EFFECTS OF ADRENOHYPOPHYSEAL HORMONES UPON URINARY EXCRETION OF GLUCURONIC ACID AND ASCORBIC ACID

SHOJI TSUTSUMI, KAZUHITO NAKAI AND HIROSHI NAKAMURA
Department of Pharmacology, Tokyo Dental College, Chiyoda-ku, Tokyo

Received for publication July 4, 1966

It was previously reported by the authors (1) that, in a normal rat with daily administration of 100 mg/kg borneol and intraperitoneal injection of 400 mg/kg glucuronolactone for the period of 13 days, the amounts of total glucuronic acid, of o-glucuronide and of l-ascorbic acid excreted in rat's urine, increased significantly, and that, in a bilaterally adrenalectomized rat administrated daily with the above noted drugs for the same period, these amount did not increase so much as in normal animal.

Based on our investigations that borneol-glucuronide formation was inhibited by bilateral adrenalectomy, we may assume, that adrenals are related to glucuronide formation in a living body.

It was already been investigated that a lot of hormone are excreted from adrenal medulla and adrenal cortex. There are adrenaline and noradrenaline as a main hormone excreted from adrenal medulla; and there are glucocorticoid including corticosterone, 17-hydroxycorticosterone, 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone and mineral corticoid including 11-desoxycorticosterone, 17-hydroxy-11-desoxycorticosterone and aldosterone, as a main hormone of adrenal cortex. That these hormones have respectively different effects on glucuronide formation is suggested from their own different actions.

In the present investigations, the authors tried to ascertain the effect of adreno-hypophysal hormones on glucuronic acid metabolism.

Adrenaline, 17-hydroxy-11-desoxycorticosterone (cortisone), 11-desoxycorticosterone (DOC) and adrenocorticotropic hormone (ACTH) which has direct influences on adrenal hormone were chosen and the changes in the amounts of total glucuronic acid, o-glucuronide and ascorbic acid in urine were examined after the administration of above drugs to a normal and a bilaterally adrenalectomized rat.

EXPERIMENTAL METHODS

Male Wistar rats aged about 3 months, weighed about 150 g were separately raised in a cage with stainless steel wire bottom. They were fed on the diet consisting of wheat
flour (85%), casein (10%) and salts mixture (5%). Food and water were administered ad libitum.

Excreted urine was collected in a bottle by mixing 5 ml of 10 per cent oxalic acid solution and 5 ml of toluene, and the total amount was measured every 24 hours.

Glucuronic acid content was measured by Fishman and Green's method (2) and ascorbic acid content by Roe's method (3).

Both normal rats and bilaterally adrenalectomized rats were injected subcutaneously with 1 unit/kg ACTH, 50 mg/kg cortisone or 5 mg/kg DOC and administered orally 100 mg/kg borneol dissolved in olive oil everyday for the period of 13 days.

Bilateral adrenalectomy was performed in one stage.

Bilaterally adrenalectomized rats were given the physiological saline instead of plain water in order to prevent the electrolyte unbalance due to the adrenalectomy.

Fig. 1. Comparison of effect of ACTH on total glucuronic acid, $\alpha$-glucuronide and ascorbic acid content excreted in normal or bilaterally adrenalectomized rat's urine.

ACTH (1 unit/kg) was subcutaneously injected every day for 13 days. Each curve is the average of the values obtained from six rats.

○: Administration of ACTH to normal rat.
●: Administration of both ACTH and borneol to normal rat.
△: Administration of ACTH to bilaterally adrenalectomized rat.
▲: Administration of both ACTH and borneol to bilaterally adrenalectomized rat.
RESULTS

1. Effect of ACTH on glucuronic acid content and ascorbic acid content excreted in rat's urine

In both normal and bilaterally adrenalectomized rats with 1 unit/kg ACTH and 100 mg/kg borneol in both, there were no changes in each urinary excretion content at earlier stage; at later stage, however, in bilaterally adrenalectomized rats with the both drugs, there was a slight increase of glucuronic acid content (Fig. 1).

2. Effect of adrenaline on glucuronic acid content and ascorbic acid content excreted in rat's urine

In normal rats injected with 0.5 mg/kg adrenaline, a very slight increase of glucuronic acid content was found at earlier stage, but in bilaterally adrenalectomized rats, there appeared no changes of each content (Fig. 2).

In both normal rats and bilaterally adrenalectomized rats with 0.5 mg/kg adrenaline and 100 mg/kg borneol in both, there were no changes of each content (Fig. 2).

---

Fig. 2. Comparison of effect of adrenaline on total glucuronic acid, α-glucuronide and ascorbic acid content excreted in normal or bilaterally adrenalectomized rat's urine.

Adrenaline (0.5 mg/kg) was subcutaneously injected every day for 13 days. Each curve is the average of values obtained from six rats.

○: Administration of adrenaline to normal rat.
•: Administration of both adrenaline and borneol to normal rat.
△: Administration of adrenaline to bilaterally adrenalectomized rat.
*: Administration of both adrenaline and borneol to bilaterally adrenalectomized rat.
3. Effect of cortisone on glucuronic acid content and ascorbic acid content excreted in rat's urine

In both normal and bilaterally adrenalectomized rats with 50 mg/kg cortisone, there were no changes in glucuronic acid content and ascorbic acid content (Fig. 3-A, -B).

In normal rats with both 50 mg/kg cortisone and 100 mg/kg borneol, however, there was a marked increase of about 5 times as much as the volume of normal glucuronic acid content, and of ascorbic acid content. But almost no changes of each content in bilaterally adrenalectomized rats with the above two drugs were observed (Fig. 3-A, -B).
4. Effect of DOC on glucuronic acid content and ascorbic acid content excreted in rats' urine

In both normal rats and bilaterally adrenalectomized rats with 5 mg/kg DOC, there was no change of glucuronic acid content, but a slight increase of ascorbic acid content (Fig. 4).

In normal rats with both 5 mg/kg DOC and 100 mg/kg borneol, no changes of each content appeared, while, in bilaterally adrenalectomized rats with the both drugs, there was a marked increase of glucuronic acid content (Fig. 4).

DISCUSSION

From our results, it seems to attract a great deal of attention that cortisone increased markedly glucuronic acid content and ascorbic acid content of normal rats, while DOC increased apparently only glucuronic acid content of bilaterally adrenalectomized rats. The mechanism of glucuronide formation postulated by Dutton and Storey (4-6), Williams (7), Smith and Mills (8) Mills, Ondarza and Smith (9) and Mills, Lochhead and Smith (10) is as follows: at first, glucose-1-phosphate is formed by the coexistence of glycogen and phosphorylase, and also by the coexistence of glucose, ATP, hexokinase and phosphoglucomutase. Glucose-1-phosphate is converted into uridine-diphospho-glucose (UDPG)
by the use of uridyl transferase in the presence of UTP, and then into uridine-diphospho-
glucuronic acid (UDPGA) by the oxidising potency of UDPG-dehydrogenase. The con-
version of UDPGA to R-glucuronide can be performed by the use of glucuronosyl trans-
ferase in the presence of aglycon. On the other hand, UDPGA is splitted into two
substances, namely uridine monophosphate and glucuronic acid l-phosphate. The latter
is finally transformed into the free form of glucuronic acid.

It has been reported by Wells (11), Lewis, Kuhlman, Delblue, Keept and Thorn (12),
Thorn, Koepf, Lewis and Olsen (13) that cortisone induces hyperglycemia and increases
of hepatic glycogen, but DOC has no such effects.

In our experiment with normal rats, it seems that the increase of glucuronic acid
content in urine due to cortisone depends upon a marked increase of glucose-l-phosphate,
derived from glucose or glycogen, as shown in the above-described pathway of glucuronide
formation.
The increase of glucuronic acid content due to DOC in the excreted urine of bilaterally adrenalectomized rats will enhance the enzyme activity of a certain kind which plays a role in the glucuronide formation, because DOC is shown to have no direct effect on the glucose and glycogen content during the glucuronide formation.

It has also been reported by Venning (14) that cortisone is the most effective in synthesizing hepatic glycogen in adrenalectomized rats. In our study, why cortisone does not increase glucuronic acid content excreted in urine of bilaterally adrenalectomized rats is the first question to be solved. The true mechanism of this phenomenon is not clear, although it seems that the increase of glucuronic acid content does not appear unless a large amount of glucose-1-phosphate is formed.

As for ascorbic acid formation, Douglas (15), Burns (16) and Hollmann (17) have confirmed that ascorbic acid is formed through the metabolic pathway from glucose or glucurono lactone to gulonolactone.

In the present study, it is resulted that the ascorbic acid content excreted in rat's urine was increased markedly in normal rats under cortisone treatment, but increased slightly in normal rats under DOC treatment. Therefore, it is assumed that the increase of ascorbic acid content may be caused by the increase of glucose in the case of cortisone administration and it depends on the activation of enzyme system through the pathway of ascorbic acid formation in the case of DOC administration.

Through our study on the effect of adrenohypophyseal hormones on the glucuronic acid and ascorbic acid formation has given no clear-cut solution for this problem, it may be true that each hormone has different effects upon glucuronic acid and ascorbic acid formation respectively.

**SUMMARY**

The authors studied on the effect of adrenohypophyseal hormones (ACTH, adrenaline, cortisone and DOC) under the administration of borneol upon glucuronic acid and ascorbic acid content excreted in the urine of normal and bilaterally adrenalectomized rats. The results obtained were as follows: neither ACTH nor adrenaline almost increased those contents. Cortisone highly increased those contents in normal rats but induced no significant changes in bilaterally adrenalectomized rats. On the contrary, DOC largely increased those contents in bilaterally adrenalectomized rats, but did not increase them in normal rats.

Acknowledgement: The authors wish to thank Prof. S. Tamura for his advice and encouragement throughout this work.

**REFERENCES**

4) DUTTON, G.J. AND STOREY, I.D.E.: *Biochem. J.* 48, 29 (1951)
14) Venning, E.A.: *Endocrinol.* 38, 79 (1946)