Table 3. RA influence on fore- and hind limbs in +/Lx progeny

Genetic background	Dose mg/kg	Forelimbs ND[a]	OD[b]	%OD	Hind limbs ND[a]	PD[c]	OD[d]	%OD + PD	Number of limbs	foetuses	litters
SHR/SHR	0	112	0	0.0	112	0	0	0.0	112	56	6
	100	140	48	25.5[e]	186	2	0	1.1[h]	188	94	10
SHR/BN	0	134	0	0.0	134	0	0	0.0	134	67	7
	100	13	173	93.0[f]	114	56	16	38.7[i]	186	93	10
SHR/BXH2	0	120	0	0.0	120	0	0	0.0	120	60	6
	100	139	57	29.1[g]	75	103	18	61.7[j]	196	98	11

[a] ND – number of normodactylous limbs
[b] OD – number of forelimbs with central oligodactyly or syndactyly
[c] PD – number of hind limbs with preaxial polydactyly
[d] OD – number of hind limbs with preaxial oligodactyly
[e,f,g] The value f differs significantly from e and g ($P < 0.001$)
[h,i,j] The values differ significantly: h from i, h from j and i from j ($P < 0.001$)

An extreme reduction of zeugopodium up to the proximal cartilage fragment, accompanied by strong reduction of femur and by severe OD (2–1 toe) was found in SHR/BXH2 and BN/BN, *Lx/Lx* groups, where marked tibial hemimelia developed in untreated controls (Figs. 9, 13). In 34 stained experimental SHR/BXH2, *Lx/Lx* foetuses (68 limbs), only 4 limbs with distinct tibia and fibula were ascertained, and 16 limbs (23%) were extremely reduced. In the BN/BN, *Lx/Lx* genotype, none of the 24 stained experimental foetuses (48 limbs) had distinct tibia and fibula, and an extreme reduction was found in 25 limbs (approximately 43 %, Fig. 16), the frequency of the extreme reduction being significantly higher in comparison with the SHR/BXH2, *Lx/Lx* group ($P < 0.01$).

Owing to the high resorption rate, only 3 experimental foetuses of the BXH2/BXH2 genotype were obtained. The number of digits in forelimbs was 4 or 5. All hind limbs had 3 toes and only one bone in zeugopodium.

Discussion

In our previous experiments the interaction of RA with mutant allele *Lx* and with its modifiers on day 11 of pregnancy was demonstrated (Bílá and Křen, 1996). The present paper confirms the interaction of RA with these genetic factors also on day 13 of pregnancy.

It is evident at first sight (Table 1) that in a homozygous dose, the genes of BN or BXH2 genetic background, respectively, markedly increase the embryolethal effect of RA. It is not clear whether this result is connected in some way with the lower average number of progeny in the litter of BN or BXH2 strains, respectively.

The interaction of RA with mutant allele *Lx* manifests itself by an occurence of hind limb preaxial PD or OD in *+/Lx* heterozygotes (Table 3), while in *Lx/Lx* homozygotes preaxial reduction of the toe number, a reduction of zeugopodium and, in the presence of BN genes in the background, an extreme reduction of stylopodium

Table 4. RA influence on forelimbs in Lx/Lx progeny

Genetic background	Dose mg/kg	Forelimbs ND[a]	OD[b]	%OD	PD 5T[c]	PD 6[d]	%PD	Number of limbs	foetuses	litters
SHR/SHR	0	104	0	0.0	0	0	0.0	104	52	6
	100	50	34	29.3[e]	31	1	27.6[i]	116	58	7
SHR/BN	0	70	0	0.0	14	0	16.7	84	42	4
	100	21	50	41.0[f]	30	21	41.8[j]	122	61	7
SHR/BXH2	0	37	0	0.0	94	9	73.6	140	70	7
	100	6	5	3.8[g]	59	62	91.7[k]	132	66	8
BN/BN	0	5	0	0.0	54	7	92.4	66	33	8
	100	2	40	69.0[h]	16	0	27.6[l]	58	29[m]	12

[a] ND – number of normodactylous limbs
[b] OD – number of limbs with preaxial or central oligodactyly or syndactyly
[c,d] - number of limbs with 5 triphalangeal digits ([c]), and 6 digits ([d])
[e,f,g,h] - the value g differs significantly from e, f, h ($P < 0.001$)
- the value h differs significantly from e, f, g ($P < 0.001$)
[i,j,k,l] - the value k differs significantly from i, j, l ($P < 0.001$)
[m] - including 6 dead foetuses

Table 5. RA influence on hind limbs in Lx/Lx progeny

Genetic background	Dose mg/kg	Hind limbs PD[a] 6-7[b]	PD[a] 5T[c]	OD[d] 4[e]	OD[d] 3[f]	OD[d] 2[g]	OD[d] 1[h]	Number of limbs	Number of foetuses	Number of litters
SHR/SHR	0	104	0	0	0	0	0	104	52	6
	100	0	35	64	17	0	0	116	58	7
SHR/BN	0	84	0	0	0	0	0	84	42	4
	100	0	7	25	85	5	0	122	61	7
SHR/BXH2	0	28	112	0	0	0	0	140	70	7
	100	0	0	3	103	13	13	132	66	8
BN/BN	0	66	0	0	0	0	0	66	33	8
	100	0	0	0	4	27	27	58	29[i]	12

[a]PD – limbs with preaxial polydactyly
[b,c] - number of limbs with 6–7 toes ([b]) and 5 triphalangeal toes ([c])
[d]OD – limbs with preaxial oligodactyly
[e,f,g,h] - number of limbs with 4 toes ([e]), 3 toes ([f]), 2 toes (g) and 1 toe ([h])
[i] - including 6 dead foetuses

develops in some cases (Table 5). Moreover, in *Lx/Lx* foetuses the interaction manifests itself also in forelimb autopodium by preaxial digital defects involving both PD and OD (Table 4).

BN strain modifying genes which support phenotypical manifestation of the *Lx* allele also promote the result of the *Lx*-RA interaction, increasing the intensity or the frequency of limb defects. In hind limbs, the promoting effect of BN genes working in homozygous state (BN/BN background) or in a special combination with SHR genes fixed in the BXH2 strain (SHR/BXH2 background) supported *Lx*-RA interaction more effectively as compared to the haploid dose of BN genes in the SHR/BN genetic background (Tables 3 and 5).

In forelimbs, BN genes support RA-induced central OD or SD even without the *Lx* allele in the genotype, the frequency of these defects being significantly higher in SHR/BN than in SHR/SHR, +/+ foetuses (Table 2). In the *+/Lx* progeny, the same is true for SHR/BN compared to the SHR/SHR, *+/Lx* group (Table 3). However, in foetuses with the SHR/BXH2 background the frequency of OD was not increased as compared to the SHR/SHR, *+/Lx* progeny. In the *Lx/Lx* progeny (Table 4), foetuses with the BN/BN background have the highest frequency of OD forelimbs following RA treatment, while SHR/BXH2, *Lx/Lx* foetuses have the highest frequency of PD limbs and the lowest frequency of OD limbs. These results suggest that genes decisive for development of forelimb OD involved in the BN strain were not passed on the BXH2 strain. On the contrary, BXH2 genes support incidence of preaxial forelimb PD.

Without the *Lx* allele in the genotype (in +/+ foetuses), the digital reductional defects were recorded following RA treatment as early as on day 13 in forelimbs and only on day 14 in hind limbs (unpublished results). It is in agreement with the cephalocaudal gradient of limb susceptibility to RA, reflecting that forelimbs precede hind limbs in development (Kwasigroch and Kochhar, 1980). In contrast to our results, in outbred Fü-albino rats digital defects in forelimbs appeared following RA treatment as late as on day 14 (Kistler, 1981). We believe that this discrepancy is caused by genetic differences between strains used in these two experiments, which differ in many genetic factors irrespective of the *Lx* allele. It follows from the results of *Lx*-RA interaction that mutant allele *Lx* exerts its influence on fore- and hind limbs on day 11 (Bílá and Křen, 1996), 12 (unpublished results) and 13 (this paper), and extends the period of limb susceptibility to RA in *Lx* carriers to an earlier developmental phase as compared to +/+ homozygotes.

Except for 2 cases of sirenomelia appearing after day 11 of treatment in SHR/BXH2, *Lx/Lx* foetuses, the results of *Lx*-RA interaction were more pronounced on day 13 than on day 11.

The mechanism of action of mutant allele *Lx* and its modifiers is not yet known, and consequently the mechanism of interaction of these genetic factors with RA is not understood. It is possible that some of the genetic factors interacting with RA can influence the activity or concentration of CRABPs or RARs and so they can change the level of RA in limb bud cells. Double nil mutation in genes for receptors (RAR α -/-, RAR γ -/-) induces in mouse, among other defects, preaxial polydactyly or ectrodactyly in forelimbs and malformation of tibia and fibula in hind limbs (Lohnes et al., 1994). On the other hand, overexpression of RAR α in transgenic mice caused fore- and hind limb preaxial and postaxial defects (Cash et al., 1997).

It could be predicted that the mutant *Lx* allele or its modifiers influence the expression of genes which are regulated by RA, and are coding for signal molecules participating in fundamental processes in the limb development.