while the results of both our and their experiments indicate that low-molecular-weight proteins are not degraded, at least during the period chosen for these experiments, but accumulate, for, as the present experiments showed, the relative radioactivity of neurospecific proteins increased after 6 and 24 h. Had degradation of the proteins taken place, this radioactivity would either have decreased or would have remained unchanged if the rates of synthesis and degradation of the protein had been equal. Since the relative radioactivity of the fraction with R_f=1.0 increased, this could have arisen through an increase of radioactivity as a result of the accumulation of neurospecific low-molecular-weight proteins.

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EFFECT OF RADIOPROTECTORS ON CYCLIC AMP-DEPENDENT PROTEIN PHOSPHORYLATION

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The ability of radioprotectors (serotonin, aminoethylisothiouronium) in radioprotective doses to stimulate cyclic AMP-dependent phosphorylation of mouse liver cytosol and nuclear and spleen cytosol proteins in vivo was demonstrated. In experiments in vitro, the radioprotectors had no direct action on protein kinase activity or its stimulation by cyclic AMP. It is postulated on the basis of these results and those of previous investigations that activation of cyclic AMP-dependent phosphorylation is due to an increase in the intracellular cyclic AMP concentration under the influence of the radioprotectors.

KEY WORDS: Radioprotectors; cyclic AMP system; protein kinases; phosphorylation of proteins.

The authors showed previously that radioprotectors directly or indirectly activate adenylate cyclase and increase the intracellular concentration of cyclic AMP in the tissues of the body [2, 4, 5]. Elevation of the cyclic AMP level is known to cause activation of cyclic AMP-stimulated protein kinases which, by phosphorylating various protein substrates, modify the course of a wide spectrum of biochemical processes [11]. The action of radioprotectors on cyclic AMP-dependent protein phosphorylation could lead to substantial changes in cell metabolism and could cause biochemical structural changes that could lead to increased radioresistance under conditions of chemical protection [1, 3, 7].

In the investigation described below the effect of radioprotective agents – serotonin and aminoethylisothiouronium (AEP) – on cyclic AMP-dependent protein phosphorylation and on protein kinase activity was investigated in animal tissues.

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TABLE 1. Incorporation of ^{32}P into Protein Fractions of Mouse Liver and Spleen under Normal Conditions (M ± m)

Tissue	Subcellular fraction	Zone of phosphorylation					
		1	2	3	4	5	6
Spleen	Cytosol	3,5—4,3 1138±68	4,4—5,2 635±69	5,4—6,2 605±60	6,4—7,2 491±44	7,3—7,9 340±37	8,2—8,8 186±16
Liver	Cytosol	3,6—4,4 8815±705	4,5—5,3 4110±246	5,4—6,2 2905±232	6,4—7,0 1758±105	7,2—7,8 1232±111	8,2—8,6 588±29
	Nucleus	3,1—3,9 2494±132	4,0—4,6 818±49	4,9—5,5 476±24	5,9—6,7 849±42	7,0—7,8 590±35	8,2—8,8 767±46

Legend. Numerator shows pH range corresponding to zone of phosphorylation; denominator, radioactivity of ^{32}P in that zone (in cpm).

TABLE 2. Effect of DB-Cyclic AMP and Radioprotectors on Protein Phosphorylation in Mouse Tissues, in % of Normal (M ± m)

Zone	Substance	Spleen (cytosol)	Liver	
			cytosol	nucleus
1	DB-cyclic AMP	122±8	160±8	165±15
	Serotonin	127±7	175±15	203±20
	AET	165±5	190±12	143±8
2	DB-cyclic AMP	135±10	136±10	171±15
	Serotonin	137±7	187±16	476±20
	AET	150±10	169±10	295±20
3	DB-cyclic AMP	97±5	131±10	323±20
	Serotonin	99±6	191±10	245±5
	AET	128±10	128±8	332±20
4	DB-cyclic AMP	108±6	148±7	276±10
	Serotonin	105±2	200±7	188±6
	AET	98±4	146±10	140±5
5	DB-cyclic AMP	104±5	100±3	152±5
	Serotonin	203±10	144±5	172±10
	AET	160±15	183±8	270±10
6	DB-cyclic AMP	195±10	155±5	104±6
	Serotonin	240±15	231±10	103±6
	AET	208±3	197±12	103±6

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-200 g and male SHK mice weighing 18-20 g. Radioactive inorganic phosphate ($NaH_2^{32}PO_4$) was injected intraperitoneally into the animals 1.5 h before the beginning of the experiment in a dose of 2 mCi/100 g. Injections of aqueous solutions of serotonin creatinine sulfate (from Reanal, Hungary) in a dose of 60 mg/kg, AET-hydrobromide (synthesized in the Department of Radiochemistry, Moscow University) in a dose of 250 mg/kg, and of N^6O^2-dibutyryl-cyclic AMP (DB-cyclic AMP), in a dose of 140 mg/kg, were given 15 min before sacrifice of the animals.

Protein kinase activity in the rat tissues were studied in preparations of both fractions of soluble cytosol protein kinases [8, 12]. The total fraction of calf thymus histones [10] was used as the phosphorylation substrate. Protein kinase activity was expressed in picomoles phosphate per milligram protein of the protein kinase preparation transferred per minute to histone. To study cyclic AMP-dependent phosphorylation in the tissues, cytosol proteins and neutral nonhistone nuclear proteins were isolated [15]. The proteins were separated by isoelectric focusing (IEF) in a thin layer of Superfine Sephadex G-75 (from Pharmacia, Sweden) [13]. After the completion of IEF the gel was cut into 0.5-cm strips and the radioactivity of ^{32}P incorporated into protein was determined in them [9].

EXPERIMENTAL RESULTS

Phosphorylation of soluble liver cytosol and mouse spleen proteins was studied. Six zones of proteins within the pH range 3.5-8.8 were isolated by the IEF method (Table 1). In the experiments with DB-cyclic AMP (Table 2) zones of proteins with cyclic AMP-dependent phosphorylation were identified (zones 1, 2 and 6 for spleen and 1, 2, 3, 4 and 6 for liver). Injection of radioprotectors into the animals in radioprotective doses stimulated the phosphorylation of these cytosol protein fractions (Table 2). Cyclic AMP-dependent protein kinases in the cytosol are connected with the functioning of many vitally important processes: glycogenolysis,

TABLE 3. Rat Liver Protein Kinase Activity in Presence of Radioprotectors (M ± m)

Radioprotector, concentration	Protein kinase activity, pmoles P/mg protein/min	
	without cyclic AMP	with cyclic AMP ($5 \cdot 10^{-6}$ M)
Control	110±8	169±10
AET, $5 \cdot 10^{-3}$ M	105±9	167±11
Serotonin, $5 \cdot 10^{-4}$ M	109±5	172±8

lipolysis, etc. [11]. Free catalytic subunits of protein kinases, activated by cyclic AMP, in the cytosol can penetrate freely into the cell nucleus [14], where they can also phosphorylate proteins. More than 90% of the cell phosphoproteins are concentrated in the nucleus, and most of them consist of neutral nonhistone proteins of chromatin, which control unique nuclear processes [6, 15]. It was this group of proteins that was next investigated.

An increase in the incorporation of ^{32}P into nonhistone proteins of liver nuclear chromatin 15 min after injection of both DB-cyclic AMP and of radioprotectors into the animals was found (Table 2, zones 1-6). The effect of the radioprotectors on cyclic AMP-dependent phosphorylation of nonhistone chromatin proteins was more marked than their effect on phosphorylation of cytosol protein fractions (Table 2). This observation is interesting in connection with the widely known fact that radioprotectors have important actions on biochemical processes in the nuclei [7]. It can be tentatively suggested that this effect of the radioprotectors is mediated through the cyclic AMP system.

To study the character of the action of radioprotectors on enzymes of the cyclic AMP system it was necessary to study the possibility that the radioprotector could act directly on the protein kinase activity. The reason for this approach is that radioprotectors can penetrate inside the cell.

The experiments showed that serotonin and AET in radioprotective concentrations, on incubation with rat liver protein kinase preparations, did not change the original (without cyclic AMP) activity of the enzyme (Table 3). The radioprotectors had no effects likewise on stimulation of the enzyme by cyclic AMP. A similar picture also was observed in the experiments with splenic protein kinases. The presence of the radioprotector was reflected in neither the original nor the cyclic AMP-stimulated protein kinase activity. The effect of the radioprotector on protein kinase activity could therefore have taken place only through cyclic AMP, the intracellular concentration of which rises in response to stimulation of adenylate cyclase by the radioprotector, as the writers showed previously [4, 5]. Increased protein kinase activity leads to intensification of the processes of cyclic AMP-dependent protein phosphorylation, which may result in essential reorganization of the cell metabolism under conditions of chemical radioprophylaxis.

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