EFFECT OF TISSUE-SPECIFIC LUNG ADHESIVE FACTOR ON INDUCTION OF ADENOMAS OF THE LUNGS WITH URETHANE IN MICE

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The effect of adhesive factor (AF), urethane, and a combination of both on the mechanical properties of the lung tissue, as reflected in isolation of the nuclei during a standard dispersion procedure, and on induction of adenomas was investigated. Doses of AF abolishing the primary action of urethane on the mechanical properties of the tissue were found to significantly reduce the frequency of adenoma formation. Larger doses of AF, with the opposite action on the mechanical properties of the tissue, did not abolish the primary effect of urethane and did not reduce the frequency of adenoma formation.

KEY WORDS: urethane; pulmonary adhesive factor; mechanical properties of tissue; adenomas of the lungs.

The prevention of tumors can develop in two fundamental directions: the discovery of carcinogenic factors and their removal from the human environment and the increase of individual resistance to carcinogenic agents. The first of these directions already rests on a solid basis [5], whereas the second is in the stage of primary research. The present stage is basically one of determining the control systems at the cell, tissue, and organism level to which action must be directed in order to increase the resistance of the organism to carcinogenic factors.

In the investigation described below the effect of a factor with the property of producing a tissue-specific increase in the strength and stability of intercellular contacts (IC) [6] on the induction of adenomas of the lungs was investigated. The following facts served as the basis for this investigation: 1) Reduction of the strength and stability of IC is a characteristic feature of tumor development [1, 7, 8]; 2) the probability of development of spontaneous tumors is inversely proportional to the strength of IC in the given tissue in animals of different strains [2]; 3) weakening of IC in the target tissue is one of the earliest signs of the action of a carcinogen on that tissue [4]. The aim was to discover whether, by affecting the strength of IC in the lungs at the moment of administration of a carcinogen (urethane), the frequency of development of adenomas of the lung can be altered.

EXPERIMENTAL METHOD

To isolate pulmonary adhesive factor (AF) mice of strain A aged 3-5 months were used; the method was that described previously [3] and only the temperature conditions were modified. The tissue was incubated at 20° C and all subsequent operations were performed at 4°C. The solutions of AF were sterilized by passing them through a glass filter G_5 with the aid of a water pump.

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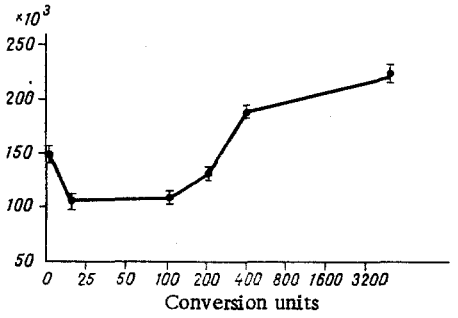

Fig. 1. Isolation of nuclei from lung tissue 6 h after injection of various doses of AF. Standard errors of means shown. Abscissa, dose of AF (in conventional units); ordinate, number of nuclei ($\times 10^3$) isolated from 1 mg lung tissue during standard dispersion procedure.

Activity of AF was estimated from its effect on isolation of nuclei from the lung tissue during dispersion. AF in 0.3 ml of Earle's solution was injected intraperitoneally into the mice. The mice were killed after various time intervals and pieces of tissue (at least five) were excised from the lungs; each piece was weighed on torsion scales and dispersed in a Potter's glass homogenizer with pestle clearance of 50 μ in Earle's solution containing 0.1% Trypan Blue. The number of isolated nuclei was counted in a Goryaev chamber. The results were expressed as the number of nuclei isolated from 1 mg tissue. The action of urethane and the combined action of urethane and AF were assessed in a similar way.

The effect of AF on induction of adenomas of the lungs with urethane was studied in mice of strain A weighing 17-22 g. Urethane in a dose of 10 ml in 0.2 ml of Earle's solution was injected subcutaneously and different doses of AF in 0.3 ml of Earle's solution were injected intraperitoneally. In the corresponding controls Earle's solution alone was injected instead of urethane and AF. The mice were killed 110-119 days after the beginning of the experiment. The lungs were fixed in 10% formalin. The number of adenomas visible with the unaided eye on the surface of each lobe of the lung was counted in the fixed material. In experiments in which four injections were given the dose of urethane was 5 mg per mouse; injections were given daily and the mice were killed on the 90th day of the experiment.

EXPERIMENTAL RESULTS

AF caused a maximal change in the degree of isolation of the nuclei from the lung tissue 6 h after injection. The magnitude and direction of its action depended on the dose of AP (Fig. 1).

It will be clear from Fig. 1 that there are two distinct regions of AF doses: Low doses caused a decrease in isolation of nuclei (the "direct" action), whereas high doses increased it ("reverse" action). Both actions were significant ($P < 0.001$). The switch from "direct" to "reversed" effects took place within a very narrow range of doses.

In this investigation AF (before sterilization) had a "direct" action in doses of 10^{-6}-10^{-7} mg/g and a "reversed" action in doses of 10^{-4}-10^{-5} mg/g. After sterilization of AF in doses of 10^{-4}-10^{-5} mg/g it no longer had a "reversed" but a "direct" action. During sterilization partial inactivation of AF evidently took place. Changes in the degree of isolation of nuclei with time during the action of AF in doses giving a "direct" and "reversed" action are shown by the curves in Fig. 2. Under the influence of urethane the degree of isolation of the nuclei during the first 24 h was greatly increased. Doses of AF with a "direct" action almost completely prevented the increase in liberation of nuclei observed following the action of urethane. When urethane was injected together with AF in doses giving a "reversed" action the degree of isolation of the nuclei during the first 10 h was about the same as following urethane alone. Later the degree of isolation of the nuclei decreased (Fig. 2).

The effect of AF on induction of adenomas of the lungs with urethane is shown in Table 1. No difference in the induction of adenomas in males and females was found and for that reason the sex of the animals is not shown in the Table. In some experiments (Nos. 2, 3, 5, and 7) the frequency of formation of adenomas induced by urethane decreased under the influence of AF. This action of AF was found within the range of doses giving "direct" effect. A significant increase in the frequency of adenoma formation was observed chiefly following the action of smaller doses from this range. In doses near the upper limits of this range, AF sometimes had

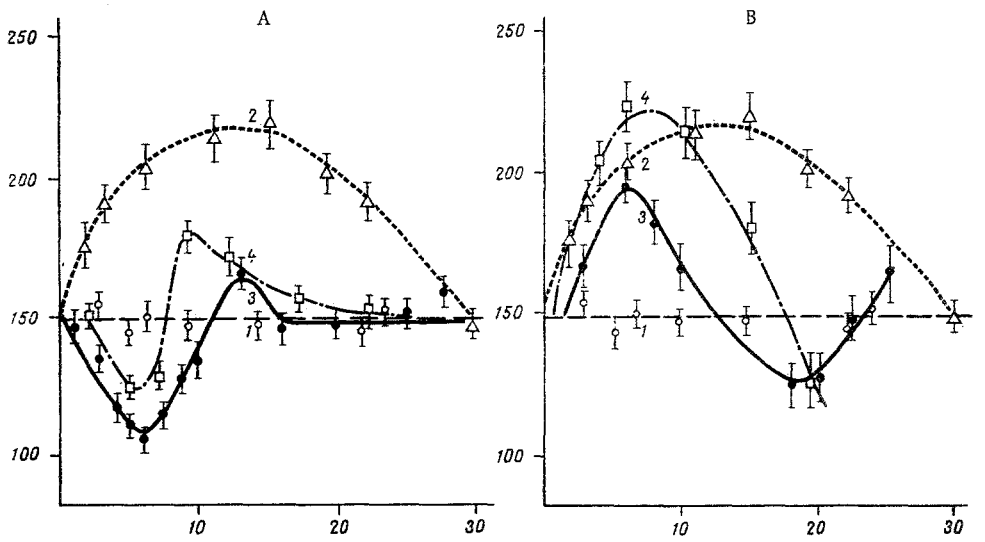

Fig. 2. Effect of AF, urethane, and a combination of both on isolation of nuclei from lung tissue: 1) isolation of nuclei in control after injection of Earle's solution; 2) effect of injection of urethane in a dose of 0.5 mg/g; 3) action of AF in doses producing "direct" (A) and "reverse" (B) effect; 4) combined action of urethane and AF. Standard errors of means shown. Abscissa, time after injection of test agents (in h); ordinate, number of nuclei ($\times 10^3$) isolated from 1 mg lung tissue during dispersion.

TABLE 1. Effect of Pulmonary AF on Induction of Adenomas of the Lungs with Urethane

Expt. No.	Administration of	Dose of AF, mg/g	Action of AF on isolation of nuclei	Number of mice in group	% of mice with adenomas	Mean number of adenomas per mouse	P
1	Urethane[2]		Reversed	28	100	6,4	
	Urethane[2] + AF[4]	10^{-5}		52	100	6,35	
	Earle's solution			18	19	0,19	
	AF[4]	10^{-5}		17	18	0,18	
2	Urethane[2]		Direct	22	100	5,95	
	Urethane[2] + AF[4]	10^{-6}		16	81	2,6	$<0,001$
	Earle's solution			16	19	0,19	
	AF[4]	10^{-6}		19	26	0,26	$>0,05$
3	Urethane[2]		Direct	31	100	5,25	
	Urethane[2] + AF[4]	10^{-6}		31	88	3,8	$>0,05$
	Urethane[2] + AF[4]	10^{-7}		35	83	3,1	$<0,01$
4	Urethane[2]		Direct	16	100	5,7	
	Urethane[2] + AF[5]	10^{-4}		30	93	5,2	$>0,05$
	Earle's solution			27	28	0,29	
	AF[5]	10^{-4}		24	29	0,29	
5	Urethane[2]		Direct	28	100	5,9	
	Urethane[2] + AF[5]	10^{-5}		36	75	2,6	$<0,001$
	Earle's solution			30	20	0,2	
	AF[5]	10^{-5}		27	15	0,15	
6	Urethane[3]		Reversed	22	100	11,2	
	Urethane[3] + AF[4]	10^{-4}		31	100	15,7	$<0,05$
	AF[4]	10^{-4}		18	22	0,28	
7	Urethane[3]		Direct	33	100	14,8	
	Urethane[3] + AF[5]	10^{-4}		30	100	9,3	$<0,01$
	Urethane[3] + AF[5]	10^{-5}		31	100	8,4	$<0,001$
	AF[5]	10^{-4}		24	8	0,08	
	AF[5]	10^{-5}		26	11,5	0,11	

[1] In experiments Nos. 1-5 urethane and AC were injected once simultaneously, in experiments Nos. 6 and 7 simultaneously on 4 consecutive days.

[2] Dose of urethane 0.5 mg/g body weight.

[3] Dose of urethane 0.25 mg/g.

[4] AF before sterilization.

[5] AF after sterilization.

no effect on the frequency of adenoma formation (experiment No. 4). Doses giving the "reversed" effect (Fig. 2B) either did not affect the frequency of adenoma formation (experiment No. 1) or actually increased it a little (experiment No. 6).

In the present experiments the affect of AF on IC was judged only with the aid of an indirect criterion – the number of nuclei isolated during dispersion of the tissue. However, it was shown previously that this index correlates very closely with more direct characteristics of IC such as the magnitude of the cohesive force measured by means of a micromanipulator and the number of cells isolated during mild dispersion [6].

The increase in the number of nuclei isolated following the action of urethane is evidently connected with weakening of the cell membranes and IC and is one of the earliest manifestations of the action of urethane on lung tissue. In doses abolishing this effect of urethane, AF reduces the frequency of adenoma formation. This correlation confirms the view that early changes in IC play a pathogenetic role in adenoma formation. However, this relationship is not simple. The primary effect of urethane is abolished almost completely by injection of AF, whereas the frequency of adenoma formation is reduced approximately by half. This indicates that the changes induced by urethane in the tissue and observed in the present experiments are not essential for adenoma induction, although they may perhaps increase the likelihood of this process.

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