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Chronic Effect of Saikosaponin on Adrenal and Thymus Growth in Normal and Dexamethasone-Treated Rats

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The effects of repeated intraperitoneal administration of total saikosaponin or saikosaponin d from Bupleuri Radix on the wet weight of the adrenals and thymus, and on the resting level of plasma corticosterone were determined in intact or hypophysectomized rats. Total saikosaponin or saikosaponin d increased the relative weight of the adrenals with respect to the initial body weight, decreased the relative weight of the thymus, and did not affect the resting evening level of plasma corticosterone in normal intact rats. A moderate, but not high, dose of saikosaponin administered with dexamethasone antagonized the atrophic effect of dexamethasone on the adrenals. Moderate and high doses of saikosaponin acted synergistically on dexamethasone-induced atrophy of the thymus, but it did not significantly affect the resting dexamethasone-depressed level of plasma corticosterone. In hypophysectomized rats, a high dose of saikosaponin d did not affect the weight of either the adrenals or the thymus, whereas exogenous adrenocorticotropin markedly increased the adrenal weight but not the thymus weight, and dexamethasone did not decrease the adrenal weight but markedly decreased the thymus weight.

Keywords——saikosaponin; Bupleurum root; adrenal hypertrophy; thymus atrophy; hypophysectomized rat; ACTH; dexamethasone; negative feedback

In a series of acute experiments, we have shown that saponins of Ginseng Radix,[1,2] saikosaponins a and d of Bupleuri Radix,[3] and other triterpenoidal saponins[4,5] increased the plasma levels of adrenocorticotropin (ACTH) and corticosterone, whereas high doses of saikosaponin c and glycyrrhizin of Glycyrrhizae Radix did not.[1] Secretion of corticosterone induced by ginseng saponin[1] and saikosaponins a and d[3] was blocked by pretreatment with dexamethasone, a potent and long-acting inhibitor of ACTH secretion[6] by a feedback mechanism. However, we found that a higher dose of ginseng saponin[7] or saikosaponins a and d[8] caused release of the blocking of corticosterone secretion partially or completely, depending on the amount and activity of the saponins in acute treatment.

The control of adrenal growth as well as corticosteroidogenesis is believed to be a consequence of stimulation by ACTH. On the other hand, chronic treatment with a high dose of glucocorticoid such as dexamethasone or betamethasone[9] results in adrenal atrophy, insufficiency of the pituitary-adrenalcortex system and involution of the thymus. Yamamoto *et al.*[10] reported that repeated administration of saikosaponin d or a did not affect adrenal weight, but showed a potent antigranulomatous action. Abe *et al.*[11] showed that chronic treatment with saikosaponin d was weakly atrophic on the adrenals, while it potentiated the antigranulomatous action of dexamethasone. The present experiments were undertaken to examine the trophic effect of saikosaponin on the adrenals in saline- and dexamethasone-treated intact and hypophysectomized rats.

Materials and Methods

"Total saikosaponin" is the saponin fraction extracted by the routine procedures from Bupleuri Radix,

Bupleurum chinense DC.[4a] It is a slightly yellowish powder containing saikosaponins a, b_1, b_2, c and d. Its ED_{50} value for corticosterone secretion-inducing activity was 2.7 mg/kg.[4a] Saikosaponin d was kindly supplied by Shionogi and Co., Osaka. These saponins and dexamethasone were dissolved in 2.5% ethanol-containing pyrogen-free saline just before use. Cortrosyn Z from N. V. Organon, the Netherlands, was diluted with saline and used as ACTH. One mg of Cortrosyn Z has an activity of 40 U.

Four-week-old male Wistar rats weighing 80—95 g initially were used. Hypophysectomized male Wistar rats weighing 135—155 g (5 weeks old) or 95—115 g (4 weeks old) were obtained from Nippon Hypox, Yamanashi, and used. These rats were fed on laboratory chow (CE-2, CLEA Japan Inc., Tokyo) and tap water *ad libitum*, and maintained at 24 °C with artificial light (light phase: 0700 to 1900 h) for more than 7 d.

Rats were "gentled" by handling and weighing every morning and evening. After 2 to 7 d of conditioning, they were injected intraperitoneally with 5 ml/kg of saline (pyrogen-free saline or 2.5% ethanol-saline) or test substance once a day at between 1800 and 1900 h, taking account of the circadian adrenocortical rhythm of the nocturnal animal, for 5 or 9 consecutive days. At 24 or 15 h after the final injection, they were decapitated with a guillotine. Trunk blood was collected in chilled heparinized tubes. Immediately after this, the adrenal glands and thymus were rapidly removed, put into ice-cold saline, and then weighed after being cleaned in cold saline and blotted onto a filter paper. Plasma corticosterone was determined by the competitive protein binding method as described in the previous paper.[1] Plasma glucose was determined by the glucose oxidase method.

Results

Effect of Saikosaponin on Body, Adrenal and Thymus Weights

Total saikosaponin was administered to rats intraperitoneally once a day after determination of body weight in the evening. As shown in Table Ia, treatment with saikosaponin tended to suppress growth over 5 d. Increase in body weight was significantly suppressed for the 1st 24 h interval, but not for the 2nd to 5th 24 h intervals (Table Ia). The reason why suppression of body weight gain was restricted to the 1st 24 h is not clear, but when isolated saikosaponin d was administered, the 1st 24 h suppression of body weight gain was again observed and was accompanied by the 1st 24 h suppression of food and water intake (unpublished observation). Thus, the cause of the 1st 24 h suppression of body weight gain may be mainly the suppression of food intake, though the mechanism of this effect is not clear.

Since treatment might affect body weight, especially in chronic experiments, the relative weight of the adrenal and thymus with respect to the initial body weight at the evening of day 0 was selected as a criterion. Increase in the wet weight of the adrenals per rat and per 100 g of initial body weight was not significant in normal rats for 5 d, but there was a significant increase in saikosaponin-treated rats (Table Ib). The wet weight of the thymus was clearly increased in these young adult rats, but saikosaponin moderately suppressed thymus growth (Table Ib).

Evening levels of plasma corticosterone and glucose were determined 24 h after the final treatment, and they were not affected by saikosaponin, though the weight of the adrenals was increased significantly (Table Ib).

Effect of Saikosaponin on Adrenal and Thymus Weights and Corticosterone Level in Dexamethasone-Treated Rats

Treatment with 0.25 mg/kg/d of dexamethasone for 5 d markedly suppressed body weight gain to one-third of normal. Total saikosaponin (5 or 10 mg/kg) co-administered with dexamethasone tended to suppress further the dexamethasone-suppressed growth. Suppression of growth due to saikosaponin was significant only for the 1st 24 h in these dexamethasone-treated rats (Table IIa), as well as in normal rats. Saikosaponin co-administered with 0.1, 0.05 or 0.025 mg/kg/d of dexamethasone significantly suppressed growth for 9 d. The saikosaponin-evoked growth suppression was again significant only for the 1st 24 h interval in the 9-day period (Table IIa).

In rats treated with 0.25 mg/kg of dexamethasone for 5 d, the adrenal weight decreased to one-half of normal (Table Ib), and the thymus weight also decreased to one-eighth of normal

TABLE Ia. Effect of Saikosaponin (Saiko) on Body Weight in Intact Rats

Treatment	Body weight (g)[a]		Weight increase (g/24 h)		
	Initial	Final	1st	2nd	2nd—final (Total)
Saline × 1 d[b]	117±2	124±3	6.6±1.5	—	—
+Saiko × 1 d[c]	113±2	116±2[d]	2.6±2.1	—	—
Saline × 3 d	116±1	140±2	7.6±0.3	6.6±0.4	8.0 (16±1)
+Saiko × 3 d	113±2	128±2[e]	0.6±1.6[e]	7.0±1.6	7.0 (14±2)
Saline × 5 d	115±1	152±2	7.3±0.6	7.0±0.4	7.3 (29±1)
+Saiko × 5 d	114±2	147±2	2.3±0.9[e]	6.3±0.6	7.8 (31±1)

a) Body weight was determined just before the 1st treatment in the evening on day 0 and 24 h after the last treatment. Figures are mean±S.E. for 6 rats. *b*) 5 ml/kg/d of 2.5% ethanol–saline. *c*) 10 mg/kg/d of total saikosaponin in 2.5% ethanol–saline. *d*) $p<0.1$, *e*) $p<0.01$ *vs.* saline.

TABLE Ib. Effect of Saikosaponin (Saiko) on Adrenal and Thymus Weights and Basal Levels of Plasma Corticosterone (Cort) and Glucose

Treatment	Adrenal weight		Thymus weight		Cort (μg/100 ml)	Glucose (mg/100 ml)
	(mg/rat)	(mg/BWi)[a]	(mg/rat)	(mg/BWi)[a]		
Saline × 1 d	21.5±1.5	18.2±1.0	447±31	380±22	20±2	151±3
+Saiko × 1 d	24.1±0.8	21.2±0.7[b]	407±41	357±32	15±4	144±1
Saline × 3 d	22.6±0.7	19.4±0.7	444±15	383±10	16±2	146±3
+Saiko × 3 d	23.9±1.4	21.1±1.3	384± 7[c]	339± 7[c]	21±2	146±2
Saline × 5 d	20.7±1.3	18.0±1.2	551±24	480±22	24±3	146±2
+Saiko × 5 d	26.6±1.2[b]	23.3±0.8[c]	495±34	435±30	21±4	145±3

a) Adrenal or thymus weight in mg/100 g of initial body weight (BWi) in the evening of day 0. *b*) $p<0.05$, *c*) $p<0.01$ *vs.* saline.

(Table Ib). Saikosaponin co-administered with dexamethasone significantly increased the relative adrenal weight, but did not affect thymus weight (Table IIb). The basal evening level of corticosterone tended to increase to the normal level (15—25 μg/100 ml) from one-fiftieth of normal (Table Ib).

In rats treated with 0.1, 0.05 or 0.025 mg/kg/d of dexamethasone for 9 d, saikosaponin co-administered with dexamethasone tended to increase the relative adrenal weight, though the increase was not statistically significant, while saikosaponin significantly decreased the relative thymus weight (Table IIb). Saikosaponin tended to restore the normal basal evening level of plasma corticosterone from the dexamethasone-suppressed level (Table IIb).

Effect of Saikosaponin d on Body Weight Gain and Adrenal Weight

The effect of isolated saikosaponin d on growth was determined in both saline- and dexamethasone-treated rats (groups of 12 rats). The corticosterone secretion-inducing activity of 2.5 mg of saikosaponin d corresponds to that of 20 mg of total saikosaponin.[4] When 2.5 mg/kg/d of saikosaponin d was administered for 9 d, saikosaponin d significantly decreased the body weight gain for the 1st 24 h interval and the 8-day period of the 2nd to 9th d, but not the 2nd to 9th 24 h intervals, in both saline- and dexamethasone-treated (0.05 mg/kg) rats (Table IIIa). When the weights of the adrenals and thymus were expressed relative to final body weight, saikosaponin d significantly increased the adrenal weight, and significantly decreased the thymus weight in both saline- and dexamethasone-treated rats.

TABLE IIa. Effect of Saikosaponin (Saiko) on Body Weight Gain in Dexamethasone-Treated (Dex) Rats

Treatment[a] (mg/kg/d)	Body weight (g)[b]		Weight increase (g/24 h)		
	Initial	Final	1st	2nd	2nd—final (Total)
Exp. 1[c]					
Dex 0.25 (5)	125±2	135±1	0 ±0.6	2.8±0.8	2.6 (10±1)
+Saiko 5	126±2	135±2	−3.3±0.8[f]	3.6±0.6	3.1 (12±1)
+Saiko 10 (4)	127±2	130±3	−4.0±2.1[e]	2.5±0.9	1.9 (8±2)
Exp. 2[d]					
Dex 0.10	122±3	160±3	−0.3±0.8	5.3±1.1	4.7 (38±3)
+Saiko 10	121±2	153±2	−4.0±1.4[e]	3.6±1.8	4.5 (36±2)
Dex 0.05	122±2	171±4	1.6±0.6	6.0±0.7	5.8 (47±2)
+Saiko 10	122±2	158±4[f]	−5.0±0.8[h]	5.0±1.1	5.2 (41±3)
Dex 0.025	121±2	177±4	2.6±0.8	7.0±0.8	6.6 (53±3)
+Saiko 10	122±2	164±4[f]	−1.3±0.8[g]	7.3±0.9	5.2 (43±4[e])

a) Total saikosaponin was administered simultaneously with dexamethasone. Groups of 6 rats (or 5 or 4, where indicated in parentheses). *b*) Body weight was determined in the evening on day 0 and 24 h after the last treatment. *c*) Five-day treatment. *d*) Nine-day treatment. *e*) $p<0.1$, *f*) $p<0.05$, *g*) $p<0.01$, *h*) $p<0.001$ *vs.* dexamethasone.

TABLE IIb. Effect of Saikosaponin (Saiko) on Adrenal and Thymus Weight and Basal Levels of Plasma Corticosterone (Cort) and Glucose

Treatment (mg/kg/d)	Adrenal weight		Thymus weight		Cort (μg/100 ml)	Glucose (mg/100 ml)
	(mg/rat)	(mg/BWi)[a]	(mg/rat)	(mg/BWi)[a]		
Dex 0.25	11.1±1.1	8.9±0.9	71± 8	57± 7	0.4±0.1	134±2
+Saiko 5	13.2±0.8	10.9±0.2[b]	89± 6	70± 4	0.3±0.1	138±4
+Saiko 10	15.6±0.5[b]	12.3±0.3[b]	78±14	62±11	0.9±0.8	145±2[c]
Dex 0.10	16.1±0.6	13.2±0.5	178±17	147±16	4.4±1.4	136±3
+Saiko 10	17.1±0.8	14.0±0.6	121± 6[b]	100± 5[b]	4.8±1.3	140±4
Dex 0.05	18.7±1.7	15.2±1.2	301±32	244±23	6.9±0.9	145±4
+Saiko 10	19.4±0.5	15.8±0.5	192±18[b]	157±15[c]	10.5±0.9[b]	143±3
Dex 0.025	19.8±2.0	16.3±1.5	457±36	378±29	9.4±2.4	156±5
+Saiko 10	23.3±1.3	19.1±0.8	319±24[b]	263±22[b]	11.6±2.8	148±2

a) Weight in mg/100 g of initial body weight in the evening of day 0. *b*) $p<0.05$, *c*) $p<0.01$ *vs.* dexamethasone.

However, when the weight was expressed relative to initial body weight, the hypertrophic effect of saikosaponin d alone was statistically insignificant in dexamethasone-treated rats (Table IIIb).

In rats treated with saikosaponin d for 5 d, saikosaponin d dose-dependently increased the adrenal weight and decreased the thymus weight (Table IV). In dexamethasone-treated (0.025 mg/kg) rats, a low dose of saikosaponin d increased the relative weight of the adrenals, restoring it to the saline-treated level, but higher doses did not. On the other hand, the effect of saikosaponin d on the thymus was dose-dependent and additive to that of dexamethasone (Table IV). This unusual dose-response relationship of saikosaponin d on the adrenals might be only apparent. In dexamethasone-treated rats, saikosaponin d may dose-dependently affect adrenal weight as well as thymus weight in the early phase of the 5-day period, but in the late phase, the effect of the increase in plasma corticosterone secreted due to saikosaponin may

TABLE IIIa. Effect of Saikosaponin d (Sd) on Body Weight in Normal and Dexamethasone-Treated (Dex) Rats

Treatment[a] (mg/kg/d × 9 d)	Body weight (g)[b]		Body weight increase (g/24 h)		
	Initial	Final	1st	2nd	2nd—9th (Total)
Saline	116±1	187±3	9.2±0.9	8.3±0.6	7.7 (62±2)
+Sd 2.5	115±1	171±4[d]	6.2±1.1[c]	7.2±0.7	6.3 (50±3[e])
Dex 0.05	115±1	169±2	5.8±1.0	6.2±0.6	6.0 (48±1)
+Sd 2.5	116±1	160±3[c]	2.5±0.7[c]	6.0±0.7	5.3 (42±2[d])

a) Groups of 12 rats. Saikosaponin d was co-administered with saline or dexamethasone. *b*) Body weight was determined in the evening on day 0 and 24 h after the last treatment. *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$ *vs.* saline or dexamethasone.

TABLE IIIb. Effect of Saikosaponin d (Sd) on the Relative Weights of the Adrenal and Thymus

Treatment (mg/kg/d × 9 d)	Weight (mg/100 g BWf)[a]		Weight (mg/100 g BWi)[b]	
	Adrenal	Thymus	Adrenal	Thymus
Saline	12.6±0.3	330±12	20.3±0.5	532±20
+Sd 2.5	16.6±0.6[e]	272± 8[e]	23.8±0.7[d]	405±16[e]
%	131	82	117	76
Dex 0.05	11.5±0.4	210±10	17.2±0.7	308±15
+Sd 2.5	12.9±0.3[c]	161± 9[d]	18.0±0.3	214±10[e]
%	112	77	105	69

a) Weight in mg/100 g of body weight 24 h after the 9th treatment. *b*) Weight in mg/100 g of body weight just before the 1st treatment. *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$ *vs.* saline or dexamethasone.

TABLE IV. Effect of Saikosaponin d (Sd) on Adrenal and Thymus Weights and Plasma Levels of Corticosterone (Cort) and Glucose in Normal and Dexamethasone-Treated (Dex) Rats

Treatment[a] (mg/kg/d × 5 d)	Weight (mg/100 g BWi)[b]				Cort		Glucose	
	Adrenal	$p<$	Thymus	$p<$	(μg/100 ml)	$p<$	(mg/100 ml)	$p<$
Saline	20.2±0.9	—	463±17	—	23.1±3.2	—	149±3	—
+Sd 1.0	21.3±1.3		441±22		15.4±1.9		145±6	
$p<$	NS		NS		NS		NS	
Dex 0.025	16.5±1.2	0.05	338±14	0.001	12.1±1.5	0.02	142±4	NS
+Sd 1.0	19.7±1.0	NS	303±20	0.001	14.1±1.4	0.05	147±5	NS
$p<$	0.1		NS		NS		NS	
Saline	23.2±0.2	—	468±28	—	17.6±0.7	—	149±4	—
+Sd 2.5 (7)	28.6±1.6		403±19		19.2±1.7		139±3	
$p<$	0.02		0.1		NS		NS	
Dex 0.025	22.0±1.3	NS	327±12	0.001	15.3±2.4	NS	143±6	NS
+Sd 2.5	21.1±0.8	0.10	250± 9	0.001	11.8±1.8	0.02	138±6	NS
$p<$	NS		0.001		NS		NS	

a) Groups of 6 rats (or 7 rats, where indicated in parentheses). *b*) Weight in mg/100 g of body weight in the evening of day 0. NS, not significant.

Table V. Effects of Adrenocorticotropin (ACTH) and Dexamethasone (Dex) on Adrenal and Thymus Weights in Intact Rats

Treatment (mg/kg/d)	Body weight increase (g/24 h)		Weight (mg/BWi)[a]	
	1st	2nd—final (Total)	Adrenal	Thymus
Saline × 4 d[b]	6.2 ± 0.5	6.2 (18.5 ± 1.1)	33.1 ± 1.1	430 ± 15
+ ACTH 0.1 × 4 d[b]	6.0 ± 0.5	6.8 (20.2 ± 1.3)	33.5 ± 0.8	459 ± 37
+ ACTH 0.2 × 4 d[b]	7.0 ± 0.7	6.1 (18.1 ± 1.7)	38.0 ± 1.0[c]	380 ± 15[c]
Saline × 5 d	5.0 ± 0.5	8.5 (34.1 ± 1.1)	33.9 ± 1.1	434 ± 29
+ ACTH 0.1 × 5 d	6.5 ± 0.3[c]	7.0 (27.8 ± 1.6[c])	32.7 ± 1.5	433 ± 21
+ Dex 0.025 × 5 d	4.6 ± 1.0	6.1 (24.5 ± 1.4[d])	29.4 ± 1.2[c]	332 ± 18[c]

a) Weight in mg/100 g of body weight in the evening of day 0. *b*) Body weight increase was determined in the morning. Adrenal and thymus weights were determined 15 h after the 4th treatment. *c*) $p < 0.05$, *d*) $p < 0.001$ *vs.* saline.

Table VI. Effects of Saikosaponin d (Sd) and Dexamethasone (Dex) on Adrenal and Thymus Weights in Hypophysectomized Rats

Treatment[a] (mg/kg/d × 5 d)	Body weight increase (g/24 h)		Weight (mg/BWi)[b]	
	1st	2nd—5th (Total)	Adrenal	Thymus
Saline (7)	1.2 ± 0.8	−0.7 (−2.7 ± 0.5)	12.3 ± 0.6	256 ± 28
+ Sd 2.5 (6)	−2.4 ± 0.8[d]	−0.9 (−3.6 ± 1.4)	12.9 ± 0.4	250 ± 8
+ ACTH 0.1 (6)	0.7 ± 0.8	−0.6 (−2.4 ± 1.0)	16.1 ± 0.6[e]	256 ± 18
+ Dex 0.025 (7)	−1.9 ± 0.9[d]	−1.6 (−6.5 ± 1.1[d])	12.2 ± 0.5	139 ± 15[e]
+ Dex + Sd (6)	−4.2 ± 1.1[e]	−1.8 (−7.0 ± 1.5[d])	12.1 ± 0.3	127 ± 16[e]
Saline (6)[c]	−0.3 ± 0.8	−0.02 (−0.1 ± 0.9)	13.9 ± 0.5	263 ± 13
+ ACTH 0.2 (5)[c]	0.3 ± 0.8	0.05 (0.2 ± 1.3)	20.8 ± 0.7[f]	238 ± 13
+ Dex 0.05 (6)[c]	−0.4 ± 0.5	−1.4 (−5.6 ± 1.6[d])	15.8 ± 0.6[d]	125 ± 8[f]

a) Figures in parentheses are numbers of rats. *b*) Weight in mg/100 g of body weight in the evening of day 0. *c*) Body weight increase was determined in the morning. Adrenal and thymus weights were determined 15 h after the 5th treatment. *d*) $p < 0.05$, *e*) $p < 0.01$, *f*) $p < 0.001$ *vs.* saline.

become effective and additive to that of dexamethasone *via* a negative feedback mechanism.

Repeated treatment with saikosaponin d did not significantly affect the basal levels of plasma corticosterone and glucose in saline- or dexamethasone-treated rats 24 h after the final treatment.

Effect of Saikosaponin d, ACTH and Dexamethasone on Adrenal and Thymus Weights in Intact and Hypophysectomized Rats

The effect of ACTH on body weight gain was not significant in intact or hypophysectomized rats (Tables V and VI). Treatment with dexamethasone tended to decrease the 1st 24 h gain of body weight, and significantly decreased the body weight gain for the last 4-day interval in intact and hypophysectomized rats. Saikosaponin d significantly suppressed the 1st 24 h body weight gain, but not the 2nd to 5th 24 h gains in hypophysectomized rats (Table VI) as well as in intact rats.

In intact rats, the low dose of ACTH did not significantly affect the weight of the adrenals or thymus, but in hypophysectomized rats the low dose of ACTH markedly increased adrenal weight, and even the high dose did not significantly affect thymus weight (Tables V and VI). In intact rats, the low dose of dexamethasone significantly decreased the weights of the adrenals and thymus, but in hypophysectomized rats even the high dose of dexamethasone did not

decrease the weight of the adrenals, though it markedly decreased thymus weight (Tables V and VI). Saikosaponin d did not affect the weights of the adrenals and thymus in hypophysectomized rats chronically treated with saline or dexamethasone (Table VI), while saikosaponin d increased adrenal weight and decreased thymus weight in intact rats. This indicates that saikosaponin d was not a stimulant, nor was it toxic to the adrenals and thymus.

Discussion

Total saikosaponin and saikosaponin d chronically administered for 5 or 9 d significantly increased the adrenal weight in normal rats, and a moderate dose of saikosaponins antagonized the atrophic effect of dexamethasone on the adrenals (Table IV). They also decreased the thymus weight in both chronically saline- or dexamethasone-treated rats (Table IIIb). On the other hand, in hypophysectomized rats saikosaponin d did not affect the weight of the adrenals or thymus. Therefore, it is clear that saikosaponin has no direct action on the adrenals and thymus, and that the trophic effect of saikosaponin on the adrenals and atrophic effect on the thymus are indirect ones, which operate *via* the function of the hypothalamic-pituitary system.

ACTH induced a marked trophic effect on the adrenals, but a weakly atrophic effect on the thymus in hypophysectomized rats. This suggests that the trophic effect of ACTH on the adrenals was a direct one, and its atrophic effect on the thymus was indirect, *via* the function of the adrenal cortex. Dexamethasone induced marked atrophy of both the adrenals and the thymus in intact rats, but it did not induce atrophy of the adrenals, though it did induce marked atrophy of the thymus, in hypophysectomized rats. This suggests that the marked atrophic effect of dexamethasone on the thymus was a direct one, whereas its weakly atrophic effect on the adrenals was not direct, but was secondarily induced *via* its direct and primary suppression of the hypothalamic-pituitary system. The trophic effect of saikosaponin on the adrenals was ACTH-mimetic, but was indirect. Further, the atrophic effect of saikosaponin on the thymus was dexamethasone-like, but was indirect. In previous acute experiments,[3] in fact, we showed that saikosaponins d and a had no direct ACTH-like action, but induced ACTH secretion, so the trophic effect of saikosaponin d on the adrenals may result from secretion of ACTH and the like, and the atrophic effect of saikosaponin on the thymus may be a secondary result of repeatedly stimulated secretion of corticosterone from the hypertrophied adrenals.

In rats chronically treated with dexamethasone, co-administration of a low dose of saikosaponin d induced hypertrophy of the adrenals and atrophy of the thymus, as in chronically saline-treated rats. In dexamethasone-treated rats, the intensity of the trophic effect of the high dose of saikosaponin d was rather weak, and was sometimes insignificant. Yamamoto *et al.*[10] reported that a high dose of a purified mixture of saikosaponins a, b and d (10 mg/kg/d × 8 d, *i.m.*), or saikosaponin d alone (2 mg/kg/d × 5 d, *i.m.*), shows a potent antigranulomatous action, but does not significantly affect body weight, adrenal weight or plasma 11-OH-corticosteroid level. Abe *et al.*[11] reported that in dexamethasone-treated (0.1 mg/kg, *i.m.*) rats saikosaponin d was rather atrophic to the adrenals on the final day, while it potentiated the antigranulomatous action of dexamethasone. In the present work, the high dose of saikosaponin d was atrophic to the thymus and additive to the atrophic effect of dexamethasone. This suggests that saikosaponin primarily stimulated the adrenal cortex to secrete corticosterone, which has antigranulomatous action and suppressive action on the hypothalamic-pituitary system in normal and dexamethasone-treated rats. Therefore, it seems clear that the trophic effect of saikosaponin on the adrenals was antagonistic to the atrophic effect due to primarily dexamethasone-induced and secondarily corticosterone-induced blocking of ACTH secretion, and saikosaponin attenuated the atrophic effect due to

dexamethasone and corticosterone. In acute experiments, in fact, we showed that saikosaponin d or a antagonized the dexamethasone-induced blocking of (ACTH and) corticosterone secretion in a competitive manner.[8] Therefore, it may be concluded that in both acute and chronic experiments, saikosaponin co-administered with dexamethasone was effective on the adrenals and thymus in essentially the same manner as in rats singly administered with saikosaponin d, and that the effect of long-term treatment with a high dose of saikosaponin d might be limited by the feedback action of endogenously saikosaponin-induced corticosterone, especially in the late phase of chronic treatment.

Chronic treatment with saikosaponin induced ACTH-like growth of the adrenals and glucocorticoid-like atrophy of the thymus, but did not significantly affect the basal evening level of plasma corticosterone. These results might suggest that the hypertrophied adrenals were active. However, it is not evident whether the hypertrophy of the adrenals reflects that of the adrenal cortex, and is accompanied by hyperplasia of the adrenal cortex. It is also not clear whether the adrenal cortex of the hypertrophied adrenals and the hypothalamic-pituitary system in rats treated with saikosaponin are fully active. Recently, N-terminal peptides of proopiomelanocortin (POC), N-POC (1—28) and (2—59), have been shown to be active in causing adrenal growth and deoxyribonucleic acid (DNA) synthesis,[12] while synthetic ACTH inhibits compensatory adrenal growth.[13] ACTH is derived from a precursor, proopiomelanocortin, which contains lipotropin and N-terminal glycopeptide.[14] In acute experiments we showed that saikosaponin induced secretion of ACTH or corticosterone in rats treated with or without dexamethasone.[3,8] Therefore, in these chronic experiments saikosaponin may also concomitantly release ACTH and N-terminal peptides of POC. Thus, increase in the adrenal weight due to saikosaponin may be mainly due to increase in the adrenal cortex, and it may be accompanied by hyperplasia of the adrenal cortex as well as hypertrophy. In order to maintain or to increase the weight and activity of the adrenal cortex, administration of saikosaponin, which endogenously induces co-secretion of ACTH and N-terminal peptides, may be better than that of ACTH alone.

From this series of experiments, it is clear that saikosaponin shows a glucocorticoid effect and has no direct blocking action on the hypothalamic-pituitary system. Therefore, co-administration of saikosaponin and glucocorticoid, or even administration of saikosaponin alone, may be useful clinically, especially in long-term treatment, to reduce the risk of glucocorticoid-induced adrenal atrophy and insufficiency without reducing glucocorticoid activity.

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