The ingestion of calcium in female and male rats

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Three facts are reported. Need-free calcium intake is greater in virgin female rats than in male rats. Adult gonadectomy does not change this phenomenon. Both male and female rats display a greater avidity for calcium when they are calcium-deprived for the first time. The enhanced need-free ingestion of calcium in the female has its roots, perhaps, in the need for calcium that the female faces during pregnancy and lactation when she suffers calcium loss.

The removal of the parathyroid gland results in chronic calcium loss, but with access to calcium solutions, both rats (Richter & Birmingham, 1941; Richter & Eckert, 1937) and monkeys (Richter, 1943) increase their calcium intake. Dietary calcium deprivation in pigs (Evvard, Dox, & Guernsey, 1914), rats (Lewis, 1968; Rodgers, 1967; Scott, Verney, & Morissey, 1950; Tordoff, Ulrich, & Schulkín, 1990; Widmark, 1944), and chickens (Hughes & Wood-Gush, 1971; Wood-Gush & Kare, 1966) results in increased calcium ingestion. Moreover, vitamin D, which leads to calcium retention, is known to influence calcium ingestion; calcium intake increases during vitamin D deficiency (Brommage & DeLuca, 1984), and it decreases when vitamin D is replaced (Richter, 1943). Thus the behavioral regulation of calcium intake is expressed in several species, under different experimental conditions.

Importantly, both calcium and sodium ingestion increase in rats during pregnancy and lactation, presumably because of the greater demands for both salts (Denton, 1982; Richter, 1943; Woodside & Millelire, 1986). And in nature it is the female ungulates that are usually sighted at salt licks, particularly during the reproductive season (Jones & Hanson, 1985). Several questions emerge regarding calcium appetite expressed in the laboratory. One question concerns the possibility that there is a sex difference with respect to the ingestion of calcium. It is known, for example, that sodium is ingested in greater amounts by virgin females than by males (see, e.g., Krecek, 1973; Krecek, Novakova, & Stribrad, 1972; Wolf, 1982). The present inquiry was conducted, first, to determine whether need-free calcium intake is greater in virgin females than in males; second, to determine whether gonadectomy interferes with this phenomenon; and third, to discern whether the calcium ingestion known to result from calcium deprivation is rapid in both male and female rats.

Figure 1. Calcium intake of male and female rats.

EXPERIMENT 1

In Experiment 1, calcium intake was monitored in male and female rats. I used the concentration of calcium that Richter used in his classic studies of calcium ingestion (Richter & Birmingham, 1941; Richter & Eckert, 1937; see also Tordoff et al., 1990).

Method

Six male and 6 female Sprague-Dawley rats 90 days old were housed in individual wire mesh cages on a 12:12-h light:dark cycle. They were offered 2.4% calcium lactate, in 100-ml bottles ad lib. They were also given tap water in addition to the calcium. Their intake of the calcium was measured at 24-h intervals over a 15-day period. Food (Purina chow) and water were given throughout this period.

Results and Discussion

Figure 1 reveals that the female rats ingested greater amounts of the calcium solution than did the male rats ($p < .05$; one-way repeated ANOVA). Thus, as with sodium ingestion (see, e.g., Krecek et al., 1972), there is a sex difference in the ingestion of calcium in female and male rats.

EXPERIMENT 2

In Experiment 2, the need-free ingestion of calcium was again observed in virgin female and male rats. But this

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Figure 2. Calcium intake before and after gonadectomy in male rats.

Figure 3. Calcium intake before and after gonadectomy in female rats.

time, it was done both before and after gonadectomy to determine whether the gonadal steroids contribute to the differences in calcium intake.

Method

A new group of rats (5 females and 5 males) were housed as described in Experiment 1. Ingestion of the calcium was monitored for 4 days before and 4 days after gonadectomy.

For the gonadectomy, the rats were briefly anesthetized (40 mg/kg of ketamine and 13 mg/kg of xylazine). The surgical region of the females was shaved, and a single midventral incision was made 1.5 cm anterior to the vaginal opening. The ovaries were then re-

moved. The testes were removed from the scrotal sac of the males after anesthesia.

Results and Discussion

Once again the female rats ingested greater amounts of calcium than did the males (cf. Figures 2 and 3). Gonadectomy did not alter this sex difference. The differences between the two sexes remained 1 month later (30 ml in the female vs. 12 ml in the males). Thus, as in the ingestion of sodium (Krecek, 1973, 1978), adult gonadectomy does not interfere with calcium ingestion.

EXPERIMENT 3

In Experiment 3, I asked whether there were any differences in calcium intake between female and male rats the very first time they were calcium-deprived.

Method

A new group of 8 male and 8 female rats, 60 days old, was given access to calcium, food, and water as described. Following baseline intakes, at 24-h intervals, of calcium for up to 2 weeks, the calcium was removed and the rats were placed on a calcium-deficient diet (0 g/kg Teklad, TD 88231) for 21 days. The rats were then given access to calcium while they remained on the diet. Another group of 8 rats was not placed on the diet. Calcium intake was measured at 5, 10, 20, 30, and 45 min, and at 1, 2, and 24 h. Testing began 6 h into the light cycle. The experimental rats were thereafter once again placed on the Purina chow normal diet, and their calcium intakes were recorded at 24-h intervals.

Results

The results show that both male and female rats increase their calcium intake when placed on a calcium deficient diet over those on the normal calcium diet (Figure 4). The ingestion was significant by 10 min ($p < .05$; one-way repeated measures ANOVA). Over the 2-h period there were no differences between the two calcium-deprived groups. Not shown in the figure is that the average intake in the male group at 24 h is elevated (40 ml), but that it thereafter returns to pre-calcium-deficiency levels of intake (Figure 5). The females' ingestion of calcium remains elevated at 24 h (46 ml).

Figure 4. Calcium intake in male rats (m) when calcium-deprived (dep) or replete (rep), and in female rats (f) when calcium-deprived (dep) or replete (rep).

Figure 5. Calcium intake in male and female rats before and following calcium deprivation.

GENERAL DISCUSSION

The results from these three studies reveal that the need-free ingestion of calcium is sexually differentiated and that the differential ingestion of calcium does not depend on the gonadal hormones during adulthood, but that there are no differences between female and male rats' ingestion of calcium when they are calcium-deprived for the first time.

These results, when taken together with data published elsewhere (Schulkin, 1986), suggest that the elevated need-free ingestion of calcium or sodium in the virgin rat may have its roots in the evolutionary demands that females face during pregnancy and lactation. Thus they are innately attracted to the salts, even though they do not need them.

One behavioral strategy that may have evolved in the calcium-deficient animal is to ingest salty or chalky tastes; calcium lactate tastes chalky to humans (Schulkin, unpublished observations). And NaCl, which is also ingested by calcium-deprived rats, tastes salty (Schulkin, 1986; Tordoff et al., 1990). One reason why calcium-deprived rats or monkeys exhibit pica may be the craving for these two taste qualities (Snowdon, 1973, 1977).

Finally, the endocrine, physiological, and neurological mechanisms for calcium ingestion are largely unknown. Although vitamin D deprivation does result in increased calcium ingestion (Brommage & Deluca, 1984), and there is greater calcium intake depending on the degree of calcium deprivation (Tordoff et al., 1990), little else is known about the underlying mechanisms. Moreover, although it is known that the enhanced ingestion of sodium in females over males is eliminated if females are gonadectomized within the first 12 days of age (Krecek, 1978), it is not known whether similar endocrine mechanisms are at work for calcium ingestion.

REFERENCES

- BROMMAGE, R., & DELUCA, H. F. (1984). Self-selection of a high calcium diet by vitamin D-deficient lactating rats increases food consumption and milk production. *Journal of Nutrition*, **114**, 1377-1385.
- DENTON, D. A. (1982). *The hunger for salt*. Berlin: Springer-Verlag.
- EVVARD, J. M., DOX, A. W., & GUERNSEY, S. C. (1914). The effect of calcium and protein fed pregnant swine upon the size, vigor, bone, coat and condition of the offspring. *American Journal of Physiology*, **34**, 312-328.
- HUGHES, B. O., & WOOD-GUSH, D. G. M. (1971). A specific appetite for calcium in domestic chickens. *Animal Behaviour*, **19**, 490-499.
- JONES, R. L., & HANSON, H. C. (1985). *Mineral licks, geophagy, and biogeochemistry of North American ungulates*. Ames, IA: Iowa State University Press.
- KRECEK, J. (1973). Sex differences in salt taste: The effect of testosterone. *Physiology & Behavior*, **10**, 683-688.
- KRECEK, J. (1978). Effect of ovariectomy of females and oestrogen administration to males during the neonatal critical period on salt intake in adulthood in rats. *Physiologia Bohemoslovaca*, **27**, 1-5.
- KRECEK, J., NOVAKOVA, V., & STRIBRAD, K. (1972). Sex differences in the test preference for a salt solution in the rat. *Physiology & Behavior*, **8**, 180-188.
- LEWIS, M. (1968). Discrimination between drives for sodium chloride and calcium. *Journal of Comparative & Physiological Psychology*, **65**, 208-212.
- RICHTER, C. P. (1943). Total self-regulatory functions in animals and human beings. *Harvey Lecture Series*, **38**, 63-103.
- RICHTER, C. P., & BIRMINGHAM, J. R. (1941). Calcium appetite of parathyroidectomized rats used to bioassay substances which affect blood calcium. *Endocrinology*, **29**, 657-666.
- RICHTER, C. P., & ECKERT, J. F. (1937). Increased calcium appetite of parathyroidectomized rats. *Endocrinology*, **21**, 50-54.
- RODGERS, W. L. (1967). The specificity of specific hungers. *Comparative & Physiological Psychology*, **64**, 49-58.
- SCHULKIN, J. (1986). The evolution and expression of salt appetite. In G. de Caro, A. N. Epstein, & M. Massi (Eds.), *The physiology of thirst and sodium appetite* (pp. 490-496). New York: Plenum.
- SCOTT, E. M., VERNEY, E. L., & MORISSEY, P. D. (1950). Self selection of diet, X1: Appetites for calcium, magnesium and potassium. *Journal of Nutrition*, **21**, 187-202.
- SNOWDON, C. T. (1973). Lead pica produced in rats. *Science*, **183**, 92-94.
- SNOWDON, C. T. (1977). A nutritional basis for lead pica. *Physiology & Behavior*, **18**, 885-893.
- TORDOFF, M. G., ULRICH, P. M., & SCHULKIN, J. (1990). Calcium deprivation increases salt intake. *American Journal of Physiology*, **28**, R411-R419.
- WIDMARK, E. M. P. (1944). The selection of food. 111. Calcium. 7. *Acta Physiologia Scandinavica*, **10**, 322-328.
- WOLF, G. (1982). Refined salt appetite methodology for rats demonstrated by assessing sex differences. *Journal of Comparative & Physiological Psychology*, **96**, 1016-1021.
- WOOD-GUSH, D. G. M., & KARE, M. R. (1966). The behaviour of calcium-deficient chickens. *British Poultry Science*, **7**, 285-290.
- WOODSIDE, B., & MILLELIRE, L. (1986). Self-selection of calcium during pregnancy and lactation in rats. *Physiology & Behavior*, **39**, 291-295.

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