laboratory rat with mutant allele *Lx* determining the polydactyly-luxate syndrome (PLS) (Křen, 1975). Our previous paper demonstrated the interaction of RA with mutant allele *Lx* and with genes modifying its phenotypical manifestation in limb development on day 11 of pregnancy (Bílá and Křen, 1996). The present results confirm the interaction of RA with mutant allele *Lx* and with its modifiers on day 13 of pregnancy. It follows from our results that modifying genes of the BN strain predisposed foetuses to maximal sensitivity to embryolethal and teratogenic RA influence. On the contrary, modifying genes of the SHR strain exhibited strong protective effect to RA affliction of foetuses. Our system of SHR.BN congenic and double-congenic strains (Křen et al., 2000) might help in hunting for individual morphogenetic modifiers involved in RA teratogenic action.

Material and Methods

Two pairs of congenic strains, SHR-SHR.*Lx* (Křen et al., 1995) and BN-BN.*Lx* (Bílá et al., 1982), and RI strain BXH2 with extreme PLS expression (Pravenec et al., 1990; Křen et al., 1996) from the congenic and RI strain system of laboratory rat with mutant allele *Lx* were used for experiments.

Mating of these strains gave rise to 11 groups of progeny, differing in the *Lx* locus genotype (+/+, +/*Lx* or *Lx*/*Lx*) as well as in the genetic background (survey in Table 1). Females with the SHR genetic background were used for mating except for the groups of foetuses BXH2/BXH2, *Lx*/*Lx* and BN/BN, +/+ and *Lx*/*Lx*, which were obtained by mating of BXH2, BN and BN.*Lx* strain females with males of the same genotype.

Without teratogenic influence all progeny of the +/+ and +/*Lx* genotypes have both pairs of limbs normodactylous (ND) with normal zeugopodium (Figs. 1, 7), as the *Lx* allele appears to be recessive in all combinations of genetic background used in +/*Lx* heterozygotes. In *Lx*/*Lx* homozygotes, however, the *Lx* allele manifestation is 100 % in one or both limb pairs, and limb morphotypes depend on gene combination in the genetic background of individual groups.

Forelimbs in the SHR/SHR, *Lx*/*Lx* group are ND (Fig. 1); in the SHR/BN, *Lx*/*Lx* group about 15 % of pollex triphalangy appears, and in SHR/BXH2, BXH2/BXH2 and BN/BN, *Lx*/*Lx* groups triphalangy of pollex or six digits prevail (Fig. 4). Hind limbs have preaxial polydactyly (PD) in all *Lx*/*Lx* groups (Figs. 9, 12, 13) except for BXH2/BXH2, where preaxial oligodactyly (OD) develops (Fig. 8) (for details see Tables 4 and 5). Except for the SHR/SHR, *Lx*/*Lx* group, tibial hemimely regularly occurs, being the most marked in BN/BN and BXH2/BXH2, *Lx*/*Lx* homozygotes (Figs. 9, 8).

The way of breeding and mating of experimental animals and the way of RA treatment were described elsewhere (Bílá and Křen, 1996). The day of finding spermatozoa in vaginal smears was considered as day 1 of pregnancy. All-trans retinoic acid (Sigma Chemical Co., St. Louis, MO) was administered by gavage on day 13 of pregnancy in a dose of 100 mg/kg body weight in corn oil (Sigma Chemical Co.) with ethanol (9 : 1, total amount of the mixture 10 ml/kg). The control females from each group received the corresponding amount of dissolving mixture. Females were sacrificed on day 22 of pregnancy; the number of resorptions, weight of foetuses and external malformations were recorded. To follow up bone malformations, one control and at least three experimental litters from each genetically different group were stained with alcian blue and alizarin red (Inouye, 1976). Statistical analyses of data were done by the t-test and χ^2 test (two-by-two table) (Robinson, 1971).

Results

The administration of RA to pregnant females significantly reduces the foetal body weight irrespective of the foetal genotype. On the contrary, the embryolethal effect of RA is significantly genotype dependent (Table 1). A significant increase of resorptions was found only in foetuses with the BN/BN and BXH2/BXH2 genetic backgrounds ($P < 0.001$) and less pronounced in SHR/BXH2, *Lx*/*Lx* foetuses ($P < 0.05$). In all the other groups the incidence of resorptions following RA treatment does not differ significantly from untreated controls (altogether 574 treated and 369 untreated foetuses). This finding strongly indicates that BN genes themselves or in a special combination with SHR genes established and fixed in the BXH2 strain sensitize the foetuses to the embryolethal effect of RA.

Following RA treatment, in all genetically different groups of foetuses we recorded facial malformations, reductional digital defects in forelimbs, shortening of radius and ulna, mild shortening of humerus and lack of tuberositas deltoidea, mild shortening of femur and malformations of the tail. However, in groups of foetuses carrying the mutant *Lx* allele, characteristic defects of hind limb autopodium, zeugopodium and stylopodium occurred depending on the genetic background. The type and frequency of forelimb autopodium malformations also depended markedly on the genotype of the foetuses.

Lx genotype of foetuses: +/+

Following RA treatment of females carrying wild-type progeny, reductional defects developed only in forelimbs, while hind limbs remained without specific malformations. Table 2 summarizes forelimb autopodium defects.

Central OD (Fig. 3) or syndactyly (SD, Fig. 2) (the 3rd digit missing or fused with the 2nd or 4th digit) occurred in foetuses with the SHR/SHR genetic background in nearly 12% of 174 limbs, while in SHR/BN foetuses in more than 92% of 166 limbs (the difference is highly significant).

Only 2 experimental foetuses BN/BN, +/+ were obtained, with 3 cases of central OD in forelimbs.