REVERSIBLE DISTURBANCE OF SYNTHESIS OF POLYAMINES AND DNA IN THE REGENERATING RAT LIVER

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It has been shown that the formation and accumulation of polyamines, primarily putrescine† and spermidine [11], increase sharply in actively proliferating tissues and other objects characterized by intensified synthesis of protein and nucleic acids. Numerous experiments conducted on a wide variety of cell-free systems have shown that polyamines can evidently participate in the regulation of the various stages of synthesis of proteins and nucleic acids, in the stabilization of the polysomal apparatus of the cell, and the performance of various other functions connected with plastic processes [13, 14]. Meanwhile, the many different manifestations of the action of polyamines and the known limitations with which interpretation of the results of experiments in vitro are associated do not permit either the mechanism of action of these physiologically active substances or their primary point of application to be identified. One promising approach to the study of the role of polyamines in vivo is the use of specific inhibitors of their synthesis, and this has enabled the metabolic effect of disturbance of the formation of putrescine, spermidine, or spermine in the cell to be traced. It has been shown that some diamines can inhibit the activity of ornithine decarboxylase – a key enzyme in the chain of polyamine synthesis [8, 9].

The aim of the present investigation was to study the effect of a diamine not found in biological objects (diaminohexane – DAH) on polyamine formation and the possible disturbances of synthesis of protein and nucleic acids arising under these circumstances. The regenerating liver after partial hepatectomy, which is characterized by a high level of plastic processes, was chosen as the test object.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-200 g, on which partial hepatectomy [4] was performed, and they were decapitated 24 h later. A single intraperitoneal injection of DAH in a dose of 100 mg/kg body weight was given to the animals of the experimental group 30, 60, 120, and 240 min before sacrifice. The rats of the control group received the same volume of 0.14 M NaCl. The concentration of di- and polyamines in the liver, and also the intensity of synthesis of spermidine and spermine were determined by Raina's method [10], using ^{14}C-methionine as the radioactive precursor, which was injected into the animals 60 min before death in a dose of 10 μCi/100 g body weight. Synthesis of DNA, RNA and protein in the liver was studied by determining incorporation of their specific precursors – ^{3}H-thymidine (20 μCi/100 g body weight), ^{14}C-uridine (10 μCi/100 g body weight), and ^{14}C-leucine (4 μCi/100 g body weight) respectively, which were injected into the animals 30 min before sacrifice. DNA and RNA were separated [5] and their concentrations determined spectrophotometrically [1]. Aliquots of digest of nucleic acids were taken for radioactivity counts. Radioactivity of total liver proteins was determined by Elwyn's method [3]. The results were subjected to statistical analysis [2].

EXPERIMENTAL RESULTS

Soon after injection the concentration of DAH in the liver reached a maximum and it was maintained at a sufficiently high level for 2 h (Table 1). Under these circumstances a sharp decline was observed in the

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†Although putrescine is a diamine, in order to simplify the terminology it is often described in the same class as spermidine and spermine.

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TABLE 1. Effect of DAH on Concentration of Diamines and Polyamines (in nmoles/g tissue) in Regenerating Rat Liver (M ± m)

Experimental conditions	Time after injection of DAH, min	DAH	Putrescine	Spermidine	Spermine
Control	—	—	140±13 (9)	1254±82 (9)	454±24 (9)
Experiment	30	586±26 (5)	117±16 (3)	1156±76 (3)	445±32 (3)
	60	529±28 (7)	27±16 (10)*	1103±176 (6)	406±23 (6)
	120	471±48 (6)	16±10 (9)*	1069±112 (6)	391±44 (6)
	240	159±18 (5)	78±17 (5)*	1282±77 (5)	439±67 (5)

Legend. Here and in Tables 2 and 3, results differing significantly from control are indicated by an asterisk; number of experiments given in parentheses.

TABLE 2. Synthesis of Polyamines (in cpm/g tissue) in Regenerating Rat Liver after Injection of DAH (M ± m)

Experimental conditions	Time after injection of DAH, min	Spermidine	Spermine
Control	—	5474±808 (9)	1661±154 (9)
Experiment	60	3353±436 (6)*	1480±155 (6)
	120	3009±620 (6)*	1636±148 (6)
	240	5101±405 (5)*	1157±125 (5)*

TABLE 3. Synthesis of DNA, RNA, and Protein (in cpm/mg substance) in Regenerating Rat Liver after Injection of DAH (M ± m)

Experimental conditions	Time after injection of DAH, min	DNA	RNA	Protein
Control	—	118358±18337 (13)	1010±73 (11)	1313±50 (12)
Experiment	30	117834±18316 (5)	920±117 (3)	1481±126 (6)
	60	69699±9620 (7)*	999±128 (7)	1107±56 (6)*
	120	68240±6349 (7)*	861±82 (7)	1254±71 (5)
	240	74125±12325 (5)	897±51 (5)	1328±78 (5)

putrescine concentration in the regenerating liver, followed by partial recovery of its level 240 min after injection of DAH. These data on the decrease in the putrescine concentration are in good agreement with the results obtained by Pegg et al. [7], and published while the present investigation was in progress. They showed, in particular, that DAH can effectively inhibit ornithine decarboxylase in the liver of animals exposed to the action of somatotrophin. By contrast with putrescine, there was practically no change in the content of spermidine and spermine in the regenerating liver after injection of DAH (Table 1). It may be that the dose of DAH used was too small to have any profound effect on synthesis of higher polyamines. These compounds also have a much longer half-life than putrescine [12] and, for that reason, the brief disturbance of synthesis of spermidine and spermine may not have been reflected significantly in the level of their reserves. Evidence that inhibition of spermidine and spermine formation under these experimental conditions does in fact take place is given by the results shown in Table 2. For instance, 60 and 120 min after injection of DAH incorporation of ^{14}C-methionine into spermidine was reduced by about 40%, but after 240 min it was already virtually back to normal. The later (after 240 min) and less marked (by 30%) decrease in spermine synthesis is in harmony with existing views on the order of the action in the chain of polyamine synthesis and it can evidently be explained by the greater distance of the spermine–synthetase reaction, metabolically speaking, from the point of application of DAH. The inhibitory action of DAH on polyamine synthesis was thus reversible in character and developed successively in the course of 30-240 min. Synthesis of putrescine was most severely disturbed, thus causing an almost complete, although temporary, disappearance of this substance from the regenerating liver. Spermidine synthesis was affected to a much lesser degree, whereas spermine formation was almost unaffected.

Disturbance of polyamine synthesis was reflected only a little in incorporation of the radioactive label into proteins of the regenerating liver and had practically no effect on RNA synthesis (Table 3). Meanwhile, a considerable (by more than 40%) inhibition of DNA synthesis was observed. This is evidence in support of the view that polyamines play an important role in DNA synthesis [14] and that they are probably essential not only for its initiation, but also for maintenance of the normal replicative function during the first 24 h of regeneration. Unlike the observations of Kallio et al. [6], who state that there is direct correlation between the spermidine concentration and DNA synthesis in the regenerating liver, we found that incorporation of ^{3}H-thymidine into DNA correlates closely (coefficient of correlation $r = 0.93$) with the putrescine concentration and, at the same time, with incorporation of ^{14}C-methionine into spermidine ($r = 0.71$). In this case spermidine synthesis probably undergoes parallel changes with DNA synthesis and evidently cannot determine it. The results suggest that maintenance of normal DNA synthesis in the course of cell proliferation is one of the functions of putrescine.

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EFFECT OF MIXED AND SEPARATE ADMINISTRATION OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON BENZPYRENE HYDROXYLASE IN THE LIVER OF SENSITIVE AND RESISTANT INBRED MICE

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The existence of two classes of inducers of monooxygenase systems in the liver, differing in their mechanism of action, has now been demonstrated. Typical representatives of one of these classes are the barbiturates, and of the other, the polycyclic aromatic hydrocarbons (PAH) [2]. One member of the first group, namely phenobarbital (PB), induces a monooxygenase which possesses low substrate specificity. Administration of PAH, on the other hand, induces de novo synthesis of an aberrant enzyme (cytochrome P-448), which selectively catalyzes the hydroxylation of PAH in microsomes [2, 4].

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