[Chem. Pharm. Bull. 29(2) 495—499 (1981)]

Stimulation of the Pituitary-adrenocortical Axis by Saikosaponin of Bupleuri Radix

Susumu Hiai,* Hiroomi Yokoyama, Tetsuro Nagasawa, and Hikokichi Oura

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

(Received July 8, 1980)

The effects of saikosaponin-a, -c and -d, isolated from the roots of *Bupleurum falcatum* L., on plasma adrenccorticotropin (ACTH) and corticosterone concentrations in unanesthetized or pentobarbital-anesthetized rats were determined by radioimmunoassay and by the competitive protein binding method. A single dose of saikosaponin-a (5 mg/kg, *i.p.*) or -d (2.5 mg/kg, *i.p.*) significantly increased plasma ACTH and corticosterone levels 30 and 60 min after the treatment, but saikosaponin-c at a dose of 100 mg/kg had no effect. Intraperitoneal administration of saikosaponin-a or -d, but not -c, induced a transient hyperglycemia and a transient hypoinsulinemia. Dexamethasone pretreatment depressed the saponin-induced increase of both corticosterone and glucose. Treatment with hexamethonium partially depressed the saponin-induced responses in plasma corticosterone, but treatment with diphenhydramine did not.

Keywords——saikosaponins; *Bupleurum falcatum* L., plasma ACTH; corticosterone; dexamethasone; glucose; immunoreactive insulin; hexamethonium; diphenhydramine

The roots of *Bupleurum falcatum* L. (Umbelliferae) (Mishimasaiko in Japanese) are an important component in various prescripitions in Chinese traditional medicine. Saikosaponins were isolated from the drug and the structures of saikosaponin-a, -c and -d were established.[1] Takagi and Shibata[2] reported that crude saikosides (saikosaponins) showed antiinflammatory and other pharmacological actions. Yamamoto *et al.*[3] reported that saikosaponin-a and -d, but not -c, had antiinflammatory and plasma cholesterol-lowering actions. Abe *et al.*[4] studied the actions of saikosaponins on biological membranes.

It is known that some triterpenoidal saponins such as glycyrrhizin and escin show antiinflammatory activities.[5] We found that ginsenoside, a saponin from the roots of *Panax ginseng*,[6] and escin[7] had adrenocorticotropin and corticosterone secretion-inducing action. These findings led us to investigate the stimulating action of saikosaponins on the pituitary-adrenocortical function in rats.

Materials and Methods

Male Wistar rats of 130—150 g body weight were used. They were fed on laboratory chow (CE-2, CLEA Japan Inc., Tokyo) and tap water *ad libitum*, and maintained at 24°, with 12 hr of artificial light (light phase: 0600 to 1800 hr) for more than 6 days. To avoid stress-stimulated corticosterone release, they were "gentled" by daily handling for 4 days, twice a day in the morning and evening. Rats were decapitated with a guillotine at between 0900 and 1000 hr. Trunk blood was collected in a chilled heparinized tube and centrifuged at 4°, and plasma was stored at −20°.

ACTH was determined by the radioimmunoassay method using the kit from the Radiochemical Centre, Amersham, which consists of anti-human ACTH serum, standard human ACTH and ^{125}I-α^{1-24}-ACTH. Immunoreactive insulin was assayed by the double antibody system with the kit from Eiken Immunochemical Laboratory, Tokyo. Corticosterone was determined by the competitive protein binding method of Murphy.[8] Human serum was used as the corticoid binding globulin. Standard corticosterone and ^{3}H-corticosterone (45 Ci/mM) were from Merck and the Radiochemical Centre, respectively. Plasma glucose was determined by the glucose oxidase method. Saikosaponins did not affect the determinations for plasma corticosterone and glucose.

Saikosaponin-a, -c and -d were kindly supplied by Dr. K. Kawasaki and Dr. H. Ishii of Shionogi and Co., Osaka. They were extracted from the roots of *B. falcatum* L. and purified by the procedure of Kubota and Hinoh.[1a] Saikosaponin-a or -d was ground in an agate mortor, and suspended in pyrogen-free saline.

Saikosaponin-c was dissolved in saline. Suspensions and solutions were made just before use. Sodium pentobarbital solution (Abbott Laboratories, Chicago) was used. Dexamethasone was dissolved in ethanol and used as ethanol-saline solution (2.5% ethanol). Hexamethonium chloride (Nakarai Chemicals, Kyoto), histamine dihydrochloride (Nakarai Chemicals, Kyoto) and diphenhydramine hydrochloride (Kongo Chemicals, Toyama) were used as saline solutions.

Results

Increase of Plasma Corticosterone and ACTH Levels by Saikosaponin-a and -d

A single intraperitoneal (*i.p.*) injection of saikosaponin-a (5 mg/kg) or -d (2.5 mg/kg) into unanesthetized rats induced a marked increase in plasma corticosterone, ACTH and glucose concentrations 30 min after the treatment (Fig. 1). The plasma corticosterone remained at this level 60 min after the treatment. Plasma ACTH had decreased somewhat at 60 min, while the plasma glucose level had decreased to the normal level 60 min after saponin treatment. Saikosaponin-c at a dose of 100 mg/kg (*i.p.*) induced no increase 30 min after the treatment. It induced a small increase of ACTH level 60 min after the treatment, but the reason for this is not clear at present. In pentobarbital-anesthetized rats (36 mg/kg, *i.p.*), saikosaponin-d (10 mg/kg) clearly induced increases of both corticosterone and glucose levels 30 min after the treatment (31±1 μg/100 ml, 212±5 mg/100 ml for 4 rats).

Dexamethasone (0.25 mg/kg, *i.p.*) or saline (0.5 ml) was administered to rats, and 3 hr after the treatment, saikosaponin-a (2.5 mg/kg) or -d (0.5 mg/kg) or saline (0.5 ml) was injected

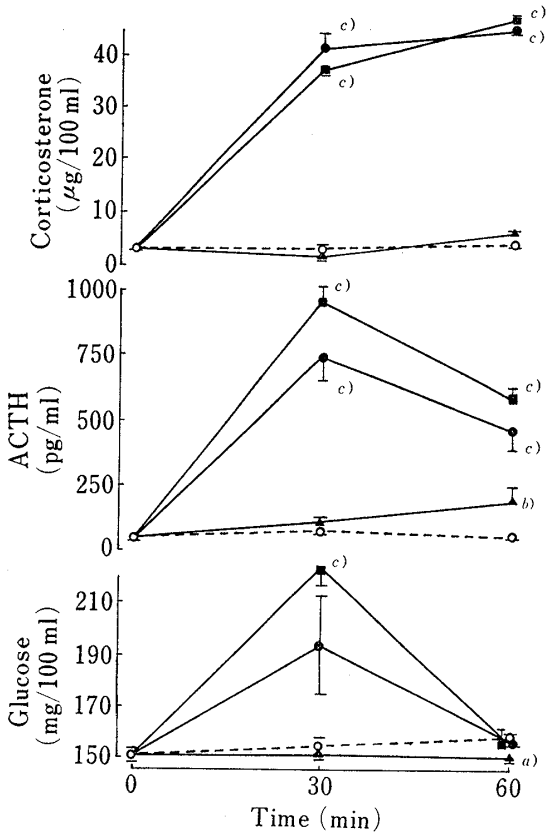

Fig. 1. Effects of Saikosaponins on Plasma Corticosterone, ACTH and Glucose Levels in Rats

Saikosaponin-a (■, 5 mg/kg), -d (●, 2.5 mg/kg), -c (▲, 100 mg/kg) or saline (○, 0.5 ml) was injected intraperitoneally. Data represent means±S.E. of 3 to 6 rats. *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$.

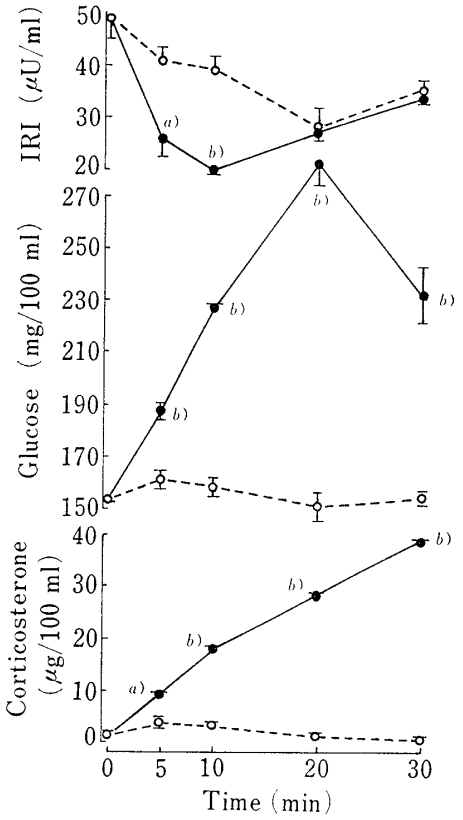

Fig. 2. Effects of Saikosaponin-d on Plasma Glucose and Immunoreactive Insulin Levels in Rats

Saikosaponin-d (●, 2.5 mg/kg, *i.p.*) or saline (○, 0.5 ml) was administered. Data represent means±S.E. of 4—5 rats. *a*) $p<0.01$; *b*) $p<0.001$.

Table I. Effect of Dexamethasone on Saikosaponin-induced Hormonal Response in Rats

Pretreatment	Corticosterone (μg/100 ml)			
	Saline	Saikosaponin-a	Saline	Saikosaponin-d
Saline	2.6 ±1.7	40 ±5	0.8±0.2	36 ±2
Dexamethasone	0.04±0.02	0.08±0.07[a)]	0.3±0.1	0.3±0.03[a)]

Dexamethasone (0.25 mg/kg, *i.p.*) or saline (0.5 ml) was administered 3 hr before the second *i.p.* treatment with saikosaponin-a (2.5 mg/kg) or -d (0.5 mg/kg), or saline (0.5 ml). Rats were sacrificed 30 min after the second treatment. Data represent means±S.E. of 4 to 6 rats. *a)* $p<0.001$ *vs.* without dexamethasone.

into the rats intraperitoneally. Treatment with dexamethasone clearly blocked saponin-induced corticosterone secretion (Table I).

Transient Hyperglycemia and Hypoinsulinemia

Acute hypoglycemia or insulin injection stimulates corticosteroid secretion.[9)] Thus, plasma glucose and immunoreactive insulin (IRI) as well as corticosterone were determined after the treatment. Fig. 2 shows that plasma corticosterone increased almost linearly up to about 30 min after the treatment, plasma glucose markedly increased, peaked at 20 min and then decreased, and plasma IRI significantly decreased and then recovered to the control level. This result suggested that the saponins induced epinephrine release.

Effect of a Ganglion Blocker and a Histamine Antagonist

The blocking effect of hexamethonium on the saponin-induced responses was examined (Fig. 3). Hexamethonium chloride at a dose of 12 mg/kg partially suppressed the saponin-induced increase of corticosterone but almost completely suppressed saponin-induced hyperglycemia. Hexamethonium at a dose of 22 mg/kg gave similar results. At higher doses, hexamethonium itself induced an increase of plasma corticosterone.

Histamine injection is known to induce corticoid secretion. We found that histamine-induced corticosterone secretion was accompanied by an increase of plasma glucose level.[6b)]

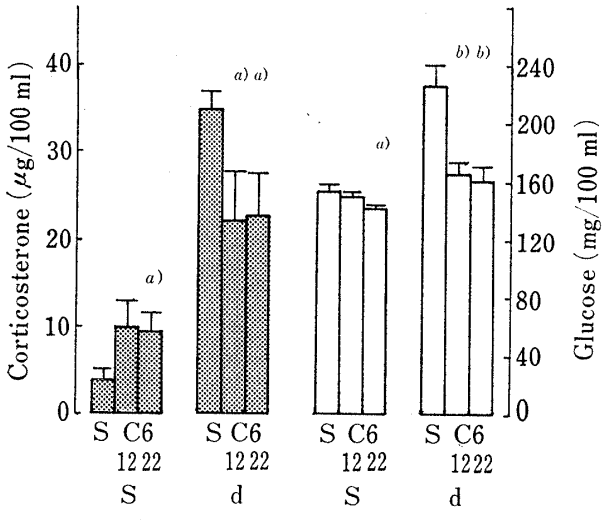

Fig. 3. Effects of Hexamethonium on Saponin-induced Plasma Corticosterone and Glucose Levels in Rats

Hexamethonium chloride (C6, 12 or 22 mg/kg, *i.p.*) or saline (S, 0.1 ml) was administered to rats 15 min before second treatment. Saikosaponin-d (d, 2.5 mg/kg, *i.p.*) or saline (S, 0.5 ml) was injected, and rats were sacrificed 30 min after this treatment. Data are means±S.E. of 8 rats. *a)* $p<0.05$; *b)* $p<0.01$. ▨, corticosterone; □, glucose.

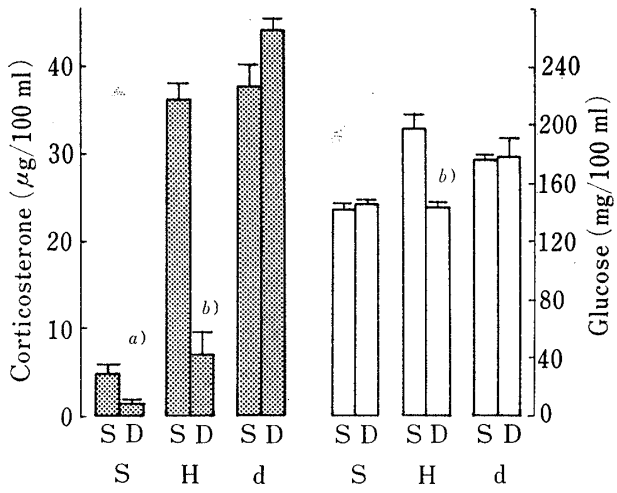

Fig. 4. Effects of Diphenhydramine on Saponin-induced Plasma Corticosterone and Glucose Levels in Rats

Diphenhydramine hydrochloride (D, 25mg/kg, *i.p.*) or saline (S, 0.1 ml) was administered to rats 45 min before second treatment. Saikosaponin-d (d, 1 mg/kg, *i.p.*), histamine dihydrochloride (H, 8 mg/kg) or saline (S, 0.5 ml) was injected, and rats were sacrificed 30 min after this treatment. Data are means±S.E. of 5—6 rats. *a)* $p<0.05$; *b)* $p<0.001$. ▨, corticosterone, □, glucose.

Thus, the effect of a histamine antagonist on the saponin-induced responses was determined (Fig. 4). The H_1 receptor antagonist diphenhydramine hydrochloride (25 mg/kg) almost completely suppressed histamine-induced responses, but it did not affect the saponin-induced ones.

Discussion

In this work we present evidence that a single dose of saikosaponin-a or -d stimulated the anterior pituitary to secrete ACTH, and the adrenal cortex to synthesize and secrete corticosterone in unanesthetized or pentobarbital-anesthetized rats. It is known that dexamethasone acts on the hypothalamus and/or the pituitary. Thus, the present blocking action of dexamethasone on saponin-induced corticosterone secretion suggested that the primary site of action of saikosaponins was probably the hypothalamus and/or the pituitary.

Takagi and Shibata[1] reported that crude saikosaponins had antiedematous and antigranulomatous actions. Yamamoto *et al.*[3] reported that repeatedly intramuscularly administered saikosaponin-a or -d showed antigranulomatous action. It is known that glucocorticoids have a strong antigranulomatous action, and therefore the antigranulomatous action, but not the antiedematous action, of saikosaponins could be explained in terms of corticosterone secretion induced by the saikosaponins.

Saikosaponin-a and -d as well as ginsenosides[6] and escin[7] induced corticosterone secretion, but both glycyrrhizin, which exerts antiinflammatory action,[10] and saikosaponin-c did not.[6a] These findings clearly suggest that the corticosterone secretion-inducing action of saikosaponins is not a general property of triterpenoidal saponins, and that some specific chemical structure, for example, the C-23 OH group of the saikogenin moiety of saikosaponin-a and -d, was responsible for the action.

Saikosaponin-a and -d were both hemolytic, but in the present *in vivo* experiments no hemolyzed plasma was found. Saikosaponin-a and -d both exhibited corticosterone secretion-inducing and hyperglycemic actions as well as hemolytic, antigranulomatous and plasma-cholesterol lowering actions, but saikosaponin-c had none of these activities. Thus, these various actions of saikosaponins might have some common mechanism.

Acute hypoglycemia or histamine injection induces corticosteroid secretion.[9] The corticosterone secretion with saikosaponins was accompanied by a transient hyperglycemia and hypoinsulinemia, so it is possible that saikosaponin induced the release of endogenous epinephrine or histamine. The blocking effect of hexamethonium on the saponin-induced corticosterone secretion suggested the possibility of release of epinephrine. However, it remains to be elucidated whether the release of epinephrine was essential for the saponin-induced corticosterone secretion. The result of a diphenhydramine blocking experiment ruled out the possibility of histamine release.

Acknowledgement This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 144074 and 557507) from the Ministry of Education, Science and Culture of Japan.

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