Inhibition of testicular androgenesis by urinary gonadotropin-inhibiting substances in rats[1]

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Summary. The effect of urinary gonadotropin-inhibiting substances (GIS) on the androgen synthesis in rat testes was studied in vitro and in vivo. GIS, which was added to the incubation medium containing teased testicular tissues and injected into rats for 2 days, showed a suppressive effect on the formation of androstenedione from pregnenolone in the testis.

Although melatonin and other pineal indoles inhibit reproductive functions[2], they are not the only substances found in the pineal that have this activity[3,4]. Urine of some mammalians, such as human beings[5], rats[6] and cattle[7], contains gonadotropin-inhibiting substances (GIS) which exert anti-LH effects. GIS can be extracted from urine specimen by the kaolin absorption method used for extraction of gonadotropins. This crude GIS consists of a thermostable large inhibitor which has a mol. wt higher than 10,000, and a small inhibitor which is a heat-labile peptide with a mol. wt less than 1000[8]. Each inhibitor exerts antiovulatory property. GIS also can be obtained from the precipitate of acidified urine by a sodium borate buffer extraction[9]. The crude GIS thus obtained contains the large inhibitor and most of the small inhibitor present in urine specimen[8]. Similar 2 substances were extracted from the bovine pineal[10,11] and there is evidence indicating that the pineal is a possible origin of GIS[12].

Although in vitro studies of a testicular level of action of melatonin have been done[13,14], little work has been performed on the action of GIS upon the reproductive organs. Damian[15] has recently reported that administration of urinary substance, which was obtained from children by Loraine and Brown's method[16] used for the extraction of gonadotropins, lowered urinary output of 17-ketosteroids and testosterone contents in the serum and testes in rats. However, it is unknown if this substance is similar to our GIS. Therefore, the present study was attempted to clarify the in vitro and in vivo effects of GIS on the testicular steroidogenesis.

Methods. a) In vitro experiment: Rats of Wistar strain, 9–10 weeks old, were placed under light for 14 h daily for 1 week prior to decapitation. The testes were quickly taken and put in a homogenizer and 4 volumes of cold 0.05 M phosphate buffer (pH 7.4) were added. The testes were crushed with a glass rod and teased to a gelatinous mass with a very loose pestle.

1 μCi (5.7 μg) of 4-^{14}C-pregnenolone (sp. act.; 55.7 mCi/mmole, The Radiochemical Centre) dissolved in ethanol was transferred into a flask and the solvent was evaporated under reduced pressure. Incubation medium contained the teased tissues (100 mg/0.5 ml), NADPH (500 μg) and nicotinamide (500 μg). The final volume was adjusted to 3.0 ml with 0.05 M phosphate buffer (pH 7.4). GIS was prepared from the precipitate of the acidified male adult urine according to Ota et al.[9]. The final lyophilized material was dissolved in a small volume of 0.05 M phosphate buffer (pH 7.4). 0.5 ml of the extract (500 ml urine-equivalent) was added to the incubation medium containing the same contents as in the control. Incubation in both cases was performed at 37 °C for 120 min under an atmosphere of 95% O_2 and 5% CO_2.

b) In vivo experiment: Rats of Wistar strain, aged 13 weeks, were maintained under light for 14 h daily for 1 week before and also during the experiment. 1 experimental group received an i.p. injection of 20 μM of melatonin (Sigma), which was dissolved in a small volume of ethanol and then diluted with 0.1 M borate buffer (pH 8.6), at

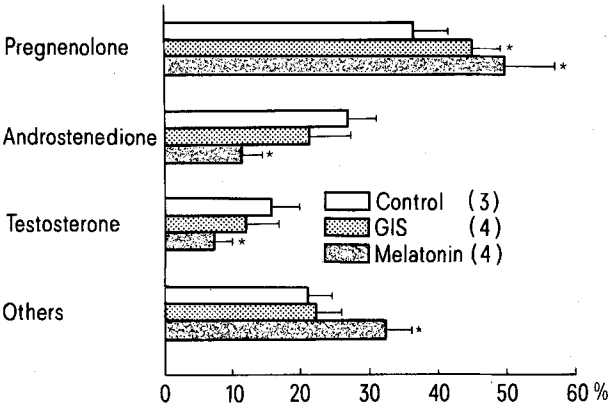

Fig. 2. Effect of the in vivo administration of GIS or melatonin on the in vitro androgen biosynthesis from pregnenolone by teased testicular tissues. Figures in parentheses are the number of animals in the groups.

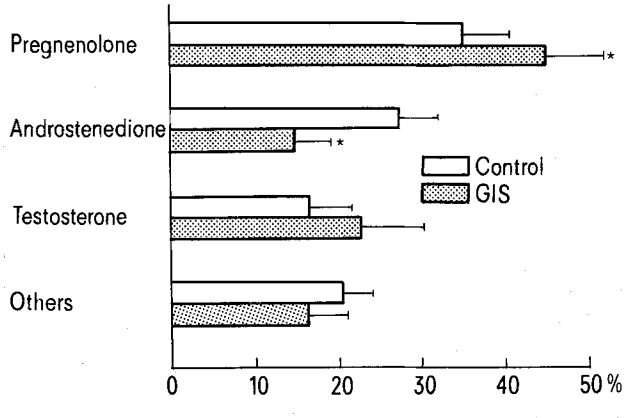

Fig. 1. The in vitro effect of GIS on the androgenesis by teased testicular tissues. Mean ±SD for triplicates. * Significantly different from the corresponding control ($p < 0.05$) using t-test.

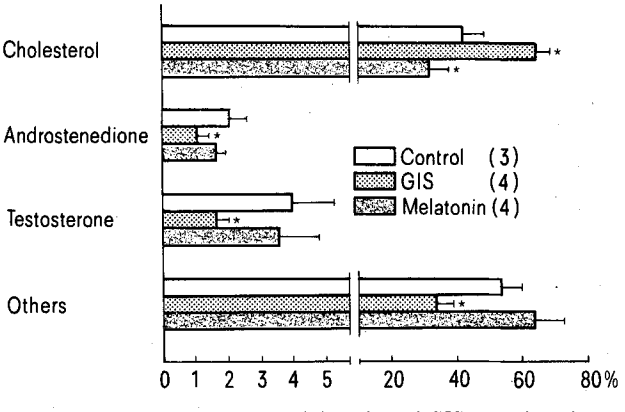

Fig. 3. Effect of the in vivo administration of GIS or melatonin on the in vitro androgen biosynthesis from cholesterol by teased testicular tissues.

14.00 h for 2 successive days. The other experimental group was injected i.p. with 500 ml urine-equivalent GIS at 14.00 h for 2 successive days. The control group received only the vehicle. The animals were decapitated 24 h after the final injection. 1 µCi of 4-^{14}C-pregnenolone or 1.6 µCi (2.5 µg) of 7α-^{3}H-cholesterol (sp. act.; 500 mCi/mmole, The Radiochemical Centre) with 1.25 µg of cold cholesterol was incubated with 100 mg of the teased tissues of the testes, prepared as described above, at 37 °C for 20 min under an atmosphere of 95% O_2 and 5% CO_2. The incubation medium (the final volume: 3.0 ml) buffered at pH 7.4 with 0.05 M phosphate buffer contained NADPH and nicotinamide (500 µg, each).

The incubation in the in vitro and in vivo experiments was terminated by adding dichloromethane. The extraction of the products was carried out by Sato et al.[17]. A suitable aliquot of the extract was chromatographed on a thin layer of silica gel according to Ota et al.[18], and the radioactive spots were detected by an autoradiographic method[17]. To identify the spots, oxidation and acetylation procedures were used[17]. Radioactivity in the spots which were scraped from the thin layer plate was measured by a liquid scintillation counter.

Results and discussion. Figure 1 shows the relative radioactivity in percent of the products of 4-^{14}C-pregnenolone by the teased tissues of rat testes with and without GIS. More residual pregnenolone was recovered from the media after the incubation with GIS than from the control media without GIS. The addition of GIS to the incubation medium resulted in a decreased formation of androstenedione. Figure 2 shows the relative radioactivity in percent of the products when 4-^{14}C-pregnenolone was incubated with the testicular teased tissues of rats treated with GIS or melatonin. The residual substrate was higher in both of the treated groups than in the control. The lowest production of androstenedione and testosterone was observed in the melatonin-treated group, followed by the GIS-treated group. The formation of products other than androstenedione and testosterone was higher in the melatonin-treated group than the other 2 groups. Figure 3 shows the effects in vivo of GIS and melatonin on the formation of testosterone and androstenedione from 7α-^{3}H-cholesterol by teased tissues of rat testes. A large amount of the unchanged substrate was recovered from the incubation media with the teased testicular tissues of the GIS-treated rats. The conversion of cholesterol into androstenedione, testosterone and other steroids was inhibited by the treatment of GIS. In the production of androstenedione and testosterone, no difference was observed between the control and the melatonin-treated groups.

The present in vitro study indicates that GIS has a direct effect on the steroidogenesis at the level of the testis. The previous study[19] revealed that GIS inhibits exogenous LH, and also exerts an effect at the hypothalamic-pituitary level to prevent LH release in mice. It may be referred from the previous[19] and present studies that GIS also has an indirect effect on the steroidogenesis, possibly through the hypothalamic-pituitary axis, which melatonin does not seem to have.

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Distribution of calcitonin cells in the thyroid glands of normal adult rhesus monkey *Macaca mulatta*

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Summary. The calcitonin cells of adult rhesus monkey *Macaca mulatta* are located in the central region of thyroid along the median axis. The anterior and posterior poles, the isthmus and peripheral regions of thyroid are completely devoid of C cells. The parathyroid also lacks C cells.

The calcitonin (C) cells have been ascribed as the site for the synthesis, storage and release of hypocalcemic, polypeptide hormone-calcitonin in a number of vertebrate species[1-4]. Several reports have been published on the morphology and distribution of these cells from the thyroid of a number of mammalian species, but there are only few reports about the C cells in primates (mostly in man)[5-10]. Since monkeys have been considered to be the best substitutes for human studies of calcium metabolism[11], we have chosen Indian rhesus monkey, *Macaca mulatta*, to study the morphology and distribution of C cells.

The thyro-parathyroid complex from 6 adult monkeys was dissected out under ether anaesthesia and fixed in Bouin and GPA mixture[12]. Serial sections of entire glands were cut at 4–6 µm and stained with haematoxylin-eosin, lead haematoxylin[12] and Davenport's silver impregnation[13].

Histologically the monkey thyroid gland consists of follicles which have a layer of follicular epithelial cells surrounding a colloid-containing lumen. Within the basement membrane of follicles, the C cells occur singly or in groups of 2 or 3. Sometimes they are also seen in the lumen (figure 1). The cells are perceivably larger than those of follicular cells