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Effects of glycyrrhetinic acid derivatives on hepatic and renal 11β-hydroxysteroid dehydrogenase activities in rats

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Abstract

The purpose of this study was to examine the structure and activity relationships of glycyrrhetinic acid derivatives on the inhibition of hepatic and renal 11β-hydroxysteroid dehydrogenases (HSDs) in rats. Furthermore, we explored whether inflammatory effect of the derivatives is involved in the inhibition of 11β-HSD activity. 18β-Glycyrrhetinic acid (**Ia**) potently inhibited 11β-HSD activity of hepatic (IC50 (concentration giving 50% inhibition of cortisone production) = 0.09 μM) and renal (IC50 = 0.36 μM) homogenate. The inhibitory effect of 18β-glycyrrhetol (**Id**) modified at the 30-position of glycyrrhetinic acid was weaker than that of glycyrrhetinic acid itself. 18β-24-Hydroxyglycyrrhetinic acid (**Ie**), oxidized at the 24-position, remarkably reduced the inhibitory activity for both enzymes. 18β-11-Deoxoglycyrrhetinic acid (**IIc**) showed the same inhibitory effect as glycyrrhetinic acid on hepatic 11β-HSD activity, but less effect on renal 11β-HSD activity. Furthermore, the inhibitory activity of 18β-deoxoglycyrrhetol (**IIa**), modified at the 11- and 30-position, was markedly decreased. Dihemiphthalate derivatives (**IIb**, **IIIb** and **IVb**) of deoxoglycyrrhetol (**IIa**), 18β-olean-9(11), 12-diene-3β, 30-diol (**IIIa**) and olean-11, 13(18)-diene-3β, 30-diol (**IVa**), which are anti-inflammatory agents, also showed weak inhibition against both hepatic and renal 11β-HSDs. While glycyrrhetinic acid (200 mg kg^{-1}, p.o.) significantly inhibited 11β-HSD activity in rat liver and kidney at 3 h after administration, compound **IVb** (100 mg kg^{-1}, p.o.) had no effect on either enzyme activity. In addition, the circulating corticosterone level was slightly increased by glycyrrhetinic acid but not by compound **IVb**. These results suggest that the anti-inflammatory effects of compound **IVb**, derived from glycyrrhetinic acid, are not due to accumulation of steroids induced by the inhibition of 11β-HSD activity. Our data also showed that the 11-, 24- and 30-positions of glycyrrhetinic acid may play important roles in the differential inhibitory effects on 11β-HSD isozyme activity.

Introduction

11β-Hydroxysteroid dehydrogenase (11β-HSD), a short-chain dehydrogenase/reductase, catalyses the interconversion of hormonally active $C_{(11)}$-hydroxylated corticosteroids (cortisol and corticosterone) and their inactive $C_{(11)}$-keto metabolites (cortisone and 11-dehydrocorticosterone). Two isozymes of 11β-HSD are designated 11β-HSD1 and 11β-HSD2 (Brown et al 1993), respectively. 11β-HSD1 shares less than 30% homology with 11β-HSD2 and uses NADP(H) as cosubstrate, but not 11β-HSD2. High expression of 11β-HSD1 is shown in the liver, lung, adipose tissue, vasculature, ovary and brain, largely localized to the cells expressing glucocorticoid receptors, suggesting that 11β-HSD1 modulates glucocorticoid access to its receptor (Kotelevtsev et al 1997). 11β-HSD1 in tissue homogenates exhibits 11β-dehydrogenase and 11β-reductase activity, with greater stability of the dehydrogenase (Lakshmi & Monder 1988). Furthermore, 11β-HSD1 is the only isozyme expressed in rat liver with predominant 11β-reductase activity reactivating 11-ketosteroid over a broad range of substrate concentrations (Jamieson et al 2000). 11β-HSD2, on the other hand, is an NAD-dependent isozyme and potently inactivates glucocorticoids (Brown et al 1993).

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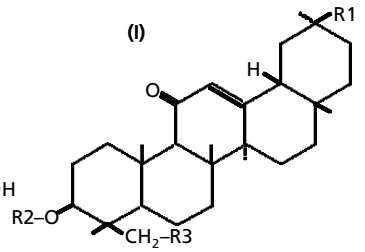
The activity of 11β-HSD2 prevents inappropriate access of cortisol to mineralocorticoid receptors and is essential for preserving the monopoly of aldosterone for mineralocorticoid receptor stimulation (Edwards et al 1988; Funder et al 1988). This isozyme is highly expressed in aldosterone target cells such as the principal cells of the kidney, colon, adrenal gland and submandibular gland (Albiston et al 1994). In general, the actions of 11β-HSDs oppose one another: 11β-HSD1 regenerates cortisol, whereas 11β-HSD2 inactivates cortisol.

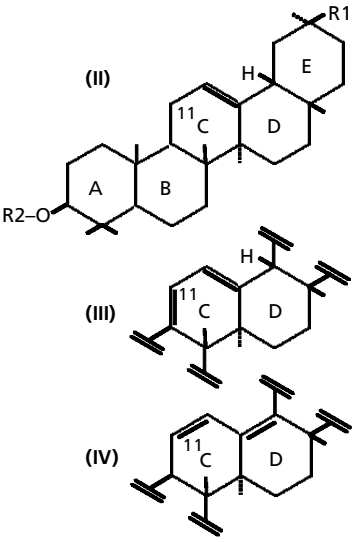
Glycyrrhetinic acid (**Ia**, Figure 1), the aglycone of glycyrrhizin isolated from liquorice root (*Glycyrrhizia* spp.), is well known to have wide pharmacological effects such as anti-inflammatory activity (Finney & Somers 1958; Capasso et al 1983), anti-tumorigenic activity (Nishino et al 1986), inhibition of intercellular gap junction communication (Davidson et al 1986), and inhibitory effect on growth of mouse melanoma (Abe et al 1987). Glycyrrhetinic acid has also been reported to inhibit steroid metabolizing enzymes such as 5β-reductase (Tamura et al 1979; Latif et al 1990) and 3β-hydroxysteroid dehydrogenase (Latif et al 1990). Studies of glucocorticoid metabolism have led to the hypothesis that glycyrrhetinic acid and other similar liquorice derivatives may express their mineralocorticoid-like actions by inhibiting 11β-HSD (Stewart et al 1987; Edwards et al 1988; Funder et al 1988). Monder et al (1989) proposed that side effects induced by liquorice, including pseudoaldosteronism with hypertension and hypokalaemia, are due to the inhibition of renal 11β-HSD by glycyrrhetinic acid.

Carbenoxolone (**Ib**), a derivative of glycyrrhetinic acid, has been used for the treatment of gastric ulcer (Doll et al 1968) but induces pseudoaldosteronism. Thus, we have prepared various glycyrrhetinic acid derivatives to enhance the therapeutic properties such as anti-inflammatory activity and suppress pseudoaldosteronism due to the inhibition of steroid metabolism by the mother compound (Shibata et al 1987). Glycyrrhetinic acid 3β-*o*-hemiphthalate (**Ic**) reduced pain at venous cannulation with transdermal 10% lidocaine (lignocaine) base gel (Kano et al 1992). Three kinds of compounds (18β-olean-12-ene-3β, 30-diol di-*o*-hemiphthalate (**IIb**), 18β-olean-9(11), 12-diene-3β, 30-diol di-*o*-hemiphthalate (**IIIb**), and olean-11, 13(18)-diene-3β, 30-diol di-*o*-hemiphthalate (**IVb**)) have an anti-inflammatory activity stronger than that of glycyrrhetinic acid. These compounds inhibited paw and

	R1	R2	R3
Glycyrrhetinic acid (GA)			
Ia:	–COOH,	–H,	–H
Carbenoxolone			
Ib:	–COOH,	–SucA,	–H
Hemiphthalate			
Ic:	–COOH,	–*o*PhA,	–H
Glycyrrhetol			
Id:	$-CH_2OH$,	–H,	–H
24-HydroxyGA			
Ie:	–COOH,	–H,	–OH



SucA: succinic acid, *o*PhA: *o*-phthalic acid



	R1	R2
Deoxoglycyrrhetol		
IIa:	$-CH_2OH$,	–H
Deoxoglycyrrhetol dihemipthalate		
IIb:	$-CH_2$–*o*PhA,	–*o*PhA
11-DeoxoGA		
IIc:	–COOH,	–H
IIIa:	$-CH_2OH$,	–H
IIIb:	$-CH_2$–*o*PhA,	–*o*PhA
IVa:	$-CH_2OH$,	–H
IVb:	$-CH_2$–*o*PhA,	–*o*PhA

Figure 1 Structure of glycyrrhetinic acid and its derivatives.

ear oedema formation in rodent, induced by inflammatory agents such as arachidonic acid (Inoue et al 1988), 12-*o*-tetradecanoylphorbol-13-acetate (TPA) (Inoue et al 1989), carrageenan (Inoue et al 1993) and capsaicin (Inoue et al 1996). Furthermore, they are also known to suppress leukotrienes and prostaglandin E_2 synthesis in-vitro (Inoue et al 1986) and in-vivo (Inoue et al 1988, 1990). However, it is unclear whether these compounds affect the metabolism of glucocorticoids. In addition, there are few reports showing which functional structure in the oleanane skeleton is responsible for inhibiting 11β-HSD activity. In this study, we have examined the effects of glycyrrhetinic acid derivatives on both hepatic 11β-HSD1 activity and renal 11β-HSD2 activity in rats, and analysed the structure and activity relationship of glycyrrhetinic acid derivatives on the inhibition of 11β-HSD activity. We also explored whether the inflammatory effect of glycyrrhetinic acid derivatives is due to the inhibition of 11β-HSD activity.

Materials and Methods

Materials

Glycyrrhetinic acid and its derivatives were prepared or synthesized by Shibata et al (1987) at the Minophagen Pharmaceutical Co. The following reagents were purchased: cortisol, cortisone, NAD^+, $NADP^+$ (Sigma Chemical Co., St Louis, MO); dimethyl sulfoxide (DMSO), polyoxyethylene (10) octylphenyl (Triton-X100, Wako Pure Chemicals Co., Osaka, Japan); polyoxyethylene sorbitan mono-oleate (Tween 80; Tokyo Kasei Chemical Industry, Tokyo, Japan). All other chemicals and solvents were of extra pure grade or HPLC grade.

Animals

Male Sprague-Dawley rats (250–270 g) obtained from SLC (Japan SLC, Shizuoka, Japan) were housed 3 or 4 per cage with free access to food and water, and maintained on a 12-h light–dark cycle in a room with controlled temperature (24 ± 1 C) and humidity ($55 \pm 10\%$). This study was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

In-vivo assay

Glycyrrhetinic acid and compound **IVb** were orally administered to rats (5 or 6 rats per group) at a dose of 200 mg kg^{-1} and 100 mg kg^{-1}, respectively. The test compounds were suspended with 1% Tween 80 in physiological saline. Control rats received the vehicle only. At 3 h after the treatment with the test compounds (Horigome et al 1999), rats were anaesthetized with diethyl ether, and blood was taken from the descending aorta for corticosterone measurement. Then, liver and kidney were removed to examine 11β-HSD activity. The serum was separated from the blood by centrifugation at 3000 *g* for 30 min (4 C) and stored at -20 C until used for corticosterone measurement.

Extraction of 11β-HSD

The tissues of rat liver or kidney were homogenized in 2 vol. of cold extraction buffer containing 0.1 M Tris-HCl (pH 8.5), 0.01% Triton-X100 and 0.25 M sucrose with a Teflon homogenizer (3000 rev min^{-1}, for 3 min). Homogenates were centrifuged at 10 000 *g* for 30 min (4 C), and the supernatant was used as 11β-HSD fractions.

Assay for 11β-HSD inhibition

The oxidative activity of 11β-HSD was measured by modified Monder's method (Monder et al 1989). Reaction buffer (450 μL) of 0.1 M Tris-HCl (pH 8.5) containing 0.01% Triton-X100, 0.3 mM cortisol as a substrate and 0.5 mM $NADP^+$ for liver or 5 mM NAD^+ for kidney, and 50 μL of the enzyme fraction was incubated for 30 min for liver or 60 min for kidney at 37 C in the presence or absence of test compounds. Test compounds were dissolved in distilled water or DMSO. The protein level of the hepatic enzyme fraction and renal fraction was 124 ± 3 mg mL^{-1} ($n = 16$) and 95 ± 2 mg mL^{-1} ($n = 16$), respectively, as measured by a protein assay kit (Bio-Rad Laboratories, Inc., CA). The enzyme reaction was terminated by the addition of 100 μL of 5% sulfuric acid. Cortisone produced from cortisol by the enzyme in the mixtures was quantitatively measured by HPLC with a Senshu pak Docosil column ($4.6\phi \times 250$ mm; Senshu Scientific Co., Tokyo, Japan). The mobile phase was a mixture of water–tetrahydrofuran–acetonitrile (69:23:8 v/v) with a flow rate of 1 mL min^{-1} at 40 C. Cortisol and cortisone were monitored by UV absorbance at 254 nm and identified by comparing their retention time with that of the standard. The recovery rate of cortisone from the incubation mixture was more than 95%, with coefficients of variation less than 5%. The inhibitory effect of the test compounds on 11β-HSD activity was expressed as a percent inhibition for control.

Measurement of serum corticosterone

Serum corticosterone levels were examined directly by the enzyme-linked immunosorbent assay (ELISA; Diagnostic System Laboratories, TX) kit according to the manufacture's direction. The limit of detection of the assay was approximately 1.6 ng mL^{-1}.

Statistical analysis

All values were expressed as the mean $\pm$ s.e.m. of at least three different experiments, and duplicate determinations were performed in each experiment. Statistical analysis using Kruskal-Wallis test was done for comparison among different concentrations of test compounds. The IC50 values (concentration giving 50% inhibition of cortisone production) were determined by regression analysis. In-vivo experiments were analysed using

Kruskal-Wallis test followed by Dunn's test. P values of less than 0.05 were considered significant.

Results

Effects of glycyrrhetinic acid derivatives on hepatic and renal 11β-HSD activity

We first examined 11β-HSD activity in rat hepatic and renal extraction mixtures by detecting chemical transformation of cortisol to cortisone in the presence of glycyrrhetinic acid (**Ia**). In this assay, cortisone levels produced from cortisol by hepatic and renal 11β-HSDs were 614 ± 20 pmol (n = 28) and 564 ± 17 pmol (n = 27), respectespectively. Glycyrrhetinic acid was the strongest inhibitor of hepatic NADP-dependent 11β-HSD1 (Figure 2A) and its IC50 value was 0.09 μM (Table 1). Similar to its effect on hepatic 11β-HSD, glycyrrhetinic acid (IC50 = 0.36 μM) was very effective in inhibiting renal NAD-dependent 11β-HSD2 activity (Figure 2B). In contrast, the IC50 value of glycyrrhizin was 59 μM for hepatic and 216 μM for renal 11β-HSD2 activity (data not shown).

Table 1 IC50 values of test compounds for hepatic 11β-HSD1 and renal 11β-HSD2 activities.

Compound	IC50 (μM)	
	Hepatic HSD1	Renal HSD2
Ia	0.09 ± 0.02	0.36 ± 0.02
Ib	0.65 ± 0.06	8.79 ± 0.65
Ic	0.23 ± 0.01	5.29 ± 0.88
Id	0.26 ± 0.03	8.10 ± 0.82
Ie	52.9 ± 5.6	216 ± 28
IIa	145 ± 20	551 ± 269
IIb	29.7 ± 1.8	32.3 ± 2.1
IIc	0.08 ± 0.01	3.24 ± 0.75
IIIa	7.97 ± 0.35	> 1000
IIIb	67.8 ± 1.9	49.5 ± 2.1
IVa	> 1000	> 1000
IVb	> 1000	92.0 ± 2.3

Values are expressed as the mean ± s.e.m. of results from 3–5 independent experiments. IC50, concentration giving 50% inhibition of cortisone production.

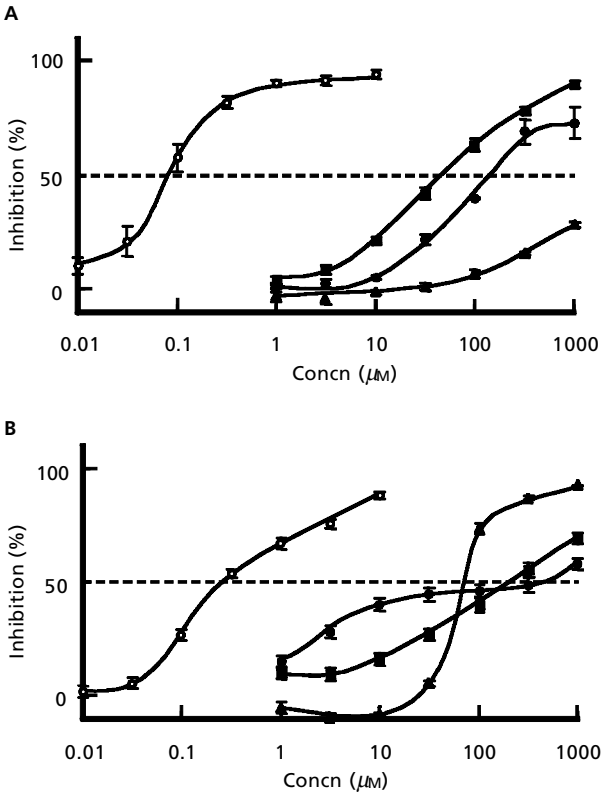


Figure 2 Inhibition of hepatic (A) and renal (B) 11β-HSD activity by glycyrrhetinic acid derivatives (**Ia**, ○; **Ie**, ■; **IIa**, ●; **IVb**, ▲). Each point represents the mean ± s.e.m. of results from 3–5 independent experiments. A. $P = 0.005$, 0.002, 0.002 and 0.002 for **Ia, Ie, IIa** and **IVb** by Kruskal-Wallis test. B. $P = 0.002$, 0.002, 0.004 and 0.002 for **Ia, Ie, IIa** and **IVb** by Kruskal-Wallis test.

As glycyrrhetinic acid strongly inhibited hepatic and renal 11β-HSD activity, we modified the 11-, 24- or 30-position of ring A and E to find an inhibitory site of glycyrrhetinic acid for the enzyme activity. All compounds dose-dependently inhibited hepatic and renal enzyme activity, and statistical significance ($P < 0.003$, Kruskal-Wallis test) was observed in comparison among different concentrations of glycyrrhetinic acid or its derivatives. Carbenoxolone (**Ib**), as well as glycyrrhetinic acid, is known to inhibit 11β-HSD activity (Akao et al 1992). Actually, this compound strongly suppressed both hepatic and renal 11β-HSD activity. Glycyrrhetinic acid 3β-*o*-hemiphthalate sodium (**Ic**) and glycyrrhetol (**Id**) also showed a potent inhibitory effect for both enzymes. However, their effects were weaker than those of glycyrrhetinic acid. 11-Deoxoglycyrrhetinic acid (**IIc**) had the same inhibitory effect as glycyrrhetinic acid on hepatic 11β-HSD activity but exhibited lower inhibitory potency on renal enzyme reaction. 24-Hydroxyglycyrrhetinic acid (**Ie**) and deoxoglycyrrhetol (**IIa**) were very weak inhibitors, and their dose–response curves markedly shifted to the right (Figure 2A, B). Furthermore, compound **IVa** and **IVb** had little effect on either enzyme activity. Compound **IIb**, derived from deoxoglycyrrhetol, showed a greater effect than the fundamental compound. Compound **IIIa**, a fundamental compound, inhibited hepatic enzyme, whereas it had no effect on renal enzyme. Many of the test compounds were more effective in inhibiting hepatic 11β-HSD than renal 11β-HSD.

Effects of glycyrrhetinic acid and compound IVb on 11β-HSD activity in-vivo and circulating corticosterone concentration

We investigated whether glycyrrhetinic acid and compound **IVb**, an anti-inflammatory agent, affect steroid

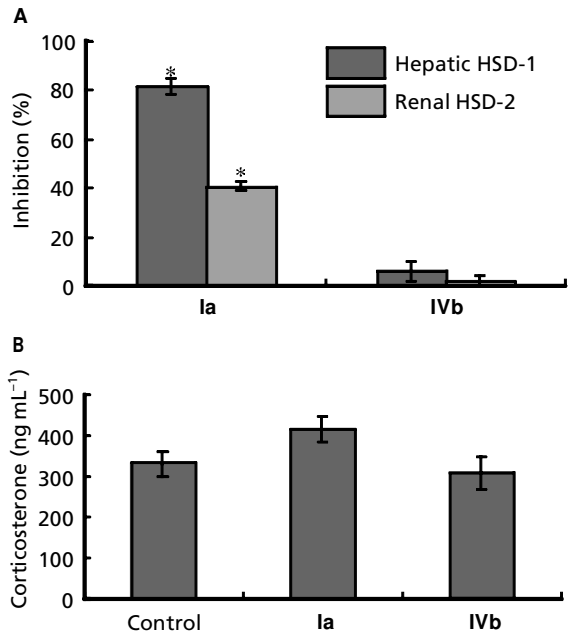


Figure 3 Effects of glycyrrhetinic acid and compound **IVb** on hepatic and renal 11β-HSD activity (A) and circulating corticosterone levels (B) in rats. Values are the mean ± s.e.m. of 5 or 6 rats. $^*P < 0.05$, compared with control (Dunn's test).

metabolism through inhibition of 11β-HSD activity in-vivo. 11β-HSD activity in rat liver and kidney was examined 3 h after the oral administration of glycyrrhetinic acid at 200 mg kg^{-1} or compound **IVb** at 100 mg kg^{-1}, which inhibited rat paw oedema induced by carrageenan (Inoue et al 1993). The cortisone level produced from cortisol by hepatic and renal 11β-HSDs in control rats was 628 ± 45 pmol (n = 5) and 484 ± 14 pmol (n = 5), respectively. Glycyrrhetinic acid significantly ($P < 0.05$) inhibited 11β-HSD activity in liver and kidney (Figure 3A). Similar to in-vitro experiments, the inhibitory effects of glycyrrhetinic acid were more potent against hepatic 11β-HSD (80% inhibition) than against renal 11B-HSD (40% inhibition). In contrast, compound **IVb** was ineffective in both enzyme reactions. Furthermore, corticosterone levels in the serum of rats treated with glycyrrhetinic acid or compound **IVb** were examined by ELISA (Figure 3B). In addition to inhibition of the 11β-HSD activity, glycyrrhetinic acid slightly enhanced corticosterone concentration from 330 ± 31 to 415 ± 32 ng mL^{-1} (n = 5 or 6), although the difference was not statistically significant. There was no difference in circulating corticosterone levels between control and compound **IVb**.

Discussion

Glycyrrhetinic acid was the strongest inhibitor of rat hepatic and renal 11β-HSDs, and its IC50 value for hepatic 11β-HSD activity was almost the same as that given in previous reports (Akao et al 1992; Homma et al 1994). We prepared various glycyrrhetinic acid derivatives to suppress the effect of the mother compound on 11β-HSD activity and examined an active site of oleanane skeleton of test compounds on the inhibition of two 11β-HSD activities in rats. Glycyrrhetinic acid was reported to strongly inhibit the reduction of the Δ^4-3-keto system of cortical steroid in rat hepatic homogenate (Kumagai et al 1957; Atherden 1958). However, 11-deoxoglycyrrhetinic acid (Atherden 1958) and deoxoglycyrrhetol (**IIa**) (Takahashi et al 1980) have little effect on 5β-reductase activity. These reports suggest that the 11-oxo-$\Delta^{12(13)}$ system in ring C of glycyrrhetinic acid competes with the 3-oxo-$\Delta^{4(5)}$ system in ring A of cortical steroids at the active site of the reducing enzyme. However, this explanation is not entirely consistent with our results. 11-Deoxoglycyrrhetinic acid (**IIc**), as well as glycyrrhetinic acid, strongly inhibited hepatic enzyme activity, as previously reported (Akao et al 1992), whereas the effect of 11-deoxoglycyrrhetinic acid (**IIc**) on renal 11β-HSD activity was much lower than that of glycyrrhetinic acid. Thus, the 11-position of ring C of glycyrrhetinic acid is a significant factor for inhibiting renal 11β-HSD activity but not the hepatic enzyme reaction. This result also implies that the active site of hepatic 11β-HSD1 may be different from that of renal 11β-HSD2.

Glycyrrhetol (**Id**) was a slightly weak inhibitor compared with 11-deoxoglycyrrhetinic acid (**IIc**) and glycyrrhetinic acid, and the inhibitory effect of 24-hydroxyglycyrrhetinic acid A (**Ie**) was much less than that of glycyrrhetinic acid, suggesting that the 24- and 30-position of ring A and E of glycyrrhetinic acid play important roles in the 11β-HSD inhibition. It has been reported that 3-keto-glycyrrhetinic acid is similarly effective to glycyrrhetinic acid in inhibiting hepatic 11β-HSD (Akao et al 1992; Irie et al 1992), indicating that the $C_{(3)}$-OH group of ring A is not a major factor. We also confirmed that there was no difference in the 11β-HSD inhibition between glycyrrhetinic acid and 3-keto-glycyrrhetinic acid (data not shown). Our present data showed that the 11β-HSD inhibitory effect was decreased by reducing both the 11-carbonyl and the 30-carboxyl of glycyrrhetinic acid, as shown with deoxoglycyrrhetol (**IIa**). This indicates that both 11-carbonyl and 30-carboxyl groups are essential for the inhibition of renal 11β-HSD activity. Compound **IIb** is known to have no inhibitory effect on 11β-HSD in rat liver microsomes (Irie et al 1992). Actually, three dihemiphthalate compounds (**IIIb** and **IVb**), including compound **IIb**, were weak inhibitors of both hepatic and renal 11β-HSDs.

Glycyrrhetinic acid, 11-deoxoglycyrrhetinic acid (**IIc**) and glycyrrhetol (**Id**) were more effective in inhibiting hepatic 11β-HSD1 than renal 11β-HSD2, although they are not selective inhibitors. Irie et al (1992) have suggested that glycyrrhetinic acid may inhibit the enzyme reaction competitively by binding to the catalytic site of hepatic 11β-HSD with high affinity. Therefore, it is conceivable that oleanane skeleton of glycyrrhetinic acid may have more affinity for 11β-HSD1 than 11β-HSD2 from rat.

The mouse and human 11β-HSD1 enzymes are known to display 78% amino acid identity. Others have reported

that carbenoxolone is more effective in inhibiting 11β-HSD1 from mouse than that from man (Barf et al 2002). In addition, glycyrrhetinic acid and carbenoxolone are less potent as inhibitors of 11β-HSD1 than 11β-HSD2 from man (Diederich et al 2000). Also, there is 79% homology for 11β-HSD1 and 86% homology for 11β-HSD2 between man and rat. Accordingly, it seems that the glycyrrhetinic acid derivatives used in this study do not inhibit 11β-HSD activity from rat and man to a similar degree. This will have to be confirmed in future studies.

11β-HSD1 mRNA is expressed in pancreatic islets isolated from man and mouse, and incubation of β-cell from ob/ob mice, an animal model of non-insulin-dependent diabetes, in the presence of 11β-hydrocorticosterone, leads to an inhibition of insulin release (Davani et al 2000). Furthermore, inhibition of 11β-HSD1 by carbenoxolone reverses inhibition of insulin release (Davani et al 2000). This indicates an important role of 11β-HSD1 in the regulation of insulin release. There is a report suggesting that the gluconeogenesis is attenuated in the 11β-HSD1 knockout mice (Kotelevtsev et al 1997). Glycyrrhizin has been reported to have an anti-diabetic effect in non-insulin-dependent diabetes model mice (Takii et al 2001). This implies that glycyrrhetinic acid metabolized from glycyrrhizin by intestinal bacteria (Akao et al 1994) may inhibit 11β-HSD in mouse pancreas. Thus, selective inhibitors of 11β-HSD1 may have a role in the therapy of patients with hepatic insulin resistance (Kotelevtsev et al 1997; Jamieson et al 1999; Davani et al 2000; Diederich et al 2000).

It has been suggested that glycyrrhetinic acid may enhance endogenous glucocorticoid activity by suppressing the metabolism of glucocorticoids (Kumagai et al 1967). Glycyrrhetinic acid administration to mice inhibits 11β-HSD activity in immune tissues (Hennebold et al 1996) and induces apoptosis in thymocytes through the increase in the levels of endogenous corticosterone by 11β-HSD inhibition (Horigome et al 1999). Indeed, oral administration of glycyrrhetinic acid at a dose of 200 mg kg^{-1}, which reduces rat paw oedema in response to carrageenan (Inoue et al 1993), inhibited the activity of both hepatic and renal 11β-HSD. In addition, the endogenous corticosterone level was slightly increased by glycyrrhetinic acid administration, suggesting that the anti-inflammatory effect of glycyrrhetinic acid may be due to the enhancement of endogenous glucocorticoid action. In contrast to glycyrrhetinic acid, oral administration of compound **IVb** at 100 mg kg^{-1}, which is a dose sufficient to induce anti-inflammatory (Inoue et al 1988, 1989, 1993, 1996) and analgesic activity (Inoue et al 1990), had no effect on either 11β-HSD activity or the endogenous corticosterone level. It appears that compound **IVb** does not induce its anti-inflammatory effect by the inhibition of 11β-HSD. Actually, the inhibitory effects of compound **IIb** on ear oedema formation in response to arachidonic acid does not involve the anti-inflammatory action of steroids mediated by the secondary formation of a reactive protein (Inoue et al 1988). Taken together, these results further support the pharmacological actions of deoxoglycyrrhetol dihemipthalate (**IIb**) and related compounds (**IIIb** and **IVb**) being quite different from that of glycyrrhetinic acid.

Conclusions

Among fourteen test compounds examined, glycyrrhetinic acid was the strongest inhibitor of rat hepatic 11β-HSD1 and renal 11β-HSD2 in in-vitro experiments. Our results suggest that the $C_{(11)}$-carbonyl, $C_{(24)}$-hydroxymethyl and $C_{(30)}$-carboxyl groups of glycyrrhetinic acid may play important roles in the 11β-HSD inhibition. Furthermore, the test compounds showed more potent inhibitory activity for hepatic 11β-HSD than for renal 11β-HSD. Oral administration of glycyrrhetinic acid (200 mg kg^{-1}) suppressed both hepatic and renal 11β-HSD activity, resulting in an accumulation of endogenous corticosterone level in-vitro, whereas glycyrrhetinic acid-derived compound **IVb** (100 mg kg^{-1}, p.o.), an anti-inflammatory agent, had no effect on either 11β-HSD activity or corticosterone level. Thus, it is suggested that the mechanism of anti-inflammatory action of compound **IIb, IIIb** and **IVb** may be different from that of glycyrrhetinic acid.

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