonic acid in immunized rat mast cells. The administration of glycyrrhizin into rats, in contrast to the *in vitro* treatment of the cells with glycyrrhizin, markedly inhibited histamine release from antigen-stimulated rat mast cells. These results suggest that glycyrrhetinic acid inhibited dexamethasone-induced changes in the histamine synthesis of mastocytoma P-815 cells, and in the histamine release from rat mast cells. On the other hand, glycyrrhizin may exert its effect after conversion to glycyrrhetinic acid *in vivo*.

Glycyrrhizin, extracted from the root of licorice (*Glycyrrhiza glabra*), and its aglycone, glycyrretinic acid, have various anti-inflammatory [1], anti-allergic [2], anti-gastric ulcer [3] and anti-viral [4, 5] activities. Glycyrrhizin and glycyrrhetinic acid have been reported to have a corticoid-like action [6, 7], and blocking effects on cortisone acetate-induced anti-granuloma action [7], or on prednisolone-induced inhibition in the growth of fibroblasts in culture [8]. Thus, the exact mechanisms of the actions of glycyrrhizin and glycyrrhetinic acid in correlation with the steroid actions involved in inflammation or allergy remain unknown.

Previously, we showed that dexamethasone increases the *de novo* synthesis of histidine decarboxylase in cultured mastocytoma P-815 cells [9] and rat stomachs [10]. In addition, Daëron *et al.* [11] showed a glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. Therefore, we examined the effects of glycyrrhizin and glycyrrhetinic acid on dexamethasone-induced changes in histamine metabolism in cultured mastocytoma P-815 cells and rat peritoneal mast cells.

MATERIALS AND METHODS

Cells and cell culture

Mastocytoma P-815 cells were maintained in a suspension culture in Fischer and Sartorelli's medium supplemented with 5% fetal calf serum. For the experiments, suspensions of cells in the logarithmic phase of growth were incubated in 125-ml Erlenmeyer flasks with or without the drugs to be tested in an atmosphere of 5% CO_2 and 95% air at 37° in a humidified incubator. The cells were harvested by centrifugation at 280 *g* for 5 min at 4° in tubes and washed twice with cold phosphate-buffered saline. Cell numbers were determined with a Coulter counter (model Z, Coulter Electronics, Hialeah, FL, U.S.A.).

Mast cells were collected from the peritoneal cavity fluid of male Wistar rats, weighing 250–300 g, and concentrated by Ficoll density gradient centrifugation [12] to 90–93% purity, as assessed by metachromasia after staining with toluidine blue. The cells were suspended in Tyrode's solution containing 0.1% gelatin at pH 7.4. Cell viability was determined by nigrosin exclusion.

Preparation of cytosol and nuclei

All operations were performed at 0–4°. Mastocytoma P-815 cells were suspended in 15 vol. of 10 mM Tris–HCl buffer containing 1 mM EDTA, 12 mM thioglycerol and 0.5 mM antipain, pH 7.4, homogenized in a Dounce type glass homogenizer and then centrifuged at 100,000 *g* for 60 min to obtain the supernatant (cytosol) fraction. For isolation of the nuclei fraction, the packed cells from separate batches were suspended for 7 min in 10 mM Tris–HCl buffer containing 1 mM EDTA and 4 mM $MgCl_2$, pH 7.80, at 2×10^6 cells/ml. The cells were resuspended in the

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same buffer at 7×10^7 cells/ml, homogenized in a Dounce-type glass homogenizer, cell rupture being monitored under a phase contrast microscope, and then centrifuged at 1000 *g* for 15 min. The pellets (nuclear fraction) obtained were washed with the same buffer.

Assay methods

Histamine assay. Histamine was extracted by heating cells suspended in a 2.1% $HClO_4$ solution at 80° for 5 min. After centrifuging at 280 *g* for 5 min, the clear supernatant solution was assayed according to the method of Shore *et al.* [13].

Histidine decarboxylase activity. Histidine decarboxylase activity was measured according to the method of Watanabe *et al.* [14]. Cells were homogenized in a solution containing 0.1 M potassium phosphate buffer, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1% polyethylene glycol (average molecular weight 300, w/w). The homogenates were centrifuged at 10,000 *g* for 20 min, and the clear supernatant fractions were dialysed three times against 100 vol. of the same solution. The dialysates were incubated at 37° for various times in an incubation mixture (1.0 ml) containing 0.25 mM histidine, and the histamine formed during incubation was fractionated on a small column of Amberlite CG-50, and measured fluorometrically using *o*-phthalaldehyde [13]. The recovery of histamine through these steps was more than 90% in all cases.

Preparation of dinitrophenylated Ascaris antiserum. Conjugated dinitrophenylated ascaris extract (DNP-Ascaris) was prepared by the method of Strejan and Campbell [15] and rat antiserum against DNP-Ascaris (DNP-Ascaris serum) was developed in Wistar rats according to the method of Tada and Okumura [16]. Male Wistar rats (180–200 g) were immunized by injection, into the forefoot pads, of a total of 1 mg of DNP-Ascaris mixed with 10^{10} *Bordetella pertussis* (adjuvant). Five days later, 0.5 mg of DNP-Ascaris alone was injected subcutaneously into the back. Eight days after the first immunization, blood was collected by aortic puncture under ether anesthesia. The antiserum titer was determined in rats by means of the homologous passive cutaneous anaphylaxis reaction 48 hr after its intradermal injection. Antisera showing titers of over 1:500 were used.

Histamine release assay. Rat mast cells were pretreated with various doses of glycyrrhetinic acid or glycyrrhizin at 37° for 20 hr in Dulbecco's Modified Eagle's Medium (DMEM), and were immunized by incubating with DNP-Ascaris antiserum at 37° for 1 hr. Immunized mast cells were then washed with Tyrode's solution containing 0.1% gelatin, and suspended in the same solution containing various doses of glycyrrhetinic acid or glycyrrhizin. Incubation was started by adding DNP-Ascaris protein (20 μg) to the cell suspension. Ten minutes later, the reaction was stopped by cooling and centrifuging the cell suspension. Amounts of histamine in the supernatant fractions and cell pellets were assayed according to the procedure of Shore *et al.* [13].

Steroid binding assays. The binding of [^{3}H]dexamethasone to cytosol and the nuclear fraction was carried out essentially according to procedures described elsewhere [9].

[^{3}H]*Arachidonic acid release and incorporation assays.* Mast cells (15×10^6 cells) were incubated for 2 hr in Dulbecco's Modified Eagle's Medium (DMEM; 30 ml) containing [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]arachidonic acid (20 μCi) and immunized by incubating with DNP-Ascaris antiserum for another 2 hr at 37°. After removing unbound [^{3}H]arachidonic acid by washing with DMEM containing 0.1% bovine serum albumin, aliquots of the [^{3}H]arachidonic acid labeled- and DNP-Ascaris-immunized-mast cells suspended in the same medium were pretreated with or without glycyrrhetinic acid for 15 min, and then challenged with DNP-Ascaris extract (15 μg protein) for 10 min at 37°. The cell mixture was centrifuged, and radioactivity in the supernatant fraction was counted in 8 ml of a Triton–toluene (3:1, v/v) mixture containing 0.5% 2,5-diphenyloxazole (PPO). After dissolving the cell pellet with an aliquot of tissue solubilizer, NCS, its radioactivity was counted.

[^{3}H]Arachidonic acid incorporation was measured using mast cells immunized with DNP-Ascaris antiserum. Aliquots of immunized mast cells suspended in Tyrode's solution containing 0.1% gelatin were incubated with various concentrations of glycyrrhizin and glycyrrhetinic acid for 15 min, and then treated with [^{3}H]arachidonic acid (0.2 μCi) for 60 min at 37°. Unbound [^{3}H]arachidonic acid was removed by washing the cells with the same solution. After dissolving the cell pellet with an aliquot of NCS, its radioactivity was counted.

Materials

The materials used were obtained from the following sources: Glycyrrhizin and glycyrrhetinic acid were supplied by the Minophagen Pharm. Co. (Tokyo, Japan). [1, 2, 4, 6, 7-^{3}H]Dexamethasone (77 Ci/mmol), [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]arachidonic acid (80–120 Ci/mmol) and a tissue solubilizer, NCS, were provided by Nuclear Chicago Scintillator, Amersham-Japan (Tokyo, Japan); Amberlite CG-50 (analytical grade) was from the Organo Chemical Co. (Tokyo, Japan), catalase from bovine liver was supplied by the Sigma Chemical Co. (St Louis, MO, U.S.A.), and dexamethasone, prednisolone, corticosterone, and hydrocortisone of reagent grade were obtained from Nakarai Pure Chemical Products.

RESULTS

Effects of glycyrrhizin and glycyrrhetinic acid on histamine synthesis in dexamethasone-stimulated mastocytoma P-815 cells

As shown in our previous paper [9], dexamethasone markedly increased the histamine content and histidine decarboxylase activity of mastocytoma P-815 cells cultured for 20 hr at 37°. When the dexamethasone-stimulated cells were incubated together with glycyrrhetinic acid, the elevated histamine levels (Fig. 1) and histidine decarboxylase activity (Fig. 2) both dose-dependently decreased to the unstimulated level. Glycyrrhetinic acid did not affect the basal levels of histamine content and histidine decarboxylase activity. In contrast to the inhibitory action of glycyrrhetinic acid, glycyrrhizin had no effect on the his-

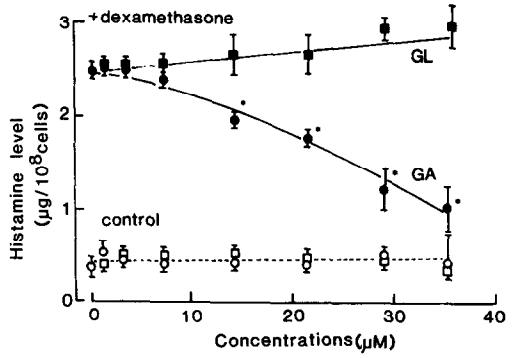

Fig. 1. Effects of glycyrrhetinic acid (GA) and glycyrrhizin (GL) on the histamine content of mastocytoma P-815 cells cultured with or without dexamethasone. Mastocytoma P-815 cells (8×10^5 cells/ml) were cultured in 100 ml of Fischer medium with (closed symbols) or without (open symbols) 1 μM dexamethasone in the presence of various doses of glycyrrhetinic acid (circles) or glycyrrhizin (squares) for 20 hr at 37°. The cells were harvested, washed twice with phosphate-buffered saline, and then assayed for histamine content. Each value is the mean ± SE of triplicate samples. *$P < 0.01$ vs 0 μM concentration value. Values at the highest concentration of GA were not significantly different from the control.

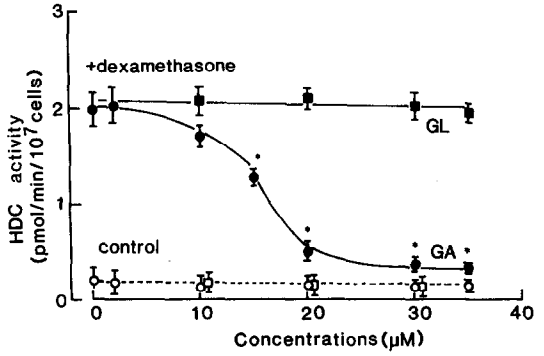

Fig. 2. Effects of glycyrrhetinic acid (GA) and glycyrrhizin (GL) on histidine decarboxylase activity of mastocytoma P-815 cells. Mastocytoma P-815 cells (6×10^5 cells/ml) were cultured in 100 ml of Fischer medium with (closed symbols) or without (open symbols) 1 μM dexamethasone in the presence of various doses of glycyrrhetinic acid (circles) or glycyrrhizin (squares) for 20 hr at 37°. The cells were harvested and then assayed for histidine decarboxylase activity. Each value (pmol/min/10^7 cells) is the mean ± SE of three determinations. *$P < 0.01$ vs 0 μM concentration value. Values at the highest concentration of GA were not significantly different from the control.

tamine level or on histidine decarboxylase activity, both of which had been elevated by dexamethasone.

As in the case of dexamethasone, glycyrrhetinic acid inhibited the elevation of cellular histamine in mastocytoma P-815 cells treated with hydrocortisone, prednisolone and corticosterone, but had no effect on their basal levels of histamine (Table 1). However, glycyrrhetinic acid did not inhibit purified histidine decarboxylase activity (data not shown).

Table 1. Effect of glycyrrhetinic acid on various steroid-induced changes in the histamine contents of mastocytoma P-815 cells

Steroid	Histamine (μg/10^8 cells)	
	Control	Glycyrrhetinic acid
None	0.526 ± 0.022	0.587 ± 0.011
Dexamethasone	2.80 ± 0.01	1.01 ± 0.02*
Hydrocortisone	2.29 ± 0.05	1.23 ± 0.05*
Prednisolone	2.31 ± 0.12	1.15 ± 0.17*
Corticosterone	2.27 ± 0.24	1.21 ± 0.05*
Progesterone	0.598 ± 0.021	0.665 ± 0.024
Testosterone	0.566 ± 0.012	0.582 ± 0.020
Estradiol	0.505 ± 0.050	0.557 ± 0.014

Mastocytoma P-815 cells were cultured in medium containing various steroids at a concentration of 1 μM with or without 20 μM glycyrrhetinic acid for 20 hr at 37°. The cells were harvested, washed and then assayed for histamine content. Each value is the mean ± SE of triplicate samples.
* $P < 0.01$.

Effects of glycyrrhizin and glycyrrhetinic acid on [^{3}H]dexamethasone binding to glucocorticoid receptors in cytosolic and nuclear fractions of mastocytoma P-815 cells

Glucocorticoids are known to act by binding to specific cytoplasmic receptors and translocating to the nuclei. The effects of glycyrrhetinic acid and glycyrrhizin were examined on [^{3}H]dexamethasone binding to cytosolic and nuclear fractions. As shown in Fig. 3, glycyrrhetinic acid at higher concentrations inhibited [^{3}H]dexamethasone binding slightly but insignificantly to both cytosolic and nuclear binding sites, whereas glycyrrhizin had no effect.

Effects of glycyrrhizin and glycyrrhetinic acid on the histamine release from rat mast cells treated with or without dexamethasone

Mouse mastocytoma P-815 cells are much less likely to release histamine by an immunological stimulation, since they contain very low levels of cellular histamine. Therefore, the effect of glycyrrhizin or glycyrrhetinic acid on histamine release was investigated by using antigen-stimulated rat peritoneal mast cells.

As shown in Fig. 4a, glycyrrhetinic acid, added to immunized mast cells when challenging with glycyrrhizin or glycyrrhetinic acid concentrations over 200 μM, inhibited the histamine release by about 50%. The histamine release from the immunized mast cells preincubated with 40 μM glycyrrhetinic acid for 20 hr was inhibited almost completely (Fig. 4b). In addition, glycyrrhetinic acid slightly potentiated the dexamethasone-induced inhibition of histamine release. On the other hand, glycyrrhizin was inactive in inhibiting histamine release *in vitro* (Fig. 4 a and b). Under these condition, incubation of mast cells with glycyrrhetinic acid or glycyrrhizin did not cause cell damage.

When mast cells were incubated with glycyrrhizin *in vitro* (Fig. 4), a significant inhibition in histamine release was not observed. However, when mast cells from rats injected for 3 days with glycyrrhizin (100 mg/kg/day) were stimulated with an antigen, histamine release was inhibited (Table 2).

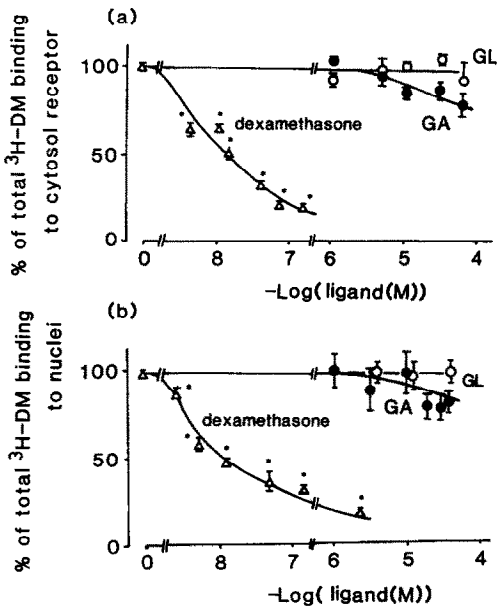

Fig. 3. Effects of glycyrrhetinic acid (GA) and glycyrrhizin (GL) on [^{3}H]dexamethasone binding to cytosolic and nuclear fractions from mastocytoma P-815 cells. (a) The cytosolic fraction (0.5 mg protein/ml) of mastocytoma P-815 cells was incubated for 2 hr at 4° with 7 nM [^{3}H]dexamethasone in the presence of various doses of non-labeled dexamethasone (△) or glycyrrhetinic acid (●) or glycyrrhizin (○). The unbound [^{3}H]dexamethasone was removed through adsorption on charcoal-dextran. (b) Mastocytoma P-815 cells (3×10^6 cells) were incubated with 7 nM [^{3}H]dexamethasone (0.22 μCi) in the presence or absence of 1 μM nonradioactive steroids for 30 min at 37°. The [^{3}H]dexamethasone binding to the nuclear fraction was assayed by using nuclei isolated by Triton treatment. Each value represents the mean ± SE of three determinations. *$P < 0.05$ vs control values.

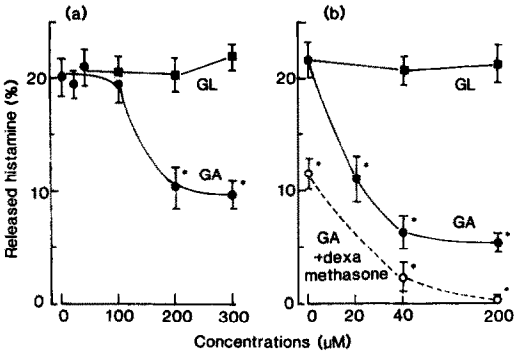

Fig. 4. Effects of glycyrrhetinic acid (GA) and glycyrrhizin (GL) on the histamine release from DNP-Ascaris-stimulated rat mast cells in the presence or absence of dexamethasone. (a) Rat mast cells were immunized by incubating with DNP-Ascaris antiserum for 1 hr at 37°. Immunized mast cells were treated with various doses of glycyrrhetinic acid or glycyrrhizin in the presence of DNP-Ascaris protein (20 μg) for 10 min. Released histamine was assayed. Each point represents the mean ± SE of three samples. *$P < 0.05$ vs 0 μM concentration value. (b) Rat mast cells (1×10^6 cells/ml) were cultured in 30 ml of Dulbecco's Modified Eagle's Medium containing glycyrrhetinic acid (●) or glycyrrhizin (■) in the presence (---) or absence (——) of 1 μM dexamethasone for 20 hr at 37°. The cells were harvested and then incubated in DNP-Ascaris antiserum. Immunized mast cells were washed, resuspended in Tyrode's solution containing 0.1% gelatin, and challenged with DNP-Ascaris protein for 10 min at 37°. After centrifugation of the cell suspension, histamine in the supernatant fraction was assayed. Released histamine is expressed as a percentage of the total histamine content of the cells (control: 0.85 ± 0.03 μg, glycyrrhetinic acid: 0.95 ± 0.06 μg, glycyrrhizin: 0.87 ± 0.05 μg per 10^5 cells). Each point is the mean ± SE of three samples. *$P < 0.05$ vs 0 μM concentration value.

Effect of glycyrrhetinic acid on [^{3}H]*arachidonic acid releasing activity of antigen-stimulated mast cells*

When [^{3}H]arachidonic acid-labeled mast cells were exposed to glycyrrhetinic acid, [^{3}H]arachidonic acid released in response to stimulation by DNP-Ascaris was inhibited markedly (Table 3). Similarly, the incorporation of [^{3}H]arachidonic acid into mast cell membranes was inhibited by pretreating the cells with glycyrrhetinic acid (Table 4). In contrast, glycyrrhizin had no effect on [^{3}H]arachidonic acid metabolism.

DISCUSSION

The administration of hydrocortisone to mice and rats is known to stimulate gastric histamine synthesis [17, 18] and the *de novo* synthesis of histidine decarboxylase [10]. In addition, daily injections of prednisolone into rats causes severe gastric ulcerative lesions, which are inhibited by H_2 antagonists [19]. The exposure of rats to cold leads to an increase in histidine decarboxylase activity in the stomach due to released corticoids [18]. Glucocorticoids have been found to increase the *de novo* synthesis of histidine decarboxylase in cultured mastocytoma P-815 cells [9]. Histidine decarboxylase activities in human, canine and pig stomachs are known to be contained entirely in mast cell-like cells [20]. On the other hand, glucocorticoids have been reported to inhibit IgE-mediated histamine release from mouse mast cells

Table 2. Histamine release by DNP-Ascaris-stimulated mast cells isolated from glycyrrhizin-pretreated rats

Treatment (*in vivo*)	Histamine released (%) DNP-Ascaris (a)	None (b)	Δ Release (a)−(b)
None	14.8 ± 1.01	1.42 ± 0.27	13.4 ± 1.30
Glycyrrhizin	3.52 ± 0.51	1.85 ± 0.35	1.43 ± 0.65*

Rats were injected with glycyrrhizin for 3 days (100 mg/kg/day). Peritoneal mast cells were isolated and incubated with DNP-Ascaris antiserum or vehicle serum for 1 hr at 37°. After washing with Tyrode's solution containing 0.1% gelatin, the cells suspended in the same solution were challenged by 20 μg of DNP-Ascaris protein. Histamine release is expressed as a percentage of the total histamine content of the cells (2.10 ± 0.02 μg/10^5 cells). Total histamine contents (cells + medium) of GL-treated mast cells did not change after the challenge with antigen (2.02 ± 0.10 μg/10^5 cells). Each value is the mean ± SE of four determinations.
* $P < 0.01$.

Table 3. Effects of glycyrrhizin and glycyrrhetinic acid on [^{3}H]arachidonic acid release from stimulated mast cells with DNP-Ascaris protein.

Addition	[^{3}H]Arachidonic acid released [dpm/10^5 cells (% of control)]	
	Supernatant fraction	Cell pellet
None	2286 ± 120 (100)	8564 ± 156
Glycyrrhetinic acid		
10 μM	1560 ± 85* (68.2)	9082 ± 320
20 μM	855 ± 35* (37.2)	9350 ± 205
30 μM	624 ± 36* (27.3)	9286 ± 225
Glycyrrhizin		
10 μM	2308 ± 150 (101)	8321 ± 352
50 μM	2150 ± 55 (94.1)	8509 ± 210
100 μM	1985 ± 124 (86.8)	8655 ± 120

[^{3}H]Arachidonic acid-labeled mast cells which were immunized by DNP-Ascaris antiserum were first incubated with or without glycyrrhizin or glycyrrhetinic acid for 15 min, and then challenged by DNP-Ascaris protein for 10 min. The radioactivity of [^{3}H]arachidonic acid in the supernatant fraction of the cell suspension and the precipitated cells was counted. The amounts of free [^{3}H]arachidonic acid in [^{3}H]arachidonic acid-labeled mast cells before or after the antigen challenge were 2.8 ± 0.3 or 3.2 ± 0.2% of the total activity respectively. Each value is the mean ± SE of triplicate samples.

*$P < 0.01$.

Table 4. Effects of glycyrrhizin and glycyrrhetinic acid on [^{3}H]arachidonic acid incorporation into mast cells stimulated with DNP-Ascaris protein

Addition	[^{3}H]Arachidonic acid incorporation [dpm/10^5 cells (% of control)]
None	15,350 ± 657 (100)
Glycyrrhetinic acid	
10 μM	6,584 ± 386* (42.9)
20 μM	1,850 ± 56* (12.0)
30 μM	1,700 ± 49* (11.1)
Glycyrrhizin	
10 μM	16,210 ± 795 (106)
50 μM	14,200 ± 358 (92.5)
100 μM	15,208 ± 335 (99.1)

Isolated mast cells were immunized by incubating them with DNP-Ascaris antiserum. Immunized mast cells were preincubated with or without various concentrations of glycyrrhizin or glycyrrhetinic acid for 15 min and then incubated with [^{3}H]arachidonic acid (0.2 μCi) for 60 min at 37°. Unbound radioactivity was removed by washing the cells with a phosphate-buffered saline solution. Each sample is the mean ± SE of triplicate samples.

* $P < 0.05$.

[11]. These results suggest that glucocorticoid affects histamine metabolism in mast cells in various tissues or histamine-forming cells in the stomach.

We showed that glycyrrhetinic acid suppressed dexamethasone-stimulated histamine synthesis in mouse mastocytoma P-815 cells and histamine release from antigen-stimulated rat mast cells. In the latter, glycyrrhetinic acid intensified the dexamethasone-induced inhibition in the histamine release. These results are likely to show that glycyrrhetinic acid and its derivatives can be used as anti-ulcer and anti-inflammatory agents. In clinical application, in fact, disodium 3-*O*-β-carboxypropionyl-glycyrrhetinate is used orally as a remedy for stomach ulcers [3], and ammonium glycyrrhizin with L-cysteine and glycine is also used intravenously as an anti-inflammatory agent [21].

The administration of a high dose of each agent for prolonged periods in patients [22, 23] and in animals [24, 25] has been observed to induce side effects such as mineralocorticoid-like actions, e.g. edema and hypertension. These side effects are suspected to be due to the inhibition of the metabolic clearance of endogenous corticoid, since glycyrrhizin inhibits 3α-hydroxysteroid dehydrogenase in the liver [5], and also glycyrrhetinic acid replaced [^{3}H]aldosterone binding in rat kidney slices. *In vitro*, at a concentration of 600 μM, glycyrrhetinic acid was reported to produce a 70% inhibition of aldosterone binding, but a 20% inhibition of dexamethasone binding to corticoid binding protein [26]. Mastocytoma P-815 cells are known to possess a considerable amount of cytosolic binding sites for dexamethasone with a K_d value of 1.26×10^{-9} M [9]. Glycyrrhetinic acid in higher doses slightly but insignificantly inhibited the [^{3}H]dexamethasone bindings to cytosolic and nuclear fractions. In contrast, glycyrrhetinic acid in concentrations as low as 20–35 μM almost completely inhibited the dexamethasone-induced increase of histamine synthesis in mastocytoma P-815 cells. This suggests that the inhibitory effect of glycyrrhetinic acid in histamine synthesis is not due to the replacement of glucocorticoids on the cytosolic receptor or to the impairment of its translocation to nuclei binding sites. Since dexamethasone is known to increase the *de novo* synthesis of histidine decarboxylase activity [9], it is necessary to study the changes in the RNA polymerase activity of histidine decarboxylase in mastocytoma P-815 cells treated with glycyrrhetinic acid and dexamethasone.

Glycyrrhetinic acid had a remarkable effect on the inhibition of antigen-stimulated histamine release from mast cells. The inhibitory effect of glycyrrhetinic acid was strengthened by incubating mast cells for a long period. Although the mechanism of the action of glycyrrhetinic acid upon the pretreatment of the cells is unclear, it may act on mast cell function in phospholipid metabolism in different ways. A short period after its addition to mast cells, glycyrrhetinic acid acted as a stabilizer for membrane phospholipid metabolism evoked by stimulation (Table 3). Unexplained is the inconsistent dose-dependency between phospholipid metabolism (Table 3) and histamine release (Fig. 4a). The change in phospholipid is generally assumed to be an important trigger for the induction of an antigen-stimulated cellular response in sensitized mast cells [26, 27]. [^{3}H]Arachidonic acid might be liberated from the phospholipid by phospholipase A_2 in antigen-stimulated sensitized mast cells [28]. The activity of phospholipase A_2 is also known to be controlled by a specific inhibitor protein, lipocortin, induced by glucocorticoid [29]. In addition, glycyrrhetinic acid and its derivatives have been reported to inhibit 5- and 12-lipoxygenase and cyclooxygenase from mastocytoma P-815 cells [20].

In contrast to glycyrrhetinic acid, glycyrrhizin had

no effects on dexamethasone-induced changes in the histamine synthesis of mastocytoma P-815 cells and the histamine release from mast cells, as well as on the affinity for [^{3}H]dexamethasone binding sites to cytosolic and nuclear receptors in mastocytoma P-815 cells. Therefore, the *in vivo* potency of glycyrrhizin may be due to its hydrolyzed product, glycyrrhetinic acid. However, glycyrrhizin was reported to have effects *in vitro* on antiviral activities [4, 5]. This needs further study.

The present results show that glycyrrhetinic acid effectively inhibited dexamethasone-stimulated histamine synthesis in mastocytoma P-815 cells and histamine release from antigen-stimulated sensitized mast cells. These actions of glycyrrhetinic acid may explain the drug actions in glucocorticoid-induced gastric ulceration and anti-inflammation.

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