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Saireito (a Chinese Herbal Drug) Decreases Inhibitory Effect of Prednisolone and Accelerates the Recovery of Rat Hypothalamic-Pituitary-Adrenal Axis

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Abstract. Saireito, a saiko agent (a Chinese herbal drug), increases the synthesis and secretion of ACTH by stimulating hypothalamic CRH release. In the present study, we examined the effect of food containing saireito (1.5%) on the recovery of the hypothalamic-pituitary-adrenal axis after treating male rats with prednisolone (PSL, 200 μM) in drinking water for 14 days. Saireito was administered during and after PSL administration. The rats were decapitated at various times after PSL administration. Tail-pinch stress had been applied to some rats. The plasma ACTH response to tail-pinch stress in the PSL + saireito group recovered to the control level on day 1, but that in the group given PSL alone recovered on day 3. The ACTH level in the anterior pituitary and the CRH level in the median eminence of the PSL + saireito group returned to the control level on day 3, and that in the group given PSL alone returned to it on day 5. These results indicate that the administration of saireito reduces the negative feedback effect of PSL on the hypothalamus and pituitary and accelerates the recovery of the hypothalamic CRH and pituitary ACTH level after glucocorticoid treatment.

Key words: Saireito, CRH, ACTH, Prednisolone

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LONG-TERM administration of glucocorticoids causes profound suppression of the hypothalamic-pituitary-adrenal (HPA) axis by negative feedback. Glucocorticoids inhibit synthesis and secretion of CRH in the hypothalamus and that of ACTH in the anterior pituitary gland (AP), causing adrenocortical atrophy. Recovery of the HPA axis in humans usually takes weeks to months [1, 2]. The pattern of recovery after long-term glucocorticoid treatment is thought to be as follows: Hypothalamic CRH synthesis and secretion presumably recover first and synthesis and secretion of ACTH recover slightly later. Finally, adrenal steroidgenesis recovers a short time after the recovery of ACTH [3–8]. In previous studies on the recovery of the HPA axis in rats after treatment with pharmacological amounts of glucocorticoids for 14 days, the responsiveness of the HPA axis was restored within 10 days [9–11].

Saiko agents, which are Chinese herbal drugs, are now believed to enhance the effect of glucocorticoids in treating nephrotic syndrome, bronchial asthma and chronic rheumatoid arthritis [10, 12, 13]. Saiko agents also stimulate ACTH secretion [14–16]. In previous studies, we found that saireito, a saiko agent, stimulated CRH release and POMC gene expression in a dose-dependent manner in rats [17, 18]. In the present study we examined whether the administration of saireito together with prednisolone (PSL) could enhance

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recovery of the suppressed HPA axis after the withdrawal of PSL treatment.

Materials and Methods

Male Wistar rats (170–190 g) were housed in an air-conditioned room under a controlled light-dark cycle (lights on at 0800 h, off at 2000 h). Food and water were available *ad libitum*. All the rats were handled and weighed daily for 1–2 min throughout the experiments and divided into 4 groups. The first group were given water containing 0.1% ethanol and normal rat food (control). The second group were given 200 μM PSL 21-sodium succinate (Sigma Chemical Co. Ltd., St. Louis, MO) in their drinking water containing 0.1% ethanol for 14 days and normal rat food (PSL alone), and mean water intake was 25 ml/rat/day. The third group were given water and rat food containing 1.5% saireito (about 1.5 g of saireito/kg BW/day, Tsumura, Co. Ltd., Tokyo, Japan) (saireito alone). The fourth group were given PSL in their water for 14 days and rat food containing saireito (PSL + saireito). After 14 days, water containing PSL was replaced with normal tap water, but food with or without saireito was not changed throughout the series of experiments. To examine the recovery of the HPA axis after 0, 1, 3, 5 and 7 withdrawal days, rats in each of the 4 groups were subdivided into 2 groups: a stress and a nonstress group. In the stress group, tail-pinch stress was performed for 15 sec in another room. In tail-pinch stress, plasma ACTH levels reached the peak level at 15 min and plateau level at 30 min, then returned to the basal level at 60 min (Fig. 1). Therefore, in the present study, the rats were decapitated either without stress (the basal level) or 20 min after tail-pinch stress. All experiments were done between 0900 h and 1100 h. Trunk blood was collected in iced tubes containing EDTA. The plasma was separated immediately and frozen until the time of assay. After decapitation, the anterior pituitary gland (AP) and median eminence (ME) were obtained as previously described [19]. Tissues were frozen on dry ice immediately after removal and kept frozen at −20°C until homogenization with 0.1 N HCl as previously reported [19]. Plasma ACTH levels were determined with an ACTH IRMA kit (Yukamedius Co. Ltd., Tokyo, Japan) as previously reported [20], CRH and ACTH levels in the tissues were determined by RIA as previously described [19, 20]. Synthetic human CRH and ACTH were purchased from Peninsula Laboratories (Belmont, CA). Experimental protocols and animal care were approved by an institutional committee responsible for animal care.

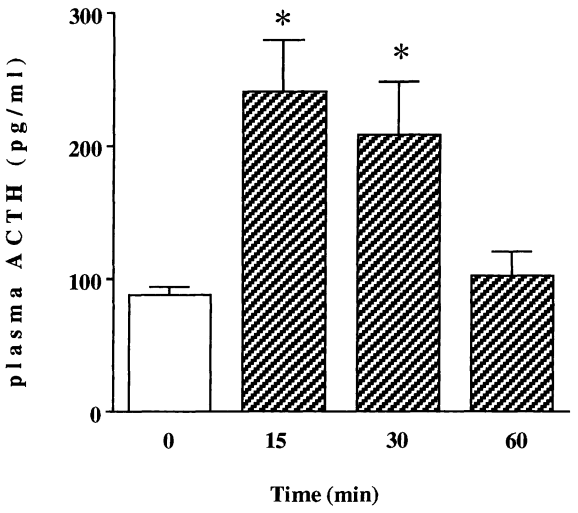

Fig. 1. Time-course study on the plasma ACTH response to tail-pinch stress in control rats. Mean ± SEM (n=6) *$P<0.05$ *vs.* basal.

Experimental results are expressed as the mean ± SEM. Statistical significance ($P<0.05$) was determined by one-way analysis of variance with Duncan's multiple range test.

Results

Body weight

On withdrawal day 0, body weight was 285 ± 34 g in the control group, 294 ± 42 g in the group given saireito alone, 195 ± 27 g in the group given PSL alone and 206 ± 37 g in the PSL+ saireito group. Body weight in the PSL-treated groups was significantly lower than that in the other 2 groups. Administration of saireito during the experiment did not affect food or water intake or the body weight of the rats.

Basal plasma ACTH levels

The basal level of plasma ACTH was 58 ± 5 pg/ml in the control group on withdrawal day 0, and

was similar to that in the group given saireito alone throughout the experimental period (Fig. 2). Plasma ACTH levels in the two PSL-treated groups were noticeably lower than those in the control group on withdrawal days 0 and 1, and then increased to the control level on day 3. In the PSL-treated groups, the basal plasma ACTH level in the group given PSL alone was significantly lower than that in the group given PSL + saireito on days 0 and 1.

Plasma ACTH response to stress

The plasma ACTH responses to tail-pinch stress were determined 20 min afterwards in all groups. The plasma ACTH level after tail-pinch stress in the group given saireito alone was slightly, but not significantly, higher than that in the control group on days 0–3 (Fig. 3). In the PSL-treated group, low plasma ACTH levels showed only a slight response to stress on day 0. In the group given PSL+saireito, the plasma ACTH level after stress increased to the control level on withdrawal day 1, and that in the group given PSL alone increased to the control level on day 3.

ACTH content in AP

ACTH content in the AP in the group given saireito alone was slightly, but not significantly, higher than that in the control group throughout the experiment (Fig. 4). In the group given PSL + saireito, ACTH content in the AP was much lower than that in the control group until withdrawal day 1 but it returned to the control level on day 3. ACTH content in the AP in the group given PSL alone was lower than that in the group given PSL + saireito on days 0–3, and returned to the control level on day 5.

CRH content in the ME

CRH content in the ME was similar in the control group and the group given saireito alone throughout the experiment (Fig. 5). In the PSL-treated groups, CRH content in the ME was significantly lower than that in the control group on withdrawal days 0–1. Nevertheless, CRH content in the ME in the PSL + saireito group was higher than that in the group given PSL alone, and returned to the control level on day 3. CRH content in the ME in the group given PSL alone returned to the control level on day 5.

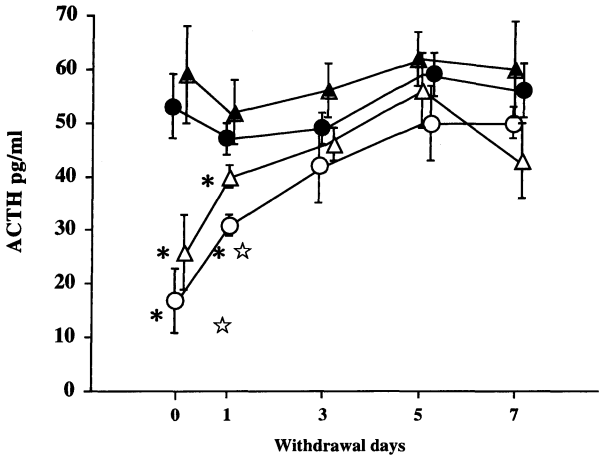

Fig. 2. Basal plasma ACTH level after PSL withdrawal. ●, control; ▲, saireito alone; △, saireito + PSL; ○, PSL alone. *$P<0.05$ *vs.* control. ☆$P<0.05$ *vs.* PSL+ saireito. Mean ± SEM (n=8 in each).

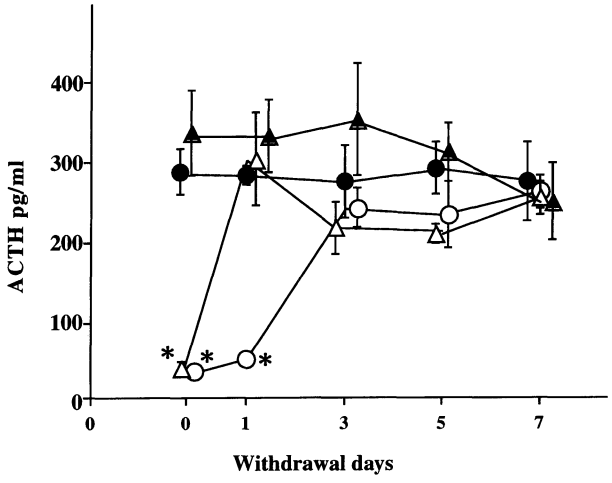

Fig. 3. Plasma ACTH levels 15 min after tail-pinch stress. ●, control; ▲, saireito alone; △, saireito + PSL; ○, PSL alone. *$P<0.05$ *vs.* control. Mean ± SEM (n=8 in each).

Discussion

Activated glucocorticoid receptor (GR) acts on transcription of glucocorticoid-response genes in two ways. A receptor homodimer binds to glucocorticoid response elements (GRE), interacts with transcription factors and enhances

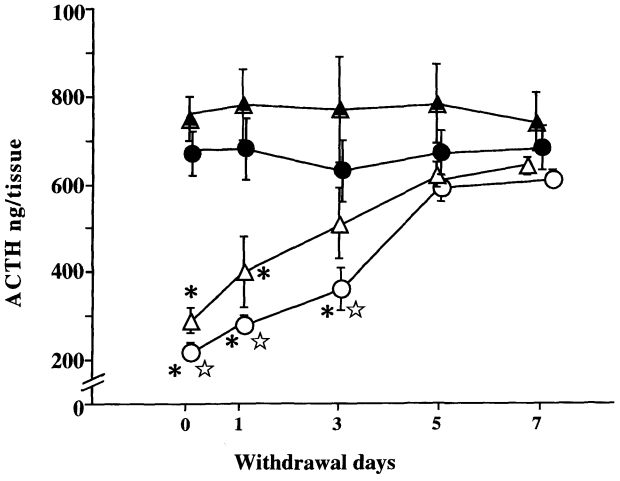

Fig. 4. AP ACTH levels after PSL withdrawal, ●, control; ▲, saireito alone; △, saireito + PSL; ○, PSL alone. *P<0.05 *vs.* control. ☆P<0.05 *vs.* PSL + saireito. Mean ± SEM (n=8 in each).

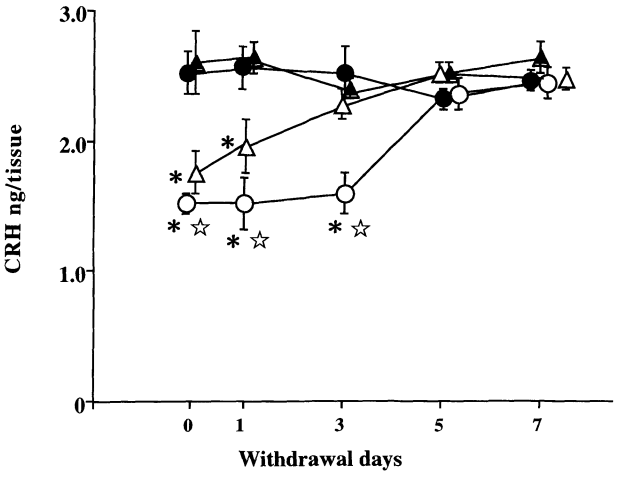

Fig. 5. ME CRH levels after PSL withdrawal. ●, control; ▲, saireito alone; △, saireito + PSL; ○, PSL alone. *P<0.05 *vs.* control. ☆P<0.05 *vs.* PSL + saireito. Mean ± SEM (n=8 in each).

transcription [21]. On the other hand, the activated receptor homotrimer binds to negative GRE (nGRE) of the target gene and represses transcription [22]. This nGRE is located in the promoter region of the POMC gene [22] and probably of the CRH gene. In a non-genomic effect of GR, the activated GR binds to transcription factors (activating protein-1, NF-kB....) and bound forms of transcription factors cannot bind to their gene binding sites.

From these points of view, differences among tissues in gucocorticoid action could be explained by following: a) A different mechanism of GR binding to GRE from GR binding to nGRE, b) The GR target gene in peripheral tissues may not contain nGRE, c) GR-affected transcription factors or GR-induced proteins in peripheral tissues may be different from those in the pituitary and in the hypothalamus.

In previous studies, glucocorticoid treatment for 7–14 days resulted in a transient suppression of the HPA axis that was followed by recovery after 5–9 days [9–11]. In the present study, the basal plasma ACTH level and plasma ACTH response to stress at 20 min recovered to the control level 3 days after PSL withdrawal in the group given PSL alone. On the other hand, the AP ACTH and ME CRH levels recovered to the control levels after 5 withdrawal days. The recovery of the AP ACTH and ME CRH levels in the present study was similar to that in previous studies [9–11]. In this study, because plasma ACTH levels were determined at one point (20 min) after stress, delayed ACTH response to stress was not detected, but such delayed response is also a kind of inhibited response. From this point of view, determination of plasma ACTH at one point after stress is fairly useful for evaluation of the HPA axis. Plasma ACTH levels and ACTH responses to stress recovered earlier than AP ACTH and ME CRH levels. This supported the previous report stating that CRH synthesis recovered earlier (after 18 h) than POMC mRNA in the AP (after 36 h) [9], and suggests that the pooled levels of ACTH in the AP and those of CRH in the ME were enough to maintain the basal level of plasma ACTH and response to a single stimulus after 3 days. These levels would probably not be sufficient for responses to repeated stimuli.

Treatment with saireito enhanced the recovery of AP ACTH and ME CRH levels in the present study. In other words, PSL-induced suppression of hypothalamic CRH and AP ACTH levels was reduced by the administration of saireito at the same time. In our previous studies, acute administration of saireito stimulated ACTH secretion and POMC gene expression in the AP in rats [17]. The levels of plasma ACTH and AP ACTH in the group given saireito alone were slightly higher than those in the control group, but were not statistically significant. This may be due

to a difference between acute and chronic administration of saireito.

Saireito-induced ACTH secretion and POMC gene expression in the AP were completely blocked by passive immunization of endogenous CRH with CRH-antiserum [18]. This indicated that saireito-induced ACTH secretion and POMC gene expression were mediated by CRH. We recently found that central administration of saikosaponin (one of the main components of saiko agents) stimulated CRH gene expression in the paraventricular nucleus of the rat hypothalamus [23]. The present results indicate that administration of saireito enhanced the recover of ME CRH levels after withdrawal. This finding and the results of our previous studies [17, 23] suggest that saireito acts on the hypothalamus to stimulate CRH synthesis as well as CRH release. In addition, saireito-induced nuclear protein may bind to GR and reduce the inhibitory effect of glucocorticoids in the hypothalamus.

In the present study, the administration of saireito seemed to be useful for enhancing the recovery of the HPA axis after treatment with glucocorticoids or surgery in patients with Cushing's syndrome.

Acknowledgments

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