ADRENERGIC COMPONENT IN THE HEPATOTROPIC CARCINOGENIC EFFECT OF DIETHYLNITROSAMINE

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The effect of the adrenomimetic mediator noradrenalin and its antagonist, the adrenergic blocking agent pyrroxan on hepatocarcinogenesis induced by diethylnitrosamine (DENA) in albino rats was investigated. Noradrenalin was found to stimulate, whereas pyrroxan inhibited the course of carcinogenesis and considerably reduced the multicentricity of origin of the liver tumors. In experiments *in vitro* on isolated rat atria it was shown that DENA, in low concentrations ($1 \cdot 10^{-6}$-$1 \cdot 10^{-8}$ M), sensitizes the adrenergic receptors of the atrium to the effects of endogenous and exogenous noradrenalin. An adrenergic component is postulated in the carcinogenic effect of DENA mediated through endogenous noradrenalin.

KEY WORDS: *hepatocarcinogenesis; diethylnitrosamine; noradrenalin; pyrroxan.*

Adaptive-trophic effects are known to play an important role in the development of tumors [1, 5]. In particular, great importance is attached to adrenergic factors regulating cell proliferation, the disturbance of which is the basis of carcinogenesis [3, 7]. However, the role of these factors in carcinogenesis has still received little study.

The object of this investigation was to study the effect of adrenergic agents modifying the tone of the sympathetic nervous system on experimental hepatocarcinogenesis induced in rats by diethylnitrosamine (DENA).

EXPERIMENTAL METHOD

Noradrenalin and pyrroxan were used as adrenergic agents. Noradrenalin is the neuromediator of the sympathetic nervous system and it differs from adrenalin by having virtually no effect on glycogenolysis in the liver. Pyrroxan is a new Soviet α-adrenergic blocking agent, a pharmacological antagonist of noradrenalin [6].

Experiments were carried out on 30 noninbred male albino rats divided into three groups: Group 1 was the control, and groups 2 and 3 received noradrenalin in bitartrate (2.5 μg/kg) and pyrroxan (25 mg/kg), respectively, three times a week subcutaneously for 6 months. All the animals received DENA with their drinking water in a concentration of 100 mg/liter.

A macroscopic and microscopic investigation was made of the liver of the experimental animals. The mechanism of the effect of DENA, noradrenalin, and pyrroxan on the adrenergic structures was investigated in model experiments *in vitro* on 30 isolated atria of intact rats as a pharmacological model with a concrete biochemical mechanism and one which can be used to study adrenergic influences.

EXPERIMENTAL RESULTS

To study the effect of adrenergic agents on hepatocarcinogenesis a low concentration of the carcinogen was administered in the drinking water, a concentration only half that described in the literature resulting in rapid induction of tumors [4].

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TABLE 1. Effect of Adrenergic Compounds on Development of Liver Tumors after Administration of DENA to Rats

Agent given	No. of animals in experiments	No. of animals surviving for 6 months		Intensity of tumor changes based on macroscopic examination (in points per rat with tumor)	Histological diagnosis		
		total	with macroscopically detectable tumor nodules		proliferation	adenoma	hepatocellular carcinoma
DENA	10	7	3	3,3±0,8	—	1	2
DENA and noradrenalin	10	9	9	3,2±0,6	1	2	6
DENA and pyrroxan	10	8	8	1,4±0,4	4	4	2

Legend. Macroscopic assessment of multiplicity and size of tumor nodules in points: 1 point) solitary (up to five in each lobe) tumor nodules measuring 0.1-0.2 cm; 2 points) multiple (more than five in each lobe) tumor nodules measuring 0.3-0.5 cm; 3 points) multiple nodules measuring 0.3-0.5 cm or single nodules measuring 0.6-1 cm; 4 points) multiple nodules measuring 0.6-1 cm or single nodules over 1 cm; 5 points) multiple nodules over 1 cm.

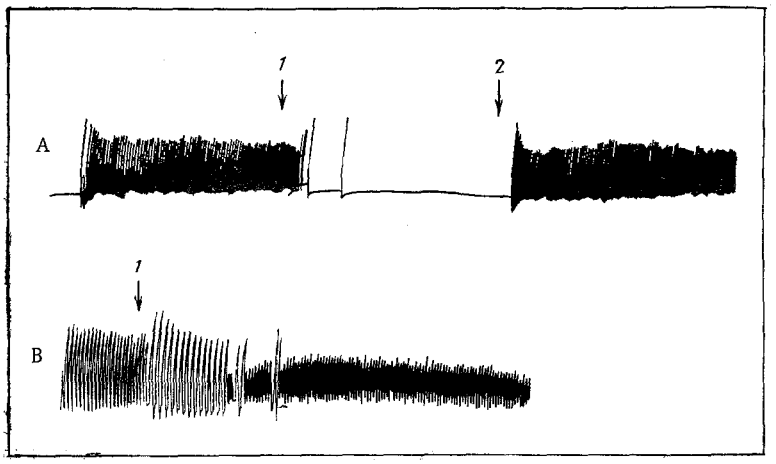

Fig. 1. Effect of DENA on contraction of isolated rat atria. A) Final concentration of DENA (1) in incubation medium $1 \cdot 10^{-4}$ M; rinsing with Ringer–Locke solution. B) Final concentration of DENA (1) $1 \cdot 10^{-8}$M.

The first liver tumors were found 4 months after the beginning of the experiment in the animals of group 2. On macroscopic examination after 6 months grayish-pink tumor nodules were found in the liver of all rats receiving the adrenergic drugs but in only three of the seven control animals (Table 1). When the multiplicity and size of the tumor nodules were assessed in points, the lowest rating was found for the animals receiving pyrroxan.

On histological investigation some of the nodules observed macroscopically consisted of foci of nodular precancerous proliferation, the rest of hepatocellular tumors (adenomas and carcinoma). Some of the nodular foci of proliferation were found only in microscopic investigation. Carcinoma was found most often in the animals receiving noradrenalin and least often in rats receiving pyrroxan (Table 1).

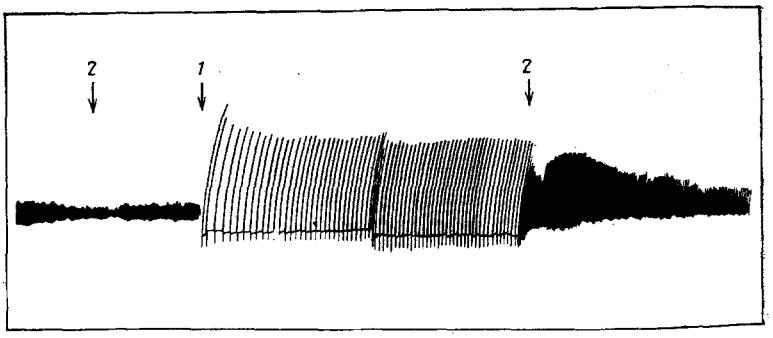

Fig. 2. Effect of DENA on response of isolated rat atrium to exogenous noradrenalin. Final concentration in incubation medium of DENA (1) $1 \cdot 10^{-7}$ M, of noradenalin (2) $1 \cdot 10^{-9}$ M.

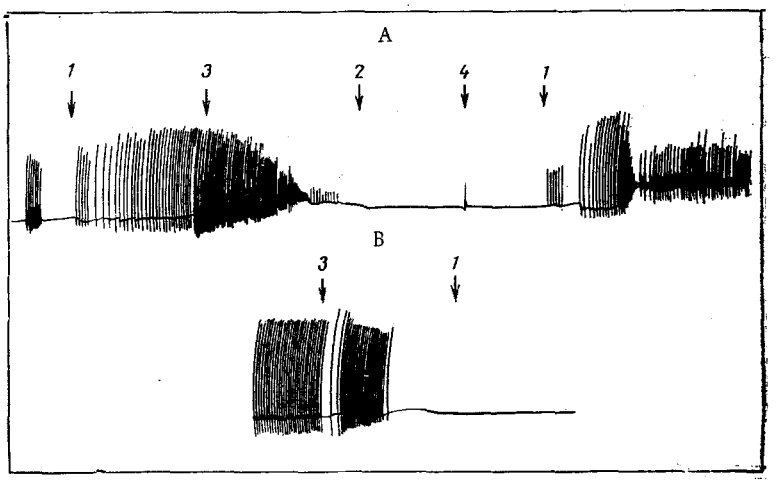

Fig. 3. Effect of pyrroxan on action of DENA stimulating contractions of isolated rat atria. A) Abolition of effect of DENA by pyrroxan; final concentration in incubation medium: of DENA (1) $1 \cdot 10^{-7}$ M, of pyrroxan (3) $0.7 \cdot 10^{-3}$ M, of noradrenalin (2) $1 \cdot 10^{-7}$ M; rinsing with Ringer–Locke solution (4). B) Prevention of DENA effect by administration of pyrroxan; final concentration in incubation medium: of pyrroxan (3) $0.7 \cdot 10^{-3}$ M, of DENA (1) $1 \cdot 10^{-7}$ M.

Toward the end of the experiment the animals of group 2 differed sharply in appearance from the animals of group 3. The rats of group 2 were grossly emaciated and their hair cover was thin; the animals of group 3 were larger, with a normal hair cover and with a well-marked layer of subcutaneous fat, thicker than in the animals of the control group.

The data in Table 1 thus show that noradrenalin stimulates and pyrroxan inhibits the course of carcinogenesis and considerably reduces the multicentricity of onset of liver tumors.

The study of the mechanism of action of DENA on the isolated rat atrium showed that the sensitivity of the atrium to the action of DENA was high in most animals. In the sensitive rats, DENA in high concentration ($1 \cdot 10^{-2}$–$1 \cdot 10^{-4}$ M) blocked atrial contraction. Lower concentrations ($1 \cdot 10^{-6}$–$1 \cdot 10^{-8}$ M), close to those observable in the liver of animals receiving the carcinogen in fractional doses with the drinking water, caused a marked increase in the frequency and amplitude of contraction (Fig. 1). Treatment of the atria with DENA enhanced their response to the action of noradrenalin, stimulating contraction (Fig. 2).

Since noradrenalin is known to change the level of cyclic AMP through activation of adenylate cyclase in the cell membranes [9] it can be postulated that the effect observed was connected with sensitization of the adrenergic receptors by DENA to the action of endogeneous noradrenalin. Pyrroxan, added to the incubation medium, abolished and prevented the sensitization effect by blocking the effect of endogenous noradrenalin (Fig. 3).

The experiments *in vitro* thus showed summation of the effects of DENA and noradrenalin and their antagonism to pyrroxan. A similar effect also was observed in the experiments *in vivo*. Of course, the functional expressions of these effects depended on the specific organization of the tissues (in one case a change in the contractile function, in the other a change in metabolism and proliferation), but the response of the cells, due in both cases to influences on adrenergic receptors, was similar in principle. Having discovered in experiments *in vitro* that the summation effect could be explained by sensitization of the adrenergic receptors by the carcinogen, it was further postulated that a similar process could take place in the experiments *in vivo* also. On this basis the hypothesis was put forward that there is an adrenergic component in hepatocarcinogenesis and that DENA acts on the hepatocytes in such a way that their sensitivity to the action of noradrenalin, both endogenous and exogenous, in stimulating proliferation is sharply increased, and it is evidently this which lies at the basis of carcinogenesis.

Comparison of the results of the experiments obtained with the adrenergic blocking agent pyrroxan and data in the literature [10] on the inhibitory action of dibenamine, another adrenoblocker, on hepatocarcinogenesis obtained under similar experimental conditions, suggests that the effect of dibenamine can be explained most probably by its adrenoblocking action rather than by other possible mechanisms (for example, the presence of alkylating groups in the molecule).

The results of the present experiments do not contradict the role of cyclic AMP in cell proliferation, examined in the literature [2], and the possible effect of noradrenalin and pyrroxan on the adenylate–cyclase system, and thus, on the whole, they confirm the view of Shabad [8] on the role of endogenous factors in carcinogenesis.

In conclusion, it should be emphasized that the concept enunciated by the present writers offers a fresh approach to the problem of anticarcinogenesis, connected with the search for effective inhibitors of carcinogenesis among the pharmacological antagonists of noradrenalin.

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