ADRENERGIC MECHANISMS OF REGULATION OF THE VARIOUS STAGES OF CHEMICAL CARCINOGENESIS: ROLE OF PRESYNAPTIC RECEPTORS

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Pharmacologic compounds affecting the tone of the adrenergic component of the autonomic nervous system (ANS) have been shown to activate (α-adrenoreceptor agonists, muscarinic cholinolytics) or inhibit (adrenoblockers, inhibitors of synthesis, reuptake, and axoplasmic transport of catecholamines, agonists of presynaptic adrenergic and dopaminergic receptors) chemical gastric and hepatic carcinogenesis [2, 3, 11]. Some chemical carcinogens have been shown to activate central adrenergic processes [10]. Together with data in the literature on the presence of adrenoreceptors on membranes of epithelial cells (hepatocytes, enterocytes, etc.) [7, 15], and their role in proliferation [1], these results are evidence that the effect of chemical resorptive carcinogens may be mediated through systemic factors and, in particular, through activation of the adrenergic component of the ANS. However, it is not yet clear at which stage of carcinogenesis (initiation or promotion) adrenergic neurotransmitters may have a modifying action. We know that adrenergic processes can be modulated by various pharmacologic agents acting on neurochemical processes in the synaptic region, including as a result of a change in the total pool of monoaminergic transmitters in response to injection of their precursors and, in particular, of dihydroxyphenylalanine (dopa). Attempts to modify carcinogenesis by injecting precursors of catecholamines into animals has not previously been undertaken.

The aim of this investigation was to estimate the importance of adrenergic activation, modified by the pharmacologic catecholamine precursor L-dopa, or different stages of hepatocarcinogenesis induced in rats by N-nitrosodiethylamine (NDEA).

EXPERIMENTAL METHOD

Experiments were carried out on 155 noninbred male albino rats weighing 100-120 g. The method of injection of the carcinogen and of evaluation of macroscopic and microscopic changes in the animals' liver was described previously [11]. Short-term (2 months) exposure of the rats to the carcinogen, aimed at obtaining the initiating effect of NDEA, was used. There were two series of experiments. In series I the modifying effect of L-dopa (20 mg/kg, intraperitoneally, 3 times a week for 2 months) was investigated on 120 rats (8 groups, 15 rats in each group). Since carcinogenesis is regarded as a two-stage process [5], L-dopa was injected together with NDEA (group 2), after NDEA (group 3, effect on promotion), and before injection of the carcinogen (group 4, effect on initiation). Animals of groups 1 and 6, receiving NDEA only, and also of groups 5 and 7, receiving L-dopa only, served as the control (the rats of groups 6 and 7 were killed after 2 months, those of groups 1 and 5 after 6 months). The animals of group 8 constituted the intact control. Animals of groups 1-5 and 8 were killed by inhalation of chloroform 6 months, and those of groups 6 and 7 two months after the beginning of the experiment. In the experiments of series II noradrenalin and dopamine concentrations in homogenates of the hypothalamus and adjacent midbrain tissues were investigated in 35 rats, either intact or receiving NDEA.

The animals were killed by decapitation. Catecholamines were determined by the method in [4]. The results were subjected to statistical analysis by Student's test.

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TABLE 1. Morphological Changes in the Liver of Rats Receiving NDEA, L-Dopa, and a Combination of Both

Group of animals	No. of animals	Characteristics of neoplastic changes								
		macroscopic assessment, conven. score in points	microscopic assessment					differential analysis of transformation of indiv. cells of the hepatic parenchyma, conventional score in points		
			no change	proliferative reaction		adenomas	carcinoma	hepatocellular epithelium	cholangiolar epithelium	endothelium
				diffuse	focal					
1	14	1,2±0,8	4	5	1	1	3	1,3±0,4	0,7±0,08	0,8±0,09
2	13	1,7±0,6	4	3	0	1	4	1,5±0,8	0,1±0,04	0,7±0,08
3	14	0,8±0,05	6	4	2	1	1	1,1±0,9	0,5±0,04	0,2±0,02*
4	14	2,4±0,9*	1	2	2	1	8	2,4±0,8*	0,8±0,07	0,9±0,09
5	15	0	15	0	0	0	0	0	0	0
6	15	0,2±0,01*	5	7	3	0	0	0,8±0,01	0,2±0,01	0,1±0,008*
7	15	0	15	0	0	0	0	0	0	0
8	15	0	15	0	0	0	0	0	0	0

Legend. On microscopic assessment the number of animals with the severest degree of morphological changes of the particular type was counted. *$p \leq 0.05$ compared with group 1 (control).

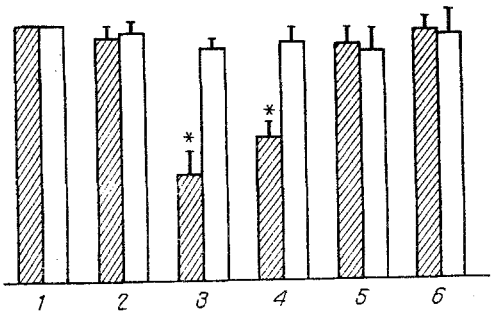

Fig. 1. Time course of noradrenalin (shaded columns) and dopamine (unshaded) concentrations in hypothalamus of rats in percent of intact control, corresponding to stages of neoplastic changes induced in the liver by NDEA. 1) Intact control, 2) animals with no morphological changes, 3) diffuse proliferation, 4) focal proliferation, 5) stage of adenomas, 6) stage of hepatocellular carcinoma, 2, 6, 7) action of NDEA. *Indicates statistically significant difference from control

EXPERIMENTAL RESULTS

Malignant tumors were found in the liver of 21% of the animals in group 1 (Table 1). Preneoplastic changes (diffuse and focal proliferation of hepatocytes) were found in the liver of some rats of group 6. In virtually all the animals of group 7 zones of dilatation of the vascular spaces, venous stasis, and well-marked foci of fatty degeneration were observed in the liver. Morphological changes in the liver of the rats of group 5 did not differ significantly from those in the liver of the intact age control (group 8).

Malignant tumors of the liver were found in 30% of the animals of group 2. The frequency of malignant tumors of the liver in the rats of group 3 was only one-third of that in the control rats of group 1. The endothelium was at least affected by neoplastic changes. Malignant neoplasms of the liver were found in 57% of the animals of group 4, and the hepatocellular epithelium was most severely involved in the process of neoplastic transformation.

In our view these phenomena can be explained by the neuropharmacologic mechanisms of the action of NDEA and L-dopa. When L-dopa enters the body it is converted into dopamine, which stimulates the system of postsynaptic and inhibitory presynaptic dopaminergic receptors [12]. The morphological manifestations of stimulation of peripheral postsynaptic receptors were found in the rats of group [6]. Stimulation of presynaptic dopaminergic receptors causes inhibition of noradrenalin secretion and reduces activity of the adrenergic

component of the ANS [8, 13]. In turn, it was shown previously that NDEA activates adrenergic processes [10]. It can be tentatively suggested that NDEA acts by a mechanism opposite to that of L-dopa, by blocking inhibitory presynaptic receptors and promoting release of noradrenalin, stimulating its turnover, and lowering its concentration in the tissues. It follows from Fig. 1 that in the early stages of action of NDEA (diffuse and focal proliferation of cells in the liver), there is actually a significant fall in the noradrenalin concentration in the hypothalamus. This hypothesis explains the mechanism of the action of L-dopa on hepatocarcinogenesis. In the rats of group 4 a depot of inactive noradrenalin is created at the beginning of injection of NDEA. Blocking inhibitory presynaptic adrenoreceptors by NDEA against the background of down-regulation of the dopaminergic system, induced by chronic administration of L-dopa [14], activates adrenergic processes and stimulates proliferation in the liver. Proliferation, however, is an essential condition for expression of neoplastic transformation, induced by a disturbance of the genetic apparatus of the cells in response to the initiating action of a carcinogen [9]. When NDEA and L-dopa are given in the opposite order (group 3) inhibition of carcinogenesis is observed. Under these conditions dopamine, formed after injection of L-dopa, activates inhibitory presynaptic receptors and reduces activity of the adrenergic component of the ANS. Under these conditions mitotic activity, an essential stage of promotion, is inhibited. After the combined injection of NDEA and L-dopa (group 2) opposite effects may be revealed, the resultant of which (from stimulation to inhibition of carcinogenesis) will be determined by the level of the doses of these compounds chosen for study. Under the present experimental conditions the dose of L-dopa was evidently insufficient for modification of hepatocarcinogenesis. As a result the frequency of the tumors in the animals of groups 1 and 2 was virtually identical.

The results of this investigation thus confirm the concept that the key stage in the mechanism of action of NDEA is evidently the membrane effect at the level of presynaptic receptors connected with catecholamine release under the influence of NDEA, and with their action (direct or indirect) on proliferation of target cells.

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