[Chem. Pharm. Bull. 32(3)1224—1227(1984)]

Effect of Saikosaponins on Dexamethasone Suppression of the Pituitary–Adrenocortical System

HIROOMI YOKOYAMA, SUSUMU HIAI* and HIKOKICHI OURA

Research Institute for WAKAN-YAKU, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930–01, Japan

(Received May 25, 1983)

The effect of saikosaponins d and a on corticosterone secretion was investigated in dexamethasone-treated rats (250 μg/kg, 0.64×10^{-6} mol/kg, *i.p.* 3 h before saponin injection). Plasma corticosterone level 30 min after saponin injection was determined by the competitive protein binding method. Saikosaponins d and a both had no effect at low doses, but elevated plasma corticosterone levels at higher doses. ED_{50} values for corticosterone response to saikosaponins d and a were 1.4 mg/kg (1.8×10^{-6} mol/kg) and 3.8 mg/kg (4.9×10^{-6} mol/kg) in the dexamethasone-treated rats, and these values were 4.2 and 4.3 times greater than the ED_{50} values determined in rats without dexamethasone treatment, respectively.

Keywords——Bupleuri Radix; saikosaponin; dexamethasone; corticosterone; pituitary–adrenocortical system; corticoid feedback

In a series of experiments, we have shown that saponins of Ginseng Radix,[1,2] Bupleuri Radix,[3] Hippocastani Semen,[4] Platycodi Radix,[5] and several other triterpenoidal saponins[6] act to stimulate corticosterone secretion in rats. It was also shown that ginseng saponin increased adrenal cyclic-adenosine-monophosphate (cAMP), a second messenger of adrenocorticotropic hormone (ACTH), to stimulate synthesis and secretion of corticosteroid in normal rats, but not in hypophysectomized rats.[7] Increases of plasma corticosterone induced by these saponins were accompanied by marked elevation of plasma ACTH levels.[1,3–6] Moreover, the plasma corticosterone-increasing activities of ginseng saponin and isolated saikosaponins d and a were blocked[1,3] by dexamethasone, a potent inhibitor of ACTH secretion. From these results, it was suggested that these saponins stimulated corticosterone secretion as a result of an increase in ACTH secretion from the pituitary grand.

On the other hand, ginseng saponin at greater doses was shown to cause some elevation of plasma corticosterone in rats treated with dexamethasone.[8] This ineffectiveness of dexamethasone in preventing corticosterone increase suggested that saponin may release the suppression of the pituitary-adrenocortical system due to dexamethasone. In this paper we further examined the effect of isolated pure saikosaponins d and a on the dexamethasone suppression of plasma corticosterone level.

Materials and Methods

Male Wistar rats weighing 130—150 g were used in this experiment. The rats had been maintained for one week under conditions of controlled lighting (illumination from 0600—1800 h) and temperature (24 °C). From four or five days before use, the rats were handled every morning and evening in order to acclimatize them to non-specific stimuli. The day before the experiment, they were transferred into individual cages. The animals were fed laboratory chow (CE-2, CLEA Japan Inc., Tokyo) and water *ad libitum*.

Dexamethasone was dissolved in ethanol and diluted with saline. The final concentration of ethanol in this solution was 2.5%. Saikosaponins d and a (kindly supplied by Shionogi and Co., Osaka) were ground and suspended in saline.

Various doses of saikosaponins d and a were injected into both saline- and dexamethasone-pretreated rats.

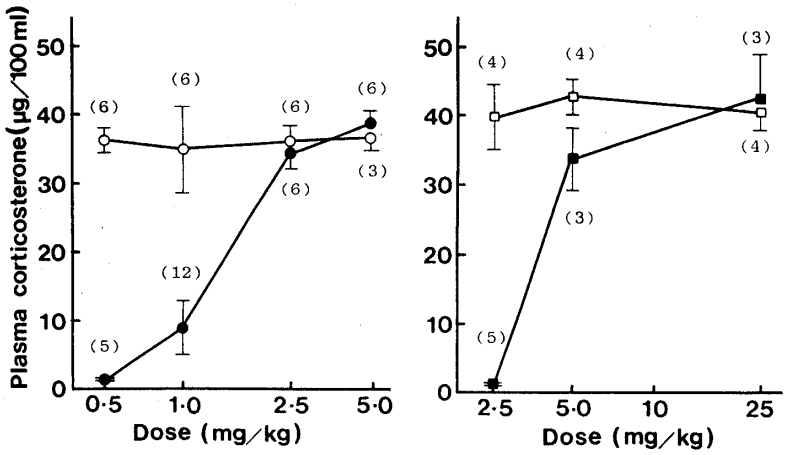

Fig. 1. Effect of Saikosaponins on Plasma Corticosterone Levels in Saline- or Dexamethasone-Pretreated Rats

The rats were given saline (1 ml/kg *i.p.*, open symbols) or dexamethasone (250 μg/kg *i.p.*, closed symbols) 3 h before saikosaponin d (circles) or saikosaponin a (squares) injection (*i.p.*). At 30 min after saikosaponin injection, 0930—1030 h, rats were decapitated and the plasma samples for corticosterone determination were collected. Each point is the mean ± S.E.M. with the number of rats used given in parentheses.

According to the report of Takebe *et al.*,[9] increasing the dose of dexamethasone prolonged the duration of inhibition of the plasma corticosterone response to laparotomy with intestinal traction, but did not increase its magnitude after stimuli. The period of effective inhibition was 3—6 h after the administration of 250 μg/kg of dexamethasone. Therefore, 3 h before injection of saikosaponins, rats received 2.5% ethanol-saline or 250 μg/kg of dexamethasone solution (1 ml/kg, *i.p.*). At 30 min after saikosaponin injection, rats were decapitated within 15 s after removal from their cages. Trunk blood was collected in chilled tubes with heparin. Determination of plasma corticosterone by the competitive protein binding method[10] was done as described in a previous paper.[1] The doses of saikosaponins d and a producing the half-maximal increase in plasma corticosterone levels (ED_{50}) and the 95% confidence limits were determined by the method of Litchfield and Wilcoxon.[11]

Results and Discussion

Figure 1 summarizes the effect of saikosaponins on plasma corticosterone response in rats pretreated with saline or dexamethasone. Saikosaponins d and a increased plasma corticosterone at all doses tested in saline-pretreated rats to about 40 μg/100 ml. This result is coincident with our previous result in non-pretreated rats.[12]

In dexamethasone-pretreated rats, the elevation of plasma corticosterone level induced by 0.5 mg/kg of saikosaponin d was completely abolished, and that by 2.5 mg/kg of saikosaponin a was also completely inhibited. It is clear that dexamethasone at a dose of 250 μg/kg (0.64×10^{-6} mol/kg) was sufficient to suppress the corticosterone secretion-inducing activity of saikosaponins.

When 1 mg/kg of saikosaponin d was administered to dexamethasone pretreated rats, plasma corticosterone was somewhat elevated, and 2.5 and 5.0 mg/kg of saikosaponin d elevated corticosterone maximally, as was the case in saline-pretreated rats. Similarly, administration of higher doses than 2.5 mg/kg of saikosaponin a, 5.0 and 25 mg/kg, elevated plasma corticosterone maximally. This result indicated that saikosaponins d and a released the dexamethasone suppression of the pituitary-adrenocortical system.

The suppressive effect of dexamethasone in the pituitary-adrenocortical system has been discussed in relation to the stress response and negative feedback mechanism. There is evidence that various types of stimuli, such as 100% nitrogen inhalation, hemorrhage, laparotomy with intestinal traction, histamine (20 mg/kg, *i.v.*), lysine vasopressin (500 mU/kg,

TABLE I. ED_{50} Values for Corticosterone Response to Saikosaponins in Dexamethasone-Pretreated Rats and in Non-Pretreated Rats

	ED_{50} (mg/kg, mol/kg)		(A)/(B)
	Dex-pretreated[a] rat (A)	Non-pretreated[b] rat (B)	
Saikosaponin d	1.4, 1.8×10^{-6} (1.1—1.8)[c]	0.33, 0.42×10^{-6} (0.25—0.43)[c]	4.2
Saikosaponin a	3.8, 4.9×10^{-6} (2.4—6.0)[c]	0.89, 1.1×10^{-6} (0.64—1.2)[c]	4.3
d : a	1 : 2.7	1 : 2.7	

a) Dexamethasone 250 μg/kg (0.64×10^{-6} mol/kg, *i.p.*) was administered 3 h before saikosaponin injection.
b) Cited from a previous paper.[12]
c) 95% confidence limits.

i.v.)[13] and urethane (1 g/kg, *i.p.*),[14] cause corticosterone secretion in rats given dexamethasone (250 μg/kg, 6000 μg/kg *i.v.*, 2—7 h before, and 4000 μg/kg *i.p.*, 4 h before). On the basis of their evidence, Dallman and Yates[13] proposed as a model of corticoid feedback control that there are two different kinds of input pathways to the adrenocortical system. One is a low threshold corticoid-sensitive pathway and the other is a high threshold corticoid-resistant pathway. They divided these stimuli into those in which the adrenal response was blocked by pretreatment with a single large dose of corticosteroids (corticoid-sensitive) and those that still give a response after pretreatment with a maximally effective dose of corticosteroids (corticoid-resistant). Our experiment showed that saikosaponins d and a caused elevation of plasma corticosterone levels in rats given a maximally effective dose of dexamethasone. Therefore, saikosaponins d and a appear to cause corticoid-resistant stimulation, like lysine vasopressin.[13]

From Fig. 1, the ED_{50} values for corticosterone response to saikosaponins d and a in dexamethasone-pretreated rats were determined to be 1.4 mg/kg (1.8×10^{-6} mol/kg) and 3.8 mg/kg (4.9×10^{-6} mol/kg), respectively. These ED_{50} values of saikosaponins d and a are 4.2 and 4.3 times greater than those determined in non-pretreated rats (saikosaponin d, 0.33 mg/kg, 0.42×10^{-6} mol/kg; saikosaponin a, 0.89 mg/kg, 1.1×10^{-6} mol/kg; Table I). These results suggest that saikosaponins d and a affect the corticoid feedback mechanism in the same manner, but with different intensities probably as a result of the differences in their chemical structures.

The site of action in feedback inhibition of glucocorticoid under the conditions which we used in this experiment is not clear. The anterior pituitary and/or hypothalamus have been suggested. In particular, the recent reports of Sakakura *et al.*[15] provided experimental evidence that there is a difference in degree of inhibition of ACTH release between rats infused with corticosterone and dexamethasone. These data seem to suggest that the main inhibitory sites of corticosterone and dexamethasone are the hypothalamus and pituitary, respectively. Our present data show that the action of lower doses of saikosaponins d and a on the pituitary-adrenocortical system was suppressed by dexamethasone, and that high doses of saikosaponins d and a released the suppressive effect of dexamethasone. Thus, the site of action of saikosaponins d and a is thought to be closely related to that of dexamethasone. Further investigation of the action mechanism of saikosaponins in relation to adrenal function is required together with a study of the mechanism of inhibitory action of dexamethasone in *in vitro* systems.

References

1) S. Hiai, H. Yokoyama, H. Oura and S. Yano, *Endocrinol. Jpn.*, **26**, 661 (1979).
2) S. Hiai, H. Yokoyama and H. Oura, *Endocrinol. Jpn.*, **26**, 737 (1979).
3) S. Hiai, H. Yokoyama, T. Nagasawa and H. Oura, *Chem. Pharm. Bull.*, **29**, 495 (1981).
4) S. Hiai, H. Yokoyama and H. Oura, *Chem. Pharm. Bull.*, **29**, 490 (1981).
5) H. Yokoyama, S. Hiai and H. Oura, *Yakugaku Zasshi*, **102**, 1191 (1982).
6) H. Yokoyama, S. Hiai, H. Oura and T. Hayashi, *Yakugaku Zasshi*, **102**, 555 (1982).
7) S. Hiai, S. Sasaki and H. Oura, *Planta Medica*, **37**, 15 (1979).
8) S. Hiai, H. Yokoyama and H. Oura, *Proc. Symp. Wakan-Yaku*, **12**, 33 (1979).
9) K. Takebe, H. Kunita, M. Sakakura, Y. Horiuchi and K. Mashimo, *Endocrinology*, **89**, 1014 (1971).
10) B. E. P. Murphy, *J. Clin. Endocrinol.*, **27**, 973 (1967).
11) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
12) H. Yokoyama, S. Hiai and H. Oura, *Chem. Pharm. Bull.*, **29**, 500 (1981).
13) M. F. Dallman and F. E. Yates, *Mem. Soc. Endocrinol.*, **17**, 39 (1968); M. F. Dallman, "Interaction within the Brain-Pituitary-Adrenocortical System," ed. by M. T. Jones, B. Gillham, M. F. Dallman and S. Chattopadhyay, Academic Press, London, 1979, p. 149.
14) N. E. Sirett and F. P. Gibbs, *Endocrinology*, **85**, 355 (1969).
15) M. Sakakura, M. Yoshioka, M. Kobayashi and K. Takebe, *Neuroendocrinology*, **32**, 38 (1981); *idem*, *ibid.*, **32**, 174 (1981).