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Combined effects of caffeine and malnutrition on the newborn rat's myocardium

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Abstract

We hypothesized that the pathological effects on the neonatal rat heart could be aggravated by Cu deficiency due to the combined effects of caffeine exposure and malnutrition. Upon birth, pups were mixed and randomly picked; 8 pups were assigned to each dam and then divided into 4 groups. Group 1 dams received a normal diet containing 20% protein. Group 2 dams were fed 20% protein diet supplemented with caffeine (4 mg/100 g BW). Group 3 dams received 6% protein diet as a malnourished group, and group 4 dams received 6% protein diet supplemented with caffeine (4 mg/100 g BW). On postnatal day 10, dams and pups were killed. Group 2 tended to have a decrease in the Cu levels of dams' plasma and milk and in pups' plasma and heart tissue compared to those of group 1. This pattern was not observed consistently between groups 3 and 4. Transmission electron microscopy of group 2 pups' hearts revealed a degree of disruption in the mitochondria compared to normal mitochondria seen in group 1. There was no consistent change in the mitochondria of group 4 compared to group 3. The caffeine level observed in all categories of group 4 (dams' plasma and milk, pups' plasma and heart tissue) was lower than those in group 2. Although malnutrition affected body weight and heart weight, combined effects of caffeine and malnutrition on Cu content in the neonatal heart was relatively minor compared to the well nourished group. This well nourished group showed that the effects of caffeine on Cu were more consistent, resulting the changes of mitochondria.

Keywords: Caffeine; Malnutrition; Heart; Newborn rat; Copper

1. Introduction

Caffeine diffuses easily into breast milk in both the human (Tyrala and Dodson, 1982) and rat (Gulberg et al., 1986). Chronic caffeine exposure during early life affects various cardiovascular functions in rat offspring (Temples et al., 1985, 1987) and causes cardiac enlargement (Temples et al., 1985).

Of the internal organs, the heart has one of the highest concentrations of Cu (Linder, 1991). Cardiac myopathy has occurred in rats with low Cu intake (Wildman et al., 1995). Copper deficiency also causes hypertrophy and mitochondria swelling in heart muscle (Goodman et al., 1970). Caffeine decreases Cu content of the

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newborn rat liver at day 22 of weaning (Rossowska and Nakamoto, 1994). Caffeine exposure during lactation decreases the serum concentration of Cu in newborn rats (Wink et al., 1999). Thus, caffeine intake and Cu status could be related.

Malnutrition has always been a public health issue. It occurs in both genders and among all races and cultures worldwide. Protein-calorie malnutrition (PCM) is an epidemic in developing countries. In developing countries the vast majority of children with PCM are malnourished because of inadequate food availability and intake. However, PCM occurs in advanced countries as well. For example, malnutrition in children living in homes with significant social chaos or in children following fad or unorthodox diets has been reported (Liu et al., 2001), and acute and chronic PCM remains common in hospitalized pediatric patients in the United States (Hendricks et al., 1995). Severely malnourished children in the US reach that state in part because they have not been involved in the existing health care services system (Listernick et al., 1985). In animal studies, malnutrition combined with caffeine during pregnancy showed a greater negative effect on the proliferation of cardiac muscle cells than malnutrition alone in vitro (Kanemaru et al., 1992). Fetal resorptions and stillborns occurred more often in the litters of malnourished, caffeine-supplemented dams (Nakamoto and Shaye, 1986).

If Cu inadequacy is common and occasionally severe (Subar et al., 1998), then it is quite likely that malnutrition observed in infants and/or children in the US could also be associated with marginal Cu deficiency. Therefore, caffeine exposure could further aggravate their nutritional status, particularly that of Cu status.

This study was done to provide first-time information regarding the correlation between caffeine intake and malnutrition during lactation and the possible Cu deficiency in the growing newborn-rat heart.

2. Materials and methods

Timed-pregnant Sprague–Dawley rats were purchased from a commercial breeder (Harlan Co., Indianapolis, IN.). They were maintained in a 12-h light/dark cycle and fed a regular laboratory diet and water ad libitum until delivery.

Within eight hours of birth, delivered pups were combined without distinguishing male and female pups, and 8 randomly picked pups were assigned to each dam. They were then designated as day 1. The dams with 8 pups each were randomly divided into four groups. Group1 dams ($N = 10$), the control, were fed a 20% protein diet. Group 2 dams ($N = 10$) were fed a 20% protein diet supplemented with caffeine (4 mg/100 g BW). Group 3 dams ($N = 10$) were fed a 6% protein diet. Group 4 dams ($N = 10$) were fed a 6% protein diet supplemented with caffeine (4 mg/100 g BW). In each group, the dams and their assigned pups were weighed. The amount of diet consumed was also measured.

The 20% diet was composed of casein, 200 g; dextrose, 192 g; sucrose, 178 g; dextrin, 192 g; Mazola corn oil, 150 ml; mineral mix, 40 g (AIN-93 G Mineral Mix, Teklad Test Diets, Madison, WI); choline chloride (w/v), 4 ml; cellulose, 35 g; and vitamin mix, 10 g (AIN Vitamin mixture 76, Teklad Test Diet, Madison, WI). The 6% diet had the above composition except for casein 60 g; dextrose, 267 g; sucrose, 172 g; dextrin, 262 g; and methionine, 1 g. Caffeine in the amount of 4 mg per 100 g of body weight was added to diets of groups 2 and 4 (Nakamoto and Shaye, 1986). The diets were made on days 1 and 6 of the experiment.

On day 10, the dams were anesthetized with ether. They were injected with 2 IU of oxytocin (Sigma Chemical), and their milk was collected directly into sterile plastic tubes. Their blood was obtained via cardiac puncture into a heparinized tube. However, we did not examine their heart. The pups were killed, and their blood was also collected and combined to get enough blood sample. The heart was removed and weighed and combined. The blood samples were centrifuged at 1000*g* for 15 min at 4 °C to obtain plasma fractions. The plasma and milk were stored at −20 °C until they were analyzed to determine caffeine and Cu content.

The Cu content of plasma, milk and heart was determined as previously described (Rossowska and Nakamoto, 1994) with a flame atomic absorption spectrophotometer (Model 2380 Perkin–Elmer Co.). Plasma, milk and heart's caffeine content for groups 2 and 4 were measured as described (Nakamoto et al., 1988).

For each group, we selected randomly pups from different dams. Hearts were removed while still beating and flooded with fixative (4% glutaraldehyde). In order to assure good fixation, pieces just deep to the epicardium of the anterior surfaces of the left ventricles were removed, minced and fixed overnight. Tissues were rinsed in 0.1 M cacodylate buffer (pH 7.2), postfixed in 2% osmium tetroxide in 0.2 M cacodylate buffer (pH 7.2) and then dehydrated and embedded in polybed 812 Araldite capsules. Sections were stained with uranyl acetate and lead citrate and observed in a Phillips CM 10 electron microscope. All electron microscopy processing and examination were blinded to minimize bias.

Our past experience has taught us that moderate amounts of caffeine exposure did not affect the appetite of rats. For example, the supplementation of caffeine up to 4 mg/100 g BW in the diet has not shown any changes in food-intake behavior compared to the noncaffeine control group. Therefore, both caffeine and noncaffeine control groups were usually fed diets ad libitum.

Data among the groups were analyzed using analysis of variance and multiple comparison (Student-New-