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Effect of Escin on Adrenocorticotropin and Corticosterone Levels in Rat Plasma

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The effect of β -escin on plasma adrenocorticotropin (ACTH) and corticosterone levels in rat plasma was determined by radioimmunoassay and by the competitive protein binding method. Intraperitoneal administration of β -escin induced increases of plasma ACTH and corticosterone in rats. The increase of plasma corticosterone was accompanied by a transient increase of plasma glucose and a transient decrease of plasma immunoreactive insulin. Hexamethonium partially suppressed the escin-induced corticosterone secretion, but diphenhydramine did not.

Keywords—escin; *Aesculus hippocastanum*; plasma corticosterone; ACTH; glucose; immunoreactive insulin; hexamethonium; diphenhydramine

Escin is a saponin occurring in the seeds of the horse chestnut tree, *Aesculus hippocastanum* L. (Hippocastanaceae), which have been used as a folk medicine for venous congestion and hemorrhoids in Europe. Escin is a complex mixture of glycosides of protoescigenin and barrintogenol C.¹⁾ Various antiinflammatory actions of escin were reported.²⁾ Antiinflammatory and other pharmacological actions of escin were blocked by adrenalectomy,³⁾ hypophysectomy^{3b)} or sympatholytic drugs.^{3a)} However the details of the blocking mechanism remain unclear. Previously we found that saponins from the roots of *Panax ginseng* stimulated the pituitary-adrenocortical system in rats.⁴⁾ In the present study we examined the action of β -escin on the adrenocortical function in rats.

Materials and Methods

Male Wistar rats weighing 130–150 g were used. They were fed on laboratory chow and tap water *ad libitum*, and maintained in a 24° room with artificial light (light on, 0600 to 1800 hr) for more than 6 days. To avoid stress-evoked release of corticosterone they were “gentled” by daily handling twice a day in the morning and evening for 4 days. Rats were decapitated by means of a guillotine at 0900 to 1000 hr. Plasma was prepared from the trunk blood, and stored at –20° until use. Plasma adrenocorticotropin (ACTH) and insulin were determined by the radioimmunoassay method using the kits from the Radiochemical Centre, Amersham, and Eiken Immunochemical Laboratory, Tokyo, respectively. Plasma corticosterone was determined essentially according to the competitive protein binding method of Murphy⁵⁾ as described previously.^{4a)} Standard corticosterone and ³H-corticosterone (45 Ci/mm) were from Merck and the Radiochemical Centre, respectively. Escin did not affect the determination for corticosterone. Glucose was measured by the glucose oxidase method with an autoanalyzer. β -Escin (Merck) was freshly suspended in pyrogen-free saline or distilled water before use.

Results

Effect of β -Escin Administration on Plasma Levels of Corticosterone, Glucose and Insulin

As shown in Fig. 1, intraperitoneal administration of β -escin (5 mg/kg) to rats increased the plasma corticosterone level 5 and 15 min after the treatment, and the corticosterone level was maximum 30 and 60 min after the treatment. It is known that insulin⁶⁾ or epinephrine⁷⁾ injection induces corticosteroid secretion. Thus, we determined the effect of escin on plasma glucose and immunoreactive insulin (IRI) levels. Fig. 1 shows that the plasma glucose level was markedly increased 5 and 15 min after escin and the maximum level was reached at about 20 min. It then rapidly decreased and was nearly at the normal level 60 min after the treat-

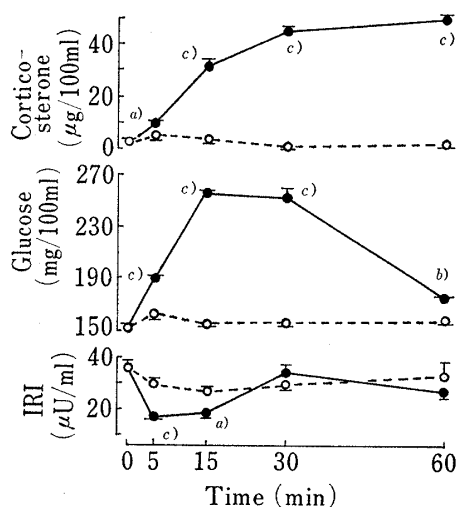


Fig. 1. Effect of β -Escin on Plasma Corticosterone, Glucose and Immunoreactive Insulin Levels

Data are means \pm S.E. of 4 to 6 rats. \circ , Saline (0.5 ml, *i.p.*); \bullet , β -escin (5 mg/kg, *i.p.*). a) $p < 0.05$; b) $p < 0.01$; c) $p < 0.001$.

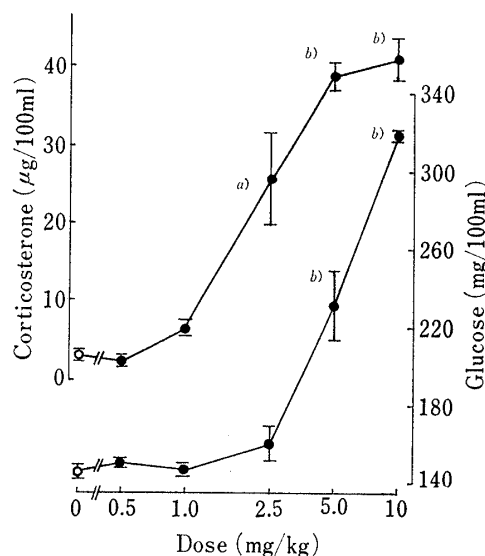


Fig. 2. Relation between β -Escin Dose and Plasma Corticosterone or Glucose Level

Rats were sacrificed 30 min after the *i.p.* treatment. Data are means \pm S.E. of 4 to 6 rats. a) $p < 0.01$; b) $p < 0.001$.

ment. Plasma IRI decreased significantly 5 and 15 min after the treatment, and the lowest level was seen at about 10 min after escin administration. In the saline-treated control rats, changes in plasma corticosterone, glucose and IRI levels were small (Fig. 1).

Intraperitoneal administration of β -escin to rats fasted for 16 hr also induced significant increases of plasma corticosterone and glucose 30 min after escin treatment (44 ± 2 vs. 19 ± 5 μ g/100 ml, $p < 0.01$; 116 ± 5 vs. 80 ± 2 mg/100 ml, $p < 0.001$, respectively). The increase of plasma glucose in fasted rats was smaller than that in fed rats.

TABLE I. Effect of Oral Administration of β -Escin to Rats

Treatment	Dose (mg/kg)	Number of rats ^{a)}	Comp. B ^{b)} (μ g/100 ml)	Glucose (mg/100 ml)
Control	(1 ml)	8	2.2 ± 0.4	144 ± 2
β -Escin	100	7	$11.8 \pm 4.2^c)$	149 ± 6
β -Escin	250	7	$24 \pm 4^d)$	168 ± 23

a) Rats were sacrificed 30 min after the treatment. Data are means \pm S.E.

b) Corticosterone.

c) $p < 0.05$.

d) $p < 0.001$.

When β -escin was administered orally, plasma corticosterone increased significantly after 30 min, but the level was not maximum at doses of 100 and 250 mg/kg (Table I). Plasma glucose concentration was not increased at 30 min after a dose of 100 mg/kg escin, but showed some increase at 250 mg/kg.

Fig. 2 shows the effect of graded doses of β -escin on plasma corticosterone and glucose levels 30 min after the treatment. Both corticosterone and glucose concentrations increased dose-dependently. The corticosterone concentration was nearly maximum at a dose of 5 mg/kg, whereas the increase of plasma glucose was not maximum at this dose.

Effect of Hexamethonium and Diphenhydramine

β -Escin-induced responses of plasma glucose and IRI suggested that escin evoked the release of epinephrine. To block the possible release of epinephrine, rats were treated with

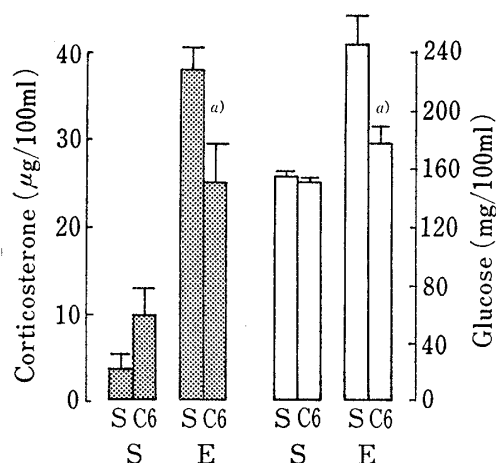


Fig. 3. Effect of Hexamethonium on β -Escin-induced Plasma Corticosterone and Glucose

Hexamethonium chloride (C6, 12 mg/kg, *i.p.*) or saline (S, 0.1 ml) was injected 15 min before β -escin (E, 5 mg/kg, *i.p.*) or saline (S, 0.5 ml) treatment. Rats were sacrificed 30 min after the second treatment. Data are means \pm S.E. of 8 rats. Corticosterone; glucose. a) $p < 0.05$.

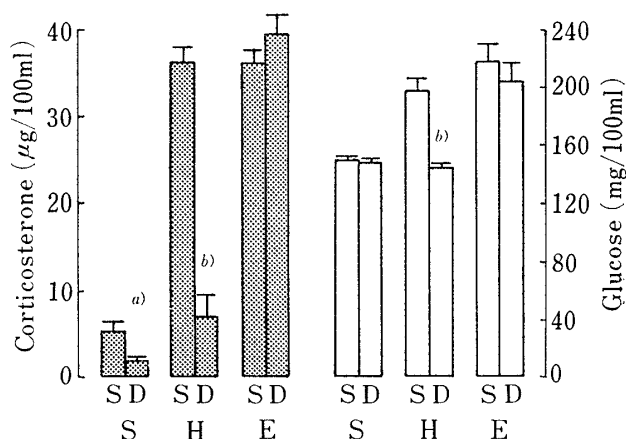


Fig. 4. Effect of Diphenhydramine on β -Escin-induced Plasma Corticosterone and Glucose

Diphenhydramine hydrochloride (D, 25 mg/kg, *i.p.*) or saline (S, 0.1 ml) was injected 45 min before β -escin (E, 5 mg/kg, *i.p.*), histamine dihydrochloride (H, 8 mg/kg) or saline (S, 0.5 ml) treatment. Rats were sacrificed 30 min after the second treatment. Data are means \pm S.E. of 5 to 6 rats. Corticosterone; glucose. a) $p < 0.05$; b) $p < 0.001$.

hexamethonium as a ganglion blocker prior to escin treatment. As shown in Fig. 3, hexamethonium chloride (12 mg/kg, *i.p.*) significantly suppressed the escin-induced increase of plasma glucose and corticosterone. However, the suppression was not complete, since β -escin still induced significant increases of plasma glucose and corticosterone (178 ± 11 vs. 151 ± 2 mg/100 ml, $p < 0.05$; 25 ± 5 vs. 10 ± 3 μ g/100 ml, $p < 0.02$, respectively). Hexamethonium itself at higher doses induced corticosterone secretion. When a higher dose of escin (10 mg/kg) was administered, the blocking effect of hexamethonium chloride (12 mg/kg) on plasma glucose was small and that on plasma corticosterone was negligible.

It is known that histamine injection also induces an increase of plasma corticosterone. Fig. 4 shows that diphenhydramine (an H_1 receptor antagonist) depressed the histamine-induced increases of plasma corticosterone and glucose, but it did not affect on the escin-induced increases.

TABLE II. Effect of Intraperitoneal Administration of β -Escin on Plasma ACTH, Corticosterone and Glucose Levels in Rats

Treatment	Dose (mg/kg)	Number of rats ^{a)}	ACTH (pg/ml)	Comp. B ^{b)} (μ g/100 ml)	Glucose (mg/100 ml)
Control	(0.5 ml)	4	103 ± 4	2.3 ± 2.0	149 ± 4
β -Escin	5.0	4	1167 ± 71 ^{c)}	46 ± 1 ^{c)}	241 ± 16 ^{d)}

a) Rats were sacrificed 30 min after the treatment. Data are means \pm S.E.

b) Corticosterone.

c) $p < 0.001$.

d) $p < 0.01$.

Effect on Plasma ACTH Level

Administration of β -escin (5 mg/kg, *i.p.*) induced an increase in plasma ACTH as well as in plasma corticosterone and glucose levels 30 min after the treatment (Table II). Therefore β -escin-induced ACTH secretion resulted in increases of corticosteroid synthesis in and secretion from the adrenal cortex.

Discussion

In this work we present evidence that escin has a stimulatory action on the anterior pituitary and the adrenal cortex. The evidence suggests that escin acted primarily on the pituitary to secrete ACTH, and that escin-induced ACTH acted on the adrenal cortex to cause the synthesis and secretion of corticosterone. The above evidence and suggestion are consistent with the results that the antiedematous and other actions of escin required the intact function of the adrenal cortex³⁾ and hypophysis.^{3b)} The mechanisms by which escin activates the pituitary are unknown and remain to be elucidated.

Escin has both anti-exudative and anti-granulomatous action.^{2b-d)} Glucocorticoid has a strong antigranulomatous action. Therefore, the antigranulomatous action of escin can probably be explained in terms of the action of escin-induced corticosterone. In view of the responses in plasma glucose and IRI levels, escin may also induce the release of epinephrine. This suggestion is in accord with the finding by Damas, *et al.*⁸⁾ that escin induced the release of epinephrine from the adrenal medulla, and with their suggestion that the antiedematous action of escin could be explained by its effect on epinephrine levels.

Escin is a surfactant and has a strong hemolytic action, but no hemolyzed plasma was found in the present *in vivo* study. Glycyrrhizin,⁹⁾ platycodin¹⁰⁾ and saikosaponin-a and -d¹¹⁾ had antiinflammatory actions. Saponins from *Gypsophyla paniculata* L., *Saponaria officinalis* L. and quillaja bark stimulated the function of the adrenal cortex.¹²⁾ However, glycyrrhizin and saikosaponin-c did not affect plasma corticosterone at a dose of 100 mg/kg, *i.p.*^{4a)} Therefore the stimulating action of escin on corticosterone secretion may not be a general property of triterpenoidal saponins.

In the previous report^{4b)} we showed that saponin from the roots of *Panax ginseng* stimulated corticosterone secretion without significantly affecting plasma glucose and IRI levels. In this study, administration of escin was found to induce a marked increase of glucose level and a significant decrease of IRI level. Thus, it was clear that the mechanism of the action of escin on corticosterone secretion did not involve acute hypoglycemia or hyperinsulinemia. Hexamethonium nearly completely blocked the escin-induced increase in plasma glucose level, while it blocked by half that in plasma corticosterone level (Fig. 3). The treatment with hexamethonium might result in a decrease in the release of epinephrine, which might in turn result in a decrease in plasma corticosterone secretion. However, hexamethonium interferes with the nervous transmission of both sympathetic and parasympathetic ganglia, so the escin-induced corticosterone secretion might involve some processes other than epinephrine release. The details of the processes are not clear at present and further studies are required. The present results clearly rule out the release of endogenous histamine, however.

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