PHARMACOLOGICAL STUDIES OF INSECT METAMORPHOSING HORMONE: PONASTERONE A, ECDYSTERONE, AND INOKOSTERONE, IN THE RAT

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The first insect moulting hormone called Ecdysone was isolated by Butenandt and Karlson (1) from the prothoracic gland of the pupa of silk worm. However, mainly due to minute supply of the compound the pharmacological study has not been made in the mammals. In 1966, Nakanishi, Takemoto et al. (2) found metamorphosing substances of the plant origin. They were closely related in chemical structure with Ecdysone. It is now possible to supply these hormones in large amounts obtained by the extractive or synthetic means. Since the demonstration by Karlson et al. (3) that the activation of certain genetic site of the insect chromosomes by Ecdysone derives from the increased synthesis of DNA-dependent RNA, the possible activation of the protein synthesis has stimulated the pharmacological studies of the hormones in the mammals. Otaka et al. (4, 5) have shown that the intraperitoneal injection of the metamorphosing plant hormone in mice activates an uptake of amino acids by the liver cells and therefore a biosynthesis of protein in the liver. However, Ecdysone itself is lacking in this effect (6). It is also reported that though long-term administration of the hormones does not affect markedly the body weight gain, some of the liver cells show a histological sign of increased metabolism (7). An activation of RNA synthesis by the metamorphosing hormones is also reported almost equipotent with that by 4-chlorotestosterone (5). By administering orally 200 to 800 µg/kg/day of Ponasterone A mixed in the diet to quail chicken, Kato et al. (8) have observed a dose-dependent activation of the molting change of feathers at the age of 17 to 20 days. In the present experiments attempts were made to observe the pharmacological effect of the metamorphosing steroids including Ponasterone A, Ecdysterone and Inokosterone in order to discuss the availability as therapeutic agent in mammals.

METHODS

Rats of SD-JCL were used. Ponasterone A, Ecdysterone and Inokosterone were supplied from Chemical Research Laboratories, Takeda Chemical Industries. They are white pulver crystals and their chemical structures are shown in Fig. 1.
A. Effects of Ponasterone A on the rats

Male and female rats, 29 days in age and weighing from 85 to 105 g were divided into one control and three treated groups, consisted of 7 males or females in each. Ponasterone A suspension in vehicle No. 17874 (0.4% polysorbate-80, 0.5% CMC and 0.9% benzyl alcohol in 0.9% physiological saline) was administered orally by use of stomach tube in the daily volume of 3 ml. The control animal was treated with the vehicle alone and the other three groups were treated with the daily doses of 2, 10 and 50 mg/rat for 5 successive days. On the next day all the animals were sacrificed by hemorrhage under ether anesthesia. Five of 7 animals in each group were subjected to the endocrinological examinations and the remaining 2 were to the histological and enzymatic ones. In addition, during the administrative term the body weight was measured before the drug administration and the behaviors were observed for about one hour after the administration. At the day of sacrifice the blood samples were collected from the abdominal aorta with injection cylinder by opening the peritoneal cavity under ether anesthesia.

I. Endocrinological examinations

Brain, pineal gland, pituitary, submaxillary glands, thyroid, thymus, heart, liver, adrenal, kidney, spleen and preputial glands were weighed. In addition, seminal vesicles, ventral and dorsal prostates, epididymides, testes, penis and levator ani muscle were weighed in male, and ovaries and uterus were in female. Uterus was weighed before and after the removal of the intrauterine fluid. All the organ weights were subjected to the t test. The parameter for the possible somatotrophin-like activity used was the cartilaginous width at the epiphyseal end of tibial bone. The proximal end of the tibial bone was divided longitudinally into half with razor blade and was stained with AgNO₃ and
Thus, the cartilaginous width was measured by use of microscope (9). The central part of the testis was transversed for the stamping of the transversed surface on the slide glass in order to observe the formation of the sperms. From the day of the vaginal opening the smear samples stained with Giemsa dye were observed daily. In the animals whose vaginas did not open until 6 day of the feeding the vaginal smears were collected after the sacrifice of the animal.

II. Hematological examinations

The parameters for the blood examinations were as follows: erythrocytes and leucocytes counts by use of Sanborn-Frommer cell counter, hemoglobin content according to Sahli method, hematocrit value following Wintrobe method, total protein content by use of DZ proteinmeter, blood glucose and alkaline phosphatase by use of autoanalyzer, total cholesterol following the Ferro-Ham direct method, urea nitrogen according to urease method, and GOT and GPT following Reitman-Frankel method.

III. Histological examinations

The liver enzymes mainly studied histochemically were D-fructose-1, 6-diphosphatase (FDPase) and glucose-6-phosphatase (G-6-Pase). The activity of the former enzyme was detected following lead citrate method (10) and that of the latter enzyme was according Wachstein-Meisel method (11). In addition, thiamine-pyrophosphatase (TPPase) in male and nonspecific adenosine-triphosphatase (ATPase) in female were also examined in their activity. The activity of ATPase was tested in an alkaline medium. All the tissues were fixed in 10% neutral formalin for preparing the tissue sections 5 μ in thickness and stained with hematoxylin-cosin or periodic acid Schiff dye (PAS). The liver and adrenal gland fixed in formalin were freeze-sectioned at the thickness of 20 μ for the staining with Sudan III. The pancreatic section previously fixed with Bouin’s solution was subjected to Gomori's aldehydefuchsin staining. The glycogen staining was also performed with the liver previously fixed in Rossman solution.

IV. Enzymatic examinations

In order to investigate the effect of Ponasterone A on the hepatic drug-metabolizing enzyme activity, the rate of aminopyrine metabolism was determined. According to the method of Fouts et al. (13), the supernatant fraction containing microsomal and soluble enzymes was prepared from the liver homogenate by centrifuging at 9000 g. To 1 ml of the supernatant (equivalent to 0.5 g of liver), 3 ml of 0.1 M phosphate buffer (pH 7.4), containing cofactors and 1 ml of aminopyrine-phosphate buffer solution were added. Then the mixture was incubated at 37°C for one hour. The enzymatic reaction was terminated by adding 15 ml of 6.7% trichloracetic acid and 4-aminopyrine in the filtrate was determined following the method of Brodie et al. (14). The final concentrations of the cofactors in the incubate were $1.1 \times 10^{-4}$ M of NADP, $2 \times 10^{-4}$ M of nicotinamide, $5 \times 10^{-3}$ M of MgSO₄, and $5 \times 10^{-3}$ M of glucose-6-phosphate·2Na and that of aminopyrine was 10 μmole.

B. Androgenic and anabolic effects of Ponasterone A and Ecdysterone in the castrated male rat

Ponasterone A prepared as mentioned above was administered orally for five days
from 8 days after the castrating procedure of male rats at the age of 21 days. The daily
dose was 50 mg/rat and the number of the animals was 5. Ecdysterone in the daily
doses of 0.06 mg and 1.92 mg/animal dissolved in vehicle No. 17874 was administered subcutaneously for 10 days from the day of castration of the male rats weighing 40 to 55 g and at the age of 21 days. The animals were sacrificed by hemorrhage under ether anesthesia next day after the termination of the administration and the isolated parenchymatous organs were weighed. The parameters of the androgenic activity were the weight increases in seminal vesicle, and ventral and dorsal prostates. The parameter of the anabolic activity was the weight increase in the levator ani muscle.

C. Antiandrogenic activities of Ponasterone A, Ecdysterone and Inokosterone in the castrated male rats

The test hormones were injected subcutaneously for 5 days from next day of the castration in the rats at the age of 21 days and weighing 40 to 55 g. Testosterone propionate in the daily dose of 0.15 mg/animal dissolved in 0.2 ml of sesame oil was used as an androgen and was injected on the dorsal neck of animal. Ponasterone A, Ecdysterone and Inokosterone in the daily doses of 1.2 and 2.4 mg/animal dissolved in vehicle No. 17874 were injected at the inguinal site of both sides alternatively. Next day after the termination of the administration the animals were exsanguinated under ether anesthesia and the androgenic target organs such as seminal vesicle and prostate were isolated for weighing. The parameter of the antiandrogenic activity was the percent inhibition of the weight increase of seminal vesicle and ventral and dorsal prostates caused by testosterone propionate alone.

RESULTS

A. Effects of Ponasterone A on the rats

The body weight gains in the control and treated animals during 5 feeding days are shown in Table 1. The treatment of the animals with Ponasterone A did not affect the body weight gain. The weight of the isolated tissues in the control and treated animals sacrificed at 5 day of the feeding are shown in Tables 2 and 3. The male animals received 50 mg/animal exhibited a significant increase in weight of the heart, kidney and levator

TABLE 1. Effect of Ponasterone A on the body weight in the rat.

| Sex | Groups   | Daily dose (mg) | No. of rats | 1st day | 2nd | 3rd | 4th | 5th | 6th | Gain |
|-----|----------|-----------------|-------------|---------|-----|-----|-----|-----|-----|------|
|     | Control  | —               | 7           | 98.0±2.2 | 102.8±2.6 | 111.9±3.7 | 117.0±3.2 | 127.1±3.7 | 138.3±3.6 | 41.3±2.0 |
| ♂   | Ponasterone A | 2 | 7     | 98.3±1.7 | 104.6±2.5 | 113.9±2.3 | 122.3±3.6 | 130.9±3.6 | 141.6±3.5 | 43.8±2.2 |
| ♂   | Ponasterone A | 10 | 7     | 95.6±1.8 | 103.5±1.0 | 109.7±2.2 | 115.1±3.7 | 125.5±3.2 | 137.8±2.9 | 42.1±1.6 |
| ♂   | Ponasterone A | 50 | 7     | 98.4±0.9 | 106.6±0.9 | 114.3±0.7 | 122.6±1.1 | 131.9±1.1 | 141.8±0.8 | 43.4±0.9 |
|     | Control  | —               | 7           | 90.4±1.6 | 96.2±1.6 | 103.5±1.5 | 107.9±1.8 | 114.8±2.6 | 124.7±2.6 | 34.4±1.6 |
| ♀   | Ponasterone A | 2 | 7     | 88.5±1.7 | 94.8±1.3 | 101.6±1.8 | 107.1±1.5 | 114.7±1.3 | 124.0±2.1 | 34.1±1.6 |
| ♀   | Ponasterone A | 10 | 7     | 91.6±1.8 | 97.0±1.5 | 102.2±1.8 | 107.4±1.7 | 114.1±1.5 | 124.5±2.2 | 32.9±1.2 |
| ♀   | Ponasterone A | 50 | 7     | 90.6±1.8 | 95.1±1.5 | 102.5±2.1 | 109.8±3.2 | 115.5±2.8 | 125.8±3.2 | 35.2±1.8 |
Table 2. Effects of Ponasterone A on the organ weights in the male rats.

| Groups   | Daily No. dose of (mg) rats | Brain (g)     | Pineal body (mg) | Pituitary (mg) | Sub-maxi. gls. (mg) | Thyroid (mg) | Thymus (mg) | Heart (mg) | Liver (g) | Adr. (L) (mg) | Kid. (L) (mg) |
|----------|-----------------------------|---------------|------------------|----------------|---------------------|---------------|-------------|-------------|------------|---------------|---------------|
| Control  | 5                           | 1.30±0.02     | 1.0±0.2          | 5.2±0.2        | 263.4±8.8          | 7.4±0.6       | 403.0±42.9  | 443.3±4.6   | 8.0±0.3    | 13.2±0.7      | 655.8±23.7    |
| Ponasterone A 2 | 5                           | 1.30±0.03     | 1.1±0.1          | 4.8±0.4        | 267.5±7.1          | 7.8±0.5       | 433.4±42.1  | 489.4±26.4  | 8.4±0.4    | 13.1±0.5      | 699.3±47.7    |
| Ponasterone A 10 | 5                           | 1.31±0.02     | 1.4±0.2          | 4.8±0.2        | 277.0±7.5          | 8.6±0.6       | 408.8±7.2   | 483.5±18.4  | 8.5±0.2    | 13.1±0.8      | 658.7±28.6    |
| Ponasterone A 50 | 5                           | 1.36±0.02     | 1.3±0.1          | 5.7±0.2        | 277.1±4.9          | 8.0±0.9       | 522.2±28.1  | 523.2±24.0  | 8.4±0.1    | 13.3±0.3      | 766.8±21.7**  |

Table continues...

| Spleen (mg) | Prep. gls. (mg) | Sem. vs. (mg) | V. pros. (mg) | D. pros. (mg) | Epididymides (mg) | L. Testes (mg) | R. Testes (mg) | T. Testes (mg) | Penis (mg) | L. ani (mg) |
|-------------|-----------------|---------------|---------------|---------------|-------------------|----------------|----------------|----------------|-------------|-------------|
| 502.9±34.0  | 39.4±7.7        | 35.2±3.7      | 62.7±9.9      | 28.1±1.9      | 117.7±6.4         | 562.4±21.5     | 578.6±23.0     | 1141.0±43.8   | 77.3±3.4    | 42.7±5.5    |
| 494.1±44.1  | 36.4±7.2        | 32.2±2.9      | 60.7±4.1      | 30.6±2.9      | 115.9±3.0         | 603.4±19.8     | 605.7±19.9     | 1209.1±39.4   | 85.4±3.4    | 44.9±3.8    |
| 555.3±45.9  | 49.0±8.7        | 33.2±2.9      | 62.6±3.6      | 29.1±2.9      | 114.4±7.2         | 560.5±25.1     | 562.9±27.6     | 1113.4±32.6   | 81.7±2.9    | 43.8±3.7    |
| 545.8±30.5  | 28.2±5.0        | 33.4±0.7      | 75.9±4.5      | 33.3±1.6      | 128.9±10.0        | 593.6±29.1     | 595.2±29.4     | 1188.8±58.0   | 85.8±2.1    | 60.5±2.3*   |

Sub-maxi. gls.: Submaxillary glands
Adr.(L): Adrenal (Left side)
Kid. (L): Kidney (Left side)
Prep. gls.: Preputial glands
Sem. vs.: Seminal vesicles
V. pros.: Ventrail prostate
D. pros.: Dorsal prostate
L. ani: Levator ani

**: Significantly different from the control group, P<0.01
*: Significantly different from the control group, P<0.05
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TABLE 3. Effects of Ponasterone A on the organ weights in the female rats.

| Groups       | Daily dose (mg) | No. of rats | Brain (g) | Pinealectomy body (mg) | Pituitary (mg) | Sub- maxi. gls. (mg) | Thyroid (mg) | Thymus (mg) | Heart (mg) |
|--------------|----------------|-------------|-----------|------------------------|----------------|----------------------|--------------|-------------|------------|
| Control      |                | 5           | 1.24 ± 0  | 1.3 ± 0.1              | 5.6 ± 0.2      | 251.8 ± 10.4         | 7.6 ± 0.4    | 392.4 ± 21.5 | 460.8 ± 22.5 |
| Ponasterone A| 2              | 5           | 1.24 ± 0  | 1.2 ± 0.1              | 6.3 ± 0.3      | 264.2 ± 9.8          | 8.0 ± 1.0    | 471.0 ± 15.5 | 468.8 ± 21.1 |
| Ponasterone A| 10             | 5           | 1.28 ± 0  | 1.0 ± 0.1              | 5.9 ± 0.3      | 265.5 ± 5.5          | 7.9 ± 0.3    | 395.9 ± 12.6 | 430.1 ± 11.9 |
| Ponasterone A| 50             | 5           | 1.23 ± 0  | 1.1 ± 0.1              | 5.8 ± 0.4      | 264.6 ± 6.8          | 7.9 ± 0.8    | 381.9 ± 24.7 | 484.4 ± 23.8 |

*Significantly different from the control group, P < 0.05

with f.: with fluid
without f.: without fluid

TABLE 4. Effects of Ponasterone A on the width of tibial cartilage in the rat.

| Groups       | Daily dose (mg) | No. of rats | Tibia cartilage width (micron) |
|--------------|----------------|-------------|-------------------------------|
| Control      |                | 5           | 412.1 ± 8.5                   | 363.3 ± 7.2    |
| Ponasterone A| 2              | 5           | 402.4 ± 12.6                  | 357.3 ± 13.5   |
| Ponasterone A| 10             | 5           | 387.6 ± 10.8                  | 370.1 ± 10.2   |
| Ponasterone A| 50             | 5           | 391.3 ± 5.3                   | 356.4 ± 5.5    |

Mean ± S.E.

TABLE 5. Effects of Ponasterone A on the vaginal smears and the related organ weights in the female rats.

| Groups       | Rat No. 4th 5th 6th day | Vaginal smear | Organ weight (mg) | Vaginal smear | Organ weight (mg) |
|--------------|-------------------------|---------------|-------------------|---------------|-------------------|
| Rat 4th 5th 6th day Ovaries With f. | | | | | |
| Control      | 1           | V            | 23.5            | 345.0         | 268.0 Ponasterone A | 1  | II | V    | 52.3 | 158.0 | 153.0 |
|              | 2           | V            | 20.6            | 134.1         | 130.0 A         | 2  | V  | V  | 24.6 | 430.3 | 307.5 |
|              | 3           | III          | 23.1            | 275.1         | 220.6 10mg/day x 5 | 3  | III | III | 22.2 | 387.7 | 252.3 |
|              | 4           | III          | 41.6            | 174.8         | 162.4            | 4  |      | I-II | 22.7 | 244.2 | 183.5 |
|              | 5           | I-III        | 29.7            | 148.1         | 137.8            | 5  |      | V   | 24.1 | 150.4 | 139.2 |
| Ponasterone A| 1           | V            | 62.0            | 209.0         | 264.0 Ponasterone A | 1  | V   | V  | 26.6 | 600.0 | 389.0 |
| 2mg/day x 5  | 2           | V            | 24.6            | 299.6         | 226.2 A          | 2  |      | V  | 24.9 | 88.6  | 84.0  |
|              | 3           | III          | 35.4            | 185.7         | 178.2 50mg/day x 5 | 3  | V   | V  | 23.8 | 153.6 | 145.7 |
|              | 4           | I-III        | 28.2            | 202.6         | 191.9            | 4  |      | V  | 26.6 | 360.5 | 260.4 |
|              | 5           | I-III        | 34.7            | 237.1         | 225.4            | 5  |      | V   | 40.6 | 130.9 | 125.0 |

I : Proestrus. II : Estrus. III : Metestrus. IV : Diestrus.
TABLE 6. Effects of Ponasterone A on the hematologic values in the rat.

| Sex | Dose mg/day/rat | No. of rats | RBC x10^6/mm³ | Ht % | Hb g/100 ml | WBC per mm³ | Differentials (%) |
|-----|-----------------|-------------|----------------|------|-------------|--------------|-------------------|
|     |                 |             |                |      |             |              |                   |
|     |                 |             |                |      |             |              | N | L | M | E | B |
|     |                 |             |                |      |             |              | 4.0 | 92.6 | 3.2 | 0.2 | 0 |
|     |                 |             |                |      |             |              | 5.2 | 91.4 | 2.8 | 0.6 | 0 |
|     |                 |             |                |      |             |              | 5.6 | 90.8 | 3.2 | 0.4 | 0 |
|     |                 |             |                |      |             |              | 3.0 | 93.0 | 3.8 | 0.2 | 0 |
|     |                 |             |                |      |             |              | 4.6 | 91.8 | 3.4 | 0.2 | 0 |
|     |                 |             |                |      |             |              | 7.4 | 89.4 | 3.0 | 0.2 | 0 |
|     |                 |             |                |      |             |              | 4.8 | 92.8 | 2.4 | 0   | 0 |
|     |                 |             |                |      |             |              | 3.8 | 93.0 | 3.2 | 0   | 0 |

Mean ± S.E.
* : Significantly different from the control group, P<0.05
** : Significantly different from the control group, P<0.01
N : Neutrophils. L : Lymphocytes. M : Monocytes. E : Eosinophils. B : Basophils.

TABLE 7. Effects of Ponasterone A on the biochemical blood components in the rats.

| Sex | Dose mg/day/rat | No. of rats | Total protein g/100 ml | Glucose mg/100 ml | Total cholesterol mg/100 ml | Urea nitrogen mg/100 ml | GOT Reitman Frankel Unit/ml | GPT King-Armstrong Unit/ml | ALP |
|-----|-----------------|-------------|------------------------|------------------|-----------------------------|-------------------------|----------------------------|----------------------------|-----|
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |
|     |                 |             |                        |                  |                             |                         |                            |                            |     |

Mean ± S.E.
* : Significantly different from the control group, P<0.05
** : Significantly different from the control group, P<0.01
ALP : Alkaline phosphatase.

ani muscle and a slight increase in weight of the ventral and dorsal prostates. However, the anabolic or androgenic effect might be accidental, because no such effect was observed in the castrated animals received the same dose, as cited below. The thymus in the female animals received 2 mg/animal and the brain in the female animals received 10 mg/animal showed a significant increase in weight. However, the effect was not dose-dependent.

The treatment of the male and female animals with Ponasterone A did not affect the cartilaginous width at the epiphyseal end of tibia. In addition, no spermatozoon was found in the stamp preparation of the transversed testis. Since the spermatogenesis is usually found from 40 days of the age, Ponasterone A is concluded not to activate the formation of sperms. As shown in Table 5, the treatment with Ponasterone A did not affect the vaginal opening and smears. In addition, the treated animals did not behave differently from the control animals.

The results obtained by the hematological examinations are shown in Table 6. Some
Fig. 2. Histochemistry of the rat liver treated with Ponasterone A (50 mg/day).

A: FDPase activity. The activity was uniformly distributed in the liver parenchymal cells, (×750).

B: G-6-Pase activity. The activity was observed in the cytoplasm of the liver parenchymal cells, (×750).

C: ATPase activity. The activity was observed in the network of bile canaliculi and the blood vessel, (×750).
The liver, kidney and adrenal showed no remarkable changes but pancreatic islet showed moderate degranulation of the B cells.

* Ponasterone A 50 mg/day.
** Ponasterone A 2 mg/day.
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A significant decrease in count of leucocytes was found in the treated female animals. However, the counts were variable according to the individual animal and the decrease was not significant. No abnormal finding was also in the hemogram. The significant increase in the hemoglobin content of the animals received 50 mg/animal was also probably accidental. Table 7 illustrates the results of the biochemical examinations of the blood in the control and treated animals. A slight decrease in the total serum protein in the animals received 50 mg/animal and the significant but slight decrease in serum urea nitrogen of the animals received 10 and 50 mg/animal seemed also to be not drug-dependent.

No significant change in pattern of distribution and activity of the hepatic FDPase and G-6-Pase, which was normally found in flecks within the cytoplasm of the liver cells, was produced by the treatment of the animals with Ponasterone A. ATPase, normally found in the bile canaliculi and TPPase demonstrated very faintly were not also affected by the treatment (Fig. 2).

No abnormality was detected in the liver, kidney and adrenal sections, stained with

**Table 8. Effects of the Ponasterone A on the tissue glycogen and fat in the male rats.**

| Dose mg/rat/day | No. | Fat staining | Glycogen staining |
|-----------------|-----|--------------|------------------|
|                 |     | Liver        | Adrenal gland    | Liver            |
| 0               | 1   | a few fat laden cells were occasionally seen | many fat droplets were found in the zona fasciculata | glycogen particles were found throughout the lobules |
|                 | 2   | a few fat laden cells were occasionally seen | many fat droplets were found in the zona fasciculata | glycogen particles were found throughout the lobules |
| 2               | 1   | no change    | no change        | no change        |
|                 | 2   | no change    | no change        | no change        |
| 10              | 1   | no change    | no change        | no change        |
|                 | 2   | no change    | no change        | no change        |
| 50              | 1   | no change    | no change        | no change        |
|                 | 2   | no change    | no change        | no change        |

**Table 9. Effect of Ponasterone A on the rat pancreatic islets.**

| Dose mg/rat/day | Rat No. | Degranulation of the beta cells |
|-----------------|---------|--------------------------------|
| 0               | 1       | ∼                               |
|                 | 2       | ∼                               |
| 2               | 1       | ∼                               |
|                 | 2       | ~                               |
| 10              | 1       | -                               |
|                 | 2       | -                               |
| 50              | 1       | -                               |
|                 | 2       | -                               |
hematoxylin-eosin, in the treated animals (Fig. 3). The mitotic index of the liver cell, though variable according to the individual animal, was within normal range. The other organs presented the normal structure. Sections stained with Sudan III showed the presence of a few number of liver cell with fatty granule and the presence of a relatively abundant fat droplet in the cells of fascicular zone of the adrenal cortex in both control and treated animals (Table 8). The staining of the pancreatic tissue with aldehyde-fuchsin revealed a marked degranulation of the beta-cells in Langerhans’ islet only in the animals received 2 mg/rat of Ponasterone A (Fig. 3 and Table 9). The liver glycogen was found to distribute diffusely in the lobular cells was not affected by the treatment (Table 8). The metabolic rate of aminopyrine in the liver homogenates of the rats received Ponasterone A was almost similar with that of the untreated animals. This suggests that

![Table 10. In vitro metabolism of aminopyrine by liver enzyme of rat treated with Ponasterone A.](image)

| Sex | Control | Ponasterone A treatment |
|-----|---------|-------------------------|
|     |         | 2 mg/rat/day | 10 mg/rat/day | 50 mg/rat/day |
| ♂   | 0.76*   | 0.80         | 0.93         | 0.76         |
|     | 0.97    | 0.58         | 1.09         | 0.90         |
| ♀   | 0.58    | 0.54         | 0.73         | 0.62         |
|     | 0.76    | 0.50         | 0.47         | 0.75         |

* μmoles of 4-aminoantipyrine/hr/g liver (wet wt.).

![Table 11. Effects of Ponasterone A on the body weight and organ weights in the orchiectomized rats.](image)

| Groups | Dose (mg/rat) of rats | No. of rats | Daily body weight (g ±S.E.) |
|--------|-----------------------|-------------|-----------------------------|
|        |                       |             | 1st day | 2nd | 3rd | 4th | 5th | 6th |
| Control| 0                     | 5           | 107.8±1.3 | 113.6±1.3 | 117.0±1.8 | 122.6±2.2 | 127.3±1.6 | 134.1±2.0 |
| Ponasterone A| 50                  | 5           | 107.2±4.0 | 111.7±3.8 | 111.9±4.0 | 118.3±3.7 | 121.6±4.0 | 128.3±4.3 |

| Gain of B.W. (g S.E.) | Brain wt. (g S.E.) | Pituitary (mg) | Pinea l body (mg) | Sub-maxi. gals. (mg) | Thyroid (mg) | Thymus (mg) | Heart (mg) |
|-----------------------|--------------------|----------------|------------------|---------------------|--------------|-------------|------------|
| 26.3±2.7 | 1.30±0.02 | 1.4±0.1 | 6.4±0.3 | 309.1±15.4 | 8.2±0.6 | 479.0±31.7 | 490.3±15.0 |
| 21.1±3.1 | 1.32±0.03 | 1.3±0.0 | 6.4±0.3 | 269.9±13.1 | 8.0±0.6 | 446.9±44.5 | 423.3±22.7** |

** Significant different from the control group, P<0.01
* Significant different from the control group, P<0.05
the long term administration of Ponasterone A does not affect the enzyme activity in the liver.

**TABLE 12. Androgenic and anabolic effects of Ecdysterone in the orchiectomized rats.**

| Rat No. | Organ weight (mg) | Control | Ecdysterone |
|---------|------------------|---------|-------------|
|         | Total dose (mg)  | Sem. vs. V. pros. | L. ani | Adr. (L) | Penis | Thymus | Thyroid | Pituitary |
| 1       | 7.4              | 10.0    | 37.9       | 15.9     | 40.6  | 519.1  | 11.5    | 6.3       |
| 2       | 7.9              | 8.5     | 44.4       | 15.0     | 42.9  | 519.7  | 12.0    | 6.3       |
| 3       | 7.8              | 7.6     | 40.4       | 16.6     | 40.6  | 502.6  | 11.2    | 6.8       |
| 1       | 7.3              | 9.0     | 37.7       | 14.6     | 43.5  | 521.1  | 10.0    | 6.4       |
| 2       | 6.6              | 9.9     | 43.5       | 13.7     | 39.2  | 483.1  | 10.1    | 5.3       |
| 1       | 8.2              | 9.7     | 43.9       | 14.8     | 40.2  | 497.5  | 9.9     | 6.3       |
| 2       | 6.9              | 7.3     | 42.7       | 14.6     | 42.9  | 380.1  | 13.4    | 6.6       |

**TABLE 13. Anti-androgenic effects of Ponasterone A, Inokosterone and Ecdysterone in the testosterone propionate-stimulated orchiectomized rats.**

| Compounds     | Organ weight (mg) | Body length (cm) | Tail length (cm) |
|---------------|-------------------|------------------|------------------|
|               | Kidney (L)        | Liver            | Heart           | Lung          | Spleen        | Brain         | Gain of B.W. (g) |
| Ponasterone A | 677.5             | 7093.9           | 488.0           | 824.2         | 502.1         | 1737.0        | 80.5            | 16.2        | 13.7        |
| Inokosterone  | 757.9             | 7025.2           | 462.2           | 780.1         | 534.9         | 1713.2        | 79.0            | 16.0        | 13.5        |
| Ecdysterone   | 737.9             | 7021.3           | 513.4           | 886.0         | 584.9         | 1805.6        | 86.0            | 16.5        | 14.2        |
| Ponasterone A | 596.0             | 7206.1           | 429.0           | 785.1         | 512.2         | 1718.2        | 71.0            | 16.3        | 12.2        |
| Inokosterone  | 674.0             | 7105.5           | 484.1           | 799.3         | 510.0         | 1695.9        | 79.5            | 16.0        | 14.5        |
| Ecdysterone   | 739.6             | 7438.1           | 511.2           | 874.5         | 648.6         | 1705.9        | 84.5            | 15.5        | 14.0        |

**TABLE 13. Anti-androgenic effects of Ponasterone A, Inokosterone and Ecdysterone in the testosterone propionate-stimulated orchiectomized rats.**

| Compounds     | Daily dose (mg) | No. of rats | Sem. vs. V. pros. | D. pros. L. ani | Penis | Prep. gts. | Thymus | Adr. (L) | Thyroid | Pituitary | Gain of B.W. (g) |
|---------------|-----------------|-------------|-------------------|-----------------|-------|------------|---------|----------|---------|-----------|-----------------|
| Negative control*1 | 5               | 8.7         | 14.7             | 8.7             | 21.3  | 40.7       | 15.1    | 307.9    | 11.0    | 7.9       | 3.6             | 382.5           | 34.0           |
| Positive control*2 | 5               | 80.1        | 75.1             | 39.3            | 50.9  | 95.4       | 41.4    | 291.6    | 9.6     | 7.0       | 3.5             | 405.1           | 41.6           |
| Ponasterone A   | 2.4             | 3           | 73.7             | 71.3            | 37.7  | 62.6       | 86.2    | 269.0    | 9.0     | 7.6       | 3.2             | 458.2           | 41.5           |
| Inokosterone    | 2.4             | 3           | 63.1             | 61.0            | 35.0  | 54.0       | 88.1    | 216.4    | 8.3     | 7.7       | 3.8             | 336.5           | 35.3           |
| Ecdysterone     | 1.2             | 1           | 70.1             | 70.2            | 36.1  | 50.3       | 102.1   | 235.3    | 8.9     | 6.7       | 4.4             | 333.4           | 41.5           |
| Negative control*1 | 5               | 7.1         | 10.6             | 7.8             | 23.8  | 35.5       | 16.7    | 322.6    | 9.1     | 6.7       | 4.1             | 372.4           | 37.4           |
| Positive control*2 | 5               | 78.2        | 78.6             | 39.6            | 54.8  | 94.5       | 29.8    | 218.3    | 9.5     | 7.2       | 3.3             | 390.4           | 38.2           |
| Inokosterone    | 2.4             | 5           | 81.4             | 77.3            | 39.9  | 38.5       | 96.8    | 263.8    | 10.1    | 7.4       | 3.5             | 444.4           | 41.3           |
| Inokosterone    | 4.8             | 5           | 79.6             | 67.1            | 34.6  | 60.6       | 89.6    | 306.1    | 7.1     | 7.4       | 3.4             | 389.8           | 37.7           |

*1 : Sesame oil (0.2 ml/rat/day × 5) + Vehicle No. 17874 (0.5 ml/rat/day × 5) injection subcutaneously.

*2 : Testosterone propionate (0.15 mg/rat/day × 5) + Vehicle No. 17874 (0.5 ml/rat/day × 5) injection subcutaneously.
B. Androgenic and anabolic activities of Ponasterone A and Ecdysterone

Table 11 shows the results obtained by the androgenic and anabolic assays of Ponasterone A. However, both activities in the castrated male rats were not confirmed. Some significant decreases in weight of the heart and spleen were present in the castrated males received Ponasterone A, but the results were controversial to those obtained in the intact but treated animals. The successive subcutaneous administration of Ecdysterone in the relatively low but usual daily doses in the castrated rats did not reveal either anabolic or androgenic activity (Table 12).

C. Antiandrogenic activity of Ponasterone A, Ecdysterone and Inokosterone in the castrated male rats

The previous observation of the anti-androgenic activity suggested the possible presence of the activity only in Inokosterone. However, the detailed study on the dose-response relationship in the more increased number of animals revealed that the dose of 4.8 mg/rat/day but not of 2.4 mg/rat/day inhibited slightly the increase in weight of prostate in response to testosterone propionate (Table 13).

DISCUSSION

The possible effect of the metamorphosing insect hormones of the plant origin in the mammalian animals is suggested to be an activation of the protein anabolism (3–7). The parameters for the anabolic activity are usually the body weight gain, the organ weight increase and other histological or enzymatic signs. However, in the present experiments the endocrinological, histological, enzymatic and hematological studies in the intact male and female rats received Ponasterone A for successively for 5 days failed to detect any pharmacological effects including an anabolic one. Since the daily doses used in the present experiments ranged from 2 to 50 mg/rat, the higher dose seemed not to be necessary. The oral route was selected because Otaka et al. (4, 5) have shown that the oral administration of Ecdysterone in mice activated the protein synthesis in the liver and because the pupa of insect ate the plant leaves containing the steroid hormones at the time of metamorphosis. However, even the subcutaneous administration of the hormones in the castrated rats failed to demonstrate any pharmacological effects.

The histological signs indicating the marked metabolic activation of the liver cell as similar as that in the young animals (7) were not observed in the liver of the rats received the hormones. The histochemical observation of the liver as well as the aminopyrine metabolism in the liver homogenates presented the negative result. In addition, the treatment of the rats with Ponasterone A did not affect the normal patterns of blood and endocrinological function. The oral administration of Ponasterone A and the subcutaneous administration of Ecdysterone in the castrated rats daily for 5 or 10 days did not produce either androgenic or anabolic effect. No apparent anti-androgenic effect of Ponasterone A, Ecdysterone and Inokosterone was observed in the castrated male rats received the various doses for 5 days. Despite the report by Otaka et al. (4, 5) that the metamorphosing hormone stimulates the protein synthesis in the mouse liver, the results obtained in the present experiments are in the same line shown by Sekeris et al. (15) that Ecdysone
does not activate the RNA polymerase in the rat liver. Imai et al. (16) in this laboratory have observed no effect of Ponasterone A on the elevated levels of cholesterol in the blood and tissues in the rats fed the high cholesterol diet as well as on the cholesterol levels of plasma and liver in the normal animals. The results obtained in the present experiments are enough to exclude some pharmacological effects of the metamorphosing insect hormones in the rats.

SUMMARY

The biological properties of the insect-molting steroids (Ponasterone A, Ecdysterone and Inokosterone) isolated from the plants were studied in the rat.

1. Any abnormalities of the general appearances (behavior, urine excretion, fur, etc.) and body weight changes of the rat were not observed.

2. Since Ponasterone A did not influenced the gain of body weight and the width of the tibial cartilage, it seems that this steroid has not the growth hormone-like activity. Also, the effects of this steroid on the advent of spermatogenesis and on the vaginal smear or vaginal opening were not observed. In the other endocrinological studies, the anabolic, androgenic effects of Ponasterone A and Ecdysterone, and the anti-androgenic effects of Ponasterone A, Ecdysterone, and Inokosterone were not observed.

3. In the hematologic values (RBC, WBC, Ht, Hb) and biochemical estimates of blood components (total protein, glucose, total cholesterol, urea nitrogen, GOT, GPT, alkaline phosphatase), we failed to detect any significant effects of Ponasterone A.

4. No significant change in the pattern of distribution and activity of the hepatic FDPase, G-6-Pase, ATPase and TPPase was produced by the treatment of the rat with Ponasterone A. The metabolic rate of aminopyrine in the liver homogenates of the rats treated with Ponasterone A was almost similar with that of the control one.

5. In the studies on weighing the general organs with special reference to endocrine glands and in the histological studies, we failed to detect any significant effects of Ponasterone A in the rat.

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