Effects of repeated administration of potassium canrenoate (SC-14266) on serum gonadotrophin, prolactin, testosterone and progesterone in male rats

T. Muraki, T. Nakadate, K. Kubota, Y. Tokunaga and R. Kato[1]

Department of Pharmacology. School of Medicine, Keio University, Shinanomachi, Shinjuku-ku, Tokyo 160 (Japan), 16 August 1979

Summary. Chronic administration of potassium canrenoate (SC-14266), a water-soluble anti-aldosterone agent, increased serum LH levels in male rats without altering serum levels of FSH, prolactin, testosterone and progesterone. The increase in serum LH may be due to the anti-androgenic effect of potassium canrenoate.

Spironolactone is an anti-aldosterone agent which inhibits the binding of aldosterone on the renal mineralocorticoid receptor. One of the side effects sometimes encountered during chronic spironolactone therapy is the occurrence of sexual disorders such as decreased libido, impotence and gynecomastia which suggests that spironolactone has an anti-androgenic action[2,3]. Potassium canrenoate (SC-14266 or Soldactone, potassium-3-[3-oxo-17β-hydroxy-4,6-androstadien-17α-yl]-propanoate) is a water-soluble anti-aldosterone agent and is suspected to have a similar anti-androgenic action[4,5]. In order to examine the possible anti-androgenic action of potassium canrenoate, we investigated whether repeated administration of potassium canrenoate affects the serum levels of gonadotrophins, prolactin, testosterone and progesterone in male rats.

Materials and methods. Male Sprague-Dawley rats, 4 weeks old on arrival, were kept in a controlled environment until 2 weeks had elapsed and then they were used for the study. The animals were housed individually in a room with constant temperature (24±2 °C) and humidity (55±5%) and alternate periods of light and dark (lights on from 06.00 to 18.00 h). The animals received food and water ad libitum. Potassium canrenoate was obtained from the Dainippon Pharmaceutical Co. Ltd. and was used after being dissolved in sterile physiological saline. Rats received saline (2 ml/kg) or potassium canrenoate (15 or 30 mg/kg) i.v. once a day in the morning for 30 days and were killed by decapitation at 30 min, 4 h and 24 h after the last injection of saline or potassium canrenoate. Another group, of 11 rats, were likewise killed by decapitation after remaining untreated for 30 days; these were used as intact controls. The trunk blood was collected and serum was kept frozen at −20 °C until the assay of hormones was performed. Gonadotrophins and prolactin were determined by radioimmunoassay as described previously[6], using the kits kindly provided by Dr A.F. Parlow for the Rat Pituitary Hormone Distribution Program, NIAMDD, NIH, and were expressed in terms of NIAMDD rat LH RP-1, FSH RP-1 and prolactin RP-1. Serum testosterone and progesterone were determined by radioimmunoassay using commercially available kits with specific antibodies: testosterone/dihydrotestosterone RIA kit (TRK 600, Amersham); and progesterone kit (Daiichi). Dihydrotestosterone was included in testosterone levels because of the omission of the procedure to measure dihydrotestosterone separately from testosterone. To eliminate the possible interference of potassium canrenoate and its metabolites on the radioimmunoassay of testosterone and progesterone, testosterone and progesterone were extracted by chloroform and purified using silica gel thin layer chromatography prior to radioimmunoassay, following the procedure of Erbler[7]. The recovery of extraction of testosterone and progesterone from serum was 80.8% and 84.5% respectively. The results were evaluated statistically by Welch and Aspin's t-test method.

Results. At 30 min after the last injection, serum LH levels of potassium canrenoate-treated rats (both 15 and 30 mg/kg) were significantly higher than those of intact controls and of saline controls. At 4 h, serum LH levels of potassium canrenoate-treated rats (15 mg/kg) were higher than those of saline controls and at 24 h serum LH levels of potassium canrenoate-treated rats (30 mg/kg) were higher than those of intact controls. At 24 h after the last injection, serum FSH levels of potassium canrenoate-treated rats (15 mg/kg) were higher than those of saline controls though they were within the levels of intact controls. No difference was seen in serum prolactin, testosterone and progesterone levels between potassium canrenoate-treated rats and intact or saline controls.

Discussion. Our study revealed that administration of potassium canrenoate for 30 successive days increased serum LH levels in male rats. The increase in the serum LH levels was most clearly seen at 30 min after the last injection of potassium canrenoate. In addition, it was shown that serum levels of other hormones studied were not altered by the administration of potassium canrenoate. The apparent increase in serum FSH at 24 h after potassium canrenoate (15 mg/kg) is probably due to the eventu-

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Treatment	Time after the last injection (h)	LH	FSH	Prolactin	Testosterone	Progesterone
None	-	26.9±2.5 (11)	308±43 (11)	5.5±2.4 (11)	4.63±1.72 (10)	48.0±11.0 (11)
Saline	0.5	29.7±3.0 (6)	249±28 (6)	6.5±1.6 (6)	4.28±0.60 (6)	17.7± 5.9 (6)
	4	23.4±2.9 (6)	221±18 (6)	1.5±0.3 (6)	4.13±0.83 (6)	14.1± 4.0 (6)
	24	31.2±6.4 (6)	190±17 (6)	4.4±1.8 (6)	4.69±0.65 (6)	23.6± 8.8 (6)
Potassium canrenoate (15 mg/kg)	0.5	46.7±4.8 (6)*,**	239±26 (6)	5.2±1.8 (6)	4.07±0.40 (6)	62.6±25.9 (6)
	4	38.3±6.0 (6)***	257±35 (6)	8.3±5.7 (6)	3.97±0.55 (6)	27.2± 6.5 (6)
	24	35.3±4.6 (6)	251±21 (6)[a]	6.7±3.4 (6)	6.10±1.29 (5)	58.9±21.6 (6)
Potassium canrenoate (30 mg/kg)	0.5	43.4±3.6 (6)*,**	266±35 (6)	2.1±0.4 (6)	6.80±3.60 (5)	30.4±10.0 (6)
	4	35.0±4.4 (6)	253±39 (6)	4.8±1.6 (6)	4.55±1.55 (5)	22.6± 7.1 (5)
	24	44.0±6.2 (6)*	215±16 (6)	11.6±3.3 (6)	2.47±1.06 (6)	14.6± 3.0 (6)

Number of rats in brackets. Values are mean ± SEM in ng/ml. * $p < 5\%$ vs none, ** $p < 5\%$ vs saline at 0.5 h, *** $p < 5\%$ vs saline at 4 h, [a] $p < 5\%$ vs saline at 24 h.

ally low value of serum FSH of saline controls at 24 h and therefore has no meaning. Elevated serum LH and normal prolactin in the potassium canrenoate-treated rats are similar to the results with spironolactone[8,9].

It was shown that spironolactone increases serum progesterone and lowers serum testosterone[10,11]. Spironolactone is believed to suppress testicular and adrenal androgen production by destroying the heme of cytochrome P-450, thereby inhibiting the enzymatic step involved in the conversion of progesterone to testosterone[12,13]. We could not confirm the previous report[5] that potassium canrenoate decreased serum testosterone levels and the cause of this discrepancy may be due to the difference in the species examined. The negligible loss of the heme of cytochrome P-450 by potassium canrenoate[13] may support the failure of potassium canrenoate to inhibit the androgen production. Potassium canrenoate may increase serum LH by its inhibitory effect on the androgenic receptor, because the possibility was shown that potassium canrenoate exerts its peripheral anti-androgenic effect via competition for the androgenic receptor[14]. The relative unresponsiveness of serum FSH to chronic potassium canrenoate may be due to the differential release of LH and FSH by endogenous LHRH. The normal serum prolactin levels in the rats chronically treated with potassium canrenoate indicate that the occurrence of gynecomastia due to hyperprolactinemia is unlikely during the chronic potassium canrenoate therapy. Although we could not obtain evidence suggesting the inhibition of the synthesis of testosterone by potassium canrenoate, the increased LH levels observed in our study suggest the anti-androgenic action of potassium canrenoate and therefore the possibility that sexual disorders similar to those due to spironolactone therapy may occur during chronic potassium canrenoate treatment.

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2 D.L. Loriaux, R. Menard, A. Taylor, J.C. Pita and R. Santen, Ann. intern. Med. *85*, 630 (1976).
3 G.T. Basinger and R.F. Gittes, J. Urol. *111*, 77 (1974).
4 C.M. Kagawa, D.J. Bouska, M.L. Anderson and W.F. Krol, Archs int. Pharmacodyn. Thér. *149*, 8 (1964).
5 J.F. Dymling, K.O. Nilsson and B. Hökfelt, Acta endocr., Copenh. *70*, 104 (1972).
6 T. Muraki, T. Nakadate, Y. Tokunaga, R. Kato and T. Makino, Neuroendocrinology *28*, 241 (1979).
7 H.C. Erbler, Naunyn-Schmiedebergs Arch. Pharmak. *285*, 403 (1974).
8 B. Stripp, A.A. Taylor, F.C. Bartter, J.R. Gillette, D.L. Loriaux, R. Easley and R.H. Menard, J. clin. Endocr. Metab. *41*, 777 (1975).
9 R.J. Santen, H.E. Kulin, D.L. Loriaux and J. Friend, J. clin. Endocr. Metab. *43*, 1386 (1976).
10 P.C. Walsh and P.K. Siiteri, J. Urol. *114*, 254 (1975).
11 S. Baba, M. Murai, S. Jitsukawa, M. Hata and H. Tazaki, J. Urol. *119*, 375 (1978).
12 R.H. Menard, B. Stripp and J.R. Gillette, Endocrinology *94*, 1628 (1974).
13 R.H. Menard, T.M. Guenthner, H. Kon and J.R. Gillette, J. biol. Chem. *254*, 1726 (1979).
14 P. Corvol, A. Michaud, J. Menard, M. Freifeld and J. Mahoudeau, Endocrinology *97*, 52 (1975).

Formation of indolyl-3-acetylaspartic acid in rats

T. Danno[1] and S. Suda

Department of Biology, Faculty of Science, Kobe University, Kobe, 657 (Japan), 7 June 1979

Summary. Indolyl-3-acetylaspartic acid (IAAsp) was detected in the urine of rats given indolyl-3-acetic acid (IAA) i.p. It was ascertained that the conversion of IAA into IAAsp could be carried out not only in plants but also in animals.

Indolyl-3-acetylaspartic acid (IAAsp) was first described as being present in the pea plant by Andreae[2] in 1955. Thereafter, its presence in many kinds of plants has been reported[3,4]. The significance of IAAsp formation was interpreted as the detoxification of higher concentrations of indolyl-3-acetic acid (IAA)[2]. IAA is generally known to be present in animal body as a metabolic product of tryptophan[5], however, the presence of IAAsp has not been known. In the present report the presence of IAAsp in the urine of rats injected with IAA is dealt with.

Materials and methods. Male rats (Wistar King strain) weighing 290–350 g were used as test animals. Rats were allowed to take water ad libitum, but no food was given for 18 h before the administration of IAA or aspartic acid. The blood samples were obtained by heart puncture using heparinized syringes, and pooled from 10 animals for 1 determination. Plasma and erythrocytes were separated by centrifugation of the heparinized blood 3 h after injection of the compound. The erythrocyte sediment was washed with saline solution 3 times and then hemolyzed in 3 times its volume of distilled water. The hemolyzate solution was used as an erythrocyte sample. Urine samples were collected 24 h after injection, from animals kept in metabolism cages. Extraction of IAA and IAAsp were made following the method of Andreae and Good[2], and the determination of these compounds was carried out using a modification of the gas chromatographic method of Seely and Powell[6]. 10 ml of plasma or 40 ml of the hemolyzate solution was acidified to pH 4 with H_3PO_4 and then extracted with 3 times its volume of n-butanol 3 times. Extraction from 10 ml of urine was also made by the same procedure. The butanol solution was washed with a small volume of water and extracted with a small amount of 0.1 N $NaHCO_3$ solution 3 times. The bicarbonate solution was acidified with H_3PO_4 to pH 2.6 and extracted with 10 ml of n-butanol 3 times. The butanol solution was washed with a small amount of water and then concentrated to 5 ml. 0.3 ml of it was applied to a sheet of Merck silica gel 60 plate, and 2-dimensional TLC was carried out with the following sol-

Table 1. IAA and IAAsp contents in urine 24 h after administration of IAA and Asp to rats

Compound applied	IAA (μmoles/animal)	IAAsp (μmoles/animal)
None	0	0
L-Asp 500 mg/kg (s.c.)	0	0
IAA 500 mg/kg (i.p.)	78.20 ± 12.59*	0.009 ± 0.002
IAA 500 mg/kg (i.p.) + L-Asp 500 mg/kg (s.c.)	89.62 ± 11.41	0.015 ± 0.006

* ± SE of 5 determinations.