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Secondary palatal closure in rats in association with relative maternofetal levels of folic acid, vitamin B12, and homocysteine

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Summary

Animal experiments are used in embryological and teratological studies of matters relevant to humans. In gravid rats, a decrease in the levels of folic acid and vitamin B12 was observed in maternal blood and in amniotic fluid. At the time of secondary palatal closure (14th day of pregnancy), the folic acid level of the amniotic fluid was 73% lower than that of the maternal blood. A drop in vitamin B12 in conjunction with an increase in amniotic homocysteine levels is seen as a risk factor for malformation of the palate. The understanding of causes of cleft generation could lead to a prophylactic treatment approach.

Introduction

The morphogenesis of the human skull is a complex process dependent upon numerous individual developmental steps (Fanghänel et al., 2006). The great variability of the head is also related to functional aspects (Breitsprecher et al., 1999, 2002). In addition to genetic and epistatic factors, nutritionally related factors such as folic acid and vitamin B12 deficiencies and hyperhomocysteinemia have been discussed as causes in the genesis of palatal clefts and neural tube defects (Fig. 1). In an experimental study on LEW.1A rats, the physiologic parameters of folic acid, vitamin B12, and homocysteine in maternal blood and fetal

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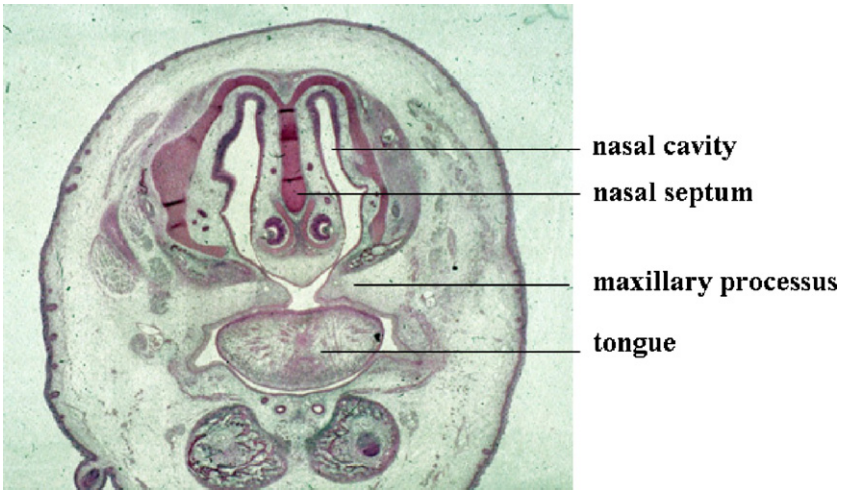

Figure 1. Open secondary palate. Histologic picture of secondary palate, thickness 5 μm, HE, × 10.

amniotic fluid were examined on days 0, 14, and 21 of gestation. The levels on gestation day 14 are of particular interest, since the secondary palate starts to close on that day.

Monogastric mammals (especially rats) can serve as models for questions relevant to humans, since their placenta hemoendotheliochorialis corresponds to the structure of the human placenta. The resorption and transport processes are therefore comparable. The objective of this study has been to examine the changes in folic acid, vitamin B12, and homocysteine levels during gestation in maternal blood and the fetal amnion. Although the importance of folic acid and vitamin B12 has been studied in detail, little is yet known about homocysteine. Thus, this equally important parameter was also examined. Because homocysteine functions as a methyl-group acceptor in the formation of methionine (Niculescu and Zeisel, 2002), an increase in its tissue level is an expression of a slower methylation rate (Fig. 2) (Mason and Miller, 1992; Naurath et al., 1995; Ngyuyen et al., 2001; Selhub, 2002). However, it can also be an expression of disturbed kidney function (Herrmann et al., 2001). A folic acid deficiency alone or in combination with vitamin B12 and B6 deficiencies is among the most frequent causes of an increased homocysteine level (Stampfer and Willett, 1993; Eskes, 1998). Increased homocysteine is therefore cause for concern, also in terms of teratology (Steegers-Theunissen et al., 1993; Eskes, 1997; Pietrzik et al., 1997; Copp, 1998; Hol et al., 1998).

Materials and methods

After copulation, female LEW.1A rats were kept in pairs in K3 cages. They were given food for breeding rats (V1326-000 ssniff R-Z, extruded; ssniff Spezialdiäten GmbH, D-59498 Soest, Germany) and drinking water ad libitum. Starting 14 days prior to the beginning of the experiment, the animals were kept at 50–60% atmospheric humidity under a standardized day–night light regime (night from 18:00 to 6:00). Live weight at the time of copulation was approx. 210 g. In this trial, 12 animals were examined prior to conception, 10 on day 14 post conceptionem (p.c.), and 12 animals on day 21 p.c. At the end of the experimental period, the animals were anesthetized with a mixture of ketamine: Rompun®: water applied intraperitoneally at a ratio of 1:1:1 and an amount of 0.1 ml/100 g body mass. Blood samples were taken via heart tap with a heparinized cannula, and amniocentesis was performed to obtain amniotic fluid. Both liquids were centrifuged 4000*g* for 4 min. under refrigeration. The supernatant fluids were poured off and examined for the following parameters: folic acid (chemiluminescence method), vitamin B12 (enzyme immunoassay), and homocysteine (HPLC procedure).

Statistical analysis consisted of comparisons of different body fluids averages using the *t*-test and the SPSS program. The significance level was defined as $p \leqslant 0.05$.

Results

All results (means and standard deviations) are presented in Table 1. The significant differences ($p \leqslant 0.05$) are shown as exponential letters after the mean. The letters denote a number of defined pregnancy days.