Hypersensitivity to lethal effect of injected calcium chloride in the third generation rats raised from parathyroidectomized mothers

TOMOKO FUJII, SHOJI MORIMOTO and HISAMOTO IKEDA
Department of Pharmacology, Teikyo University School of Medicine, Itabashiku, Tokyo 173, Japan

ABSTRACT
The LD₅₀ values for calcium chloride in F₃ adult male and female rats raised from mothers parathyroidectomized on the 15th day of pregnancy (15d-PTx) were much lower than in normal adult rats. Parathyroidectomy on the 5th day of pregnancy (5d-PTx) failed to induce the hypersensitivity to a lethal dose of calcium in the F₃ rats. Despite the low LD₅₀ values for calcium chloride in the 15d-PTx F₃ rats, their serum calcium level at 1.5-2 min after the injection of calcium solution did not differ from the levels in normal and 5d-PTx F₃ rats.

KEY WORDS calcium hypersensitivity / LD₅₀ / offspring / parathyroidectomy

When the parathyroid glands of pregnant rats were removed on the 5th day of gestation, the first generation rats born to these mothers exhibited a less marked decline in serum calcium levels following parathyroidectomy (PTx) than in normal controls and this characteristic feature was transmitted down to the 3rd or 4th generation rats (3). We report here that the LD₅₀ for calcium chloride solution in the F₃ adult rats raised from mothers parathyroidectomized on the 15th day of gestation was much lower than that obtained in the rats raised from normal mothers or from mothers parathyroidectomized on the 5th day of gestation.

Adult male and female (SPF) rats of the inbred Wistar-Imamichi strain (Animal Breeding Laboratory, Ohmiya, Saitama Prefecture) were mated. The day on which sperms were present in the vaginal smears was designated as day 0 of pregnancy. The parathyroid glands were surgically removed on the 5th (5d-PTx) or 15th (15d-PTx) day of gestation under ether anesthesia. The mothers were given 1% calcium lactate solution as drinking water for 3 days before parturition and for the first 3 days of lactation. When calcium was not given they died of tetanic seizures on the 21st or 22nd day of pregnancy. Their infants were weaned at day 21 after birth and mated by brother-sister mating when 3 months old to develop subsequent generations. The animals were supplied with commercial stock diet (calcium, 1.01%; phosphorus, 1.17%, Oriental Yeast Co., Ltd.) and water ad libitum. No special care was taken regarding the food and drinking water of subsequent generations of offspring after the first generation.

All rats were kept in an air conditioned room (22±2°C; humidity, 55±5%) with a lighting schedule of 14 hr light (0600-2000) and 10 hr darkness. Anhydrous calcium chloride (special grade, Kanto Chemical Co., Ltd.) was dissolved in deionized and distilled water to give a final concentration ranging from 0.54 M to 1.56 M, with the spacing of dose levels of 1.1. The volume injected was 0.1 ml per 100 g body weight. LD₅₀ values for calcium chloride given intravenously were estimated by the 'up-and-down' method (1). Calcium chloride solution was injected intravenously into a branch of the saphenous vein without anesthesia within 10-20 sec for 2.5-month-old or 6-month-old males weighing 300-700 g, and 10-12 sec for females weighing 270-440 g. Duplicate or triplicate trials were run for each experimental group, and one trial consisted of at least 10 rats. Death was defined by respiratory arrest; all rats showed the first respiratory arrest within 35 to 45 sec.
after the injection and the respiratory movement of the chest reappeared approximately 30 sec after the first arrest. The second respiratory arrest occurred within 1.5–2 min after the injection, leading to death. Blood sampling by decapitation began within 10 sec following death and the blood was collected into a centrifuge tube for approximately 30 sec. When the rats survived, they were decapitated 1.5–2 min after the injection and the blood was collected in a similar manner as for the dead rats. The basal serum calcium level was measured for the blood taken from the jugular vein before the injection of calcium solution without anesthesia. The isolated serum was kept frozen at −20°C. Serum calcium concentration was determined using o-cresolphthalein complexone (7).

As shown in Table 1, the calculated values of the LD50 for calcium chloride in the 15d-PTx F3 rats were much lower than those in the normal (P<0.01) or 5d-PTx F3 rats. The LD50 values in the 5d-PTx F3 rats tended to be slightly higher than those in normal controls. Their basal calcium levels were in a normal range. There was no sex difference in the LD50 in the three groups tested. It is of interest to note that there was no significant difference in the serum calcium level determined 1.5–2 min after the injection of calcium chloride between the normal group and 15d-PTx group, despite the different values of LD50 for calcium. The serum calcium level was roughly correlated with either the death or survival of animals in the three groups.

In rats, the 15th day of gestation has been considered as the time of completion of fetal organogenesis and the functional differentiation of various organs begins (5). In our previous work (3), parathyroidectomy on the 5th day of pregnancy which has been considered in rats as the time of gestation of fertilized eggs modified somehow the phenotypic expression in the F1–F4 offspring of the organs which regulate serum calcium level.

The present results clearly indicate that exposure of fetal rats to a low calcium environment and/or the lack of parathyroid hormone during the period of functional differentiation, from the 15th day of pregnancy afterward, affects the responsiveness of tissues, probably of the heart and of the part of the central nervous system which controls the respiratory and cardiovascular system, to calcium; and that exposure to the same maternal environment from the time of ontogenesis, the 5th day of pregnancy, was less effective in developing hypersensitivity to calcium. The relatively high level of serum calcium measured 1.5–2 min after the injection of calcium in the 15d-PTx F3 rats which died with a lower dosage of calcium suggests an alteration in the homeostatic control system (2, 4, 6) for a rapid regulation of serum calcium level. The mechanism for the development of a lower

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<th>Table 1</th>
<th>LD50 Values for Calcium Chloride, and Serum Calcium Concentrations before and after the Intravenous Injection of Calcium Chloride Solution in Normal Adult Rats and in F3 Adult Rats Raised from Parathyroidectomized Mothers</th>
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<td>Group</td>
<td>Serum calcium (mg/100 ml)</td>
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Parathyroidectomy was performed on the 5th day (5d-PTx) or 15th day (15d-PTx) of pregnancy.

a, the values represent the result of 2 trials; b, the rats were decapitated 1.5–2 min after the injection and the blood was collected for approximately 30 sec; c, mean ± SEM; d, vs. the values of normal controls. Analysis of variance was applied. Numbers of rats used for the Ca determination in parentheses.
threshold to a lethal dose of calcium in 15d-PTx F₃ rats remains to be clarified.

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REFERENCES