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ACTION OF ISONIAZID AND NIALAMIDE ON BRAIN CORTEX RIBOSOMES

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Abstract—The action of isoniazid and nialamide at the ribosomal level of goat brain cortex tissue has been studied. The drugs do not affect the chemical and nucleotide compositions or the ultraviolet absorption characteristics of the ribosomal particles. During the action of the drugs on brain cortex slices the ribosomal particles become more susceptible to breakdown, releasing protein, ribonucleic acid and acid soluble nucleotides in the media, resulting in the loss of ribosomal enzyme activity.

IN RECENT years considerable interest has developed in hydrazine derivatives which are widely used in the treatment of depression. Although most of these drugs are known to act as general inhibitors of monoamine oxidase system in brain, little is known about their action at the sub-cellular levels of brain tissue. It has been known that ribonucleoprotein particles associated with the Nissl granules in nerve cells undergo rapid chromatolytic changes during increased functional activities in brain.[1–6] Previous work from this laboratory has demonstrated that different convulsant drugs bring about significant changes in the ribonucleoprotein constituents of nerve tissue, studied histologically and biochemically.[7] It has been further observed that when brain cortex slices are incubated in presence of different convulsant and anti-depressant drugs both acid soluble nucleotides and inorganic phosphate are released to a greater extent indicating the degradation of nucleic acid constituents of brain cortex tissue.[8]

The anti-depressant drugs are well-known central nervous system stimulants and it is expected that during the action of anti-depressant drugs similar changes in the ribonucleoprotein constituents of brain tissue may occur. It is, therefore, of interest to study the action of anti-depressant drugs on the ribonucleoprotein constituents of brain cortex tissue under similar conditions. In the present study the action of two anti-depressant drugs, isoniazid (isonicotinyl (acid) hydrazide) and nialamide (1-(2-(benzylcarbamyl)ethyl)-2-isonicotinylhydrazine) on the ribosomal particles of goat brain cortex tissue has been studied. The brain cortex slices were incubated in presence of these two drugs and the changes observed in the ribosomal particles are presented in the present communication which suggest that stability of the ribosomal particles is affected as a result of drug-treatment of brain cortex slices.

METHODS AND MATERIALS

Drug-treatment of brain cortex slices

The cortex portions of brain tissue obtained from freshly slaughtered goats were removed and cut into thin slices, 0·5 mm thick, in the cold. Approximately 10 g wet

weight of cortex slices were suspended in 100 ml Krebs–Ringer buffer with 0·05 M glucose (pH 7·0) which was previously saturated with a gas mixture of 95% oxygen and 5% carbon dioxide. Drugs (1 mg/g wet tissue) were added in the flasks containing the suspensions of slices. They were incubated on shaking for 2 hr at 37°. The respiring conditions of the slices were checked by separate manometric experiments carried out at the same time under similar conditions. After incubation the slices were collected by centrifugation and washed twice with 100 ml portions of Krebs–Ringer buffer (pH 7·0).

Unless otherwise stated, 'drug treated' cortex slice indicates glucose plus drug treated slice, whereas untreated (normal) slice means slice treated with glucose only.

Preparation of ribosomes

Ribosomes were prepared from the cortex slices, after homogenization, by the method of Datta and Ghosh.[9]

Extraction of ribonucleic acid (RNA)

RNA was extracted from ribosomes by the method of Kirby[10] as described by Littauer and Eisenberg.[11]

Chemical estimations

Nitrogen—The modified micro-Kjeldahl method of Ma and Zuazaga[12] was used. *Phosphorus*—The organic phosphorus compounds were digested according to the method of King[13] and the inorganic phosphorus was estimated by the method described by Lowry *et al.*[14] *Protein*—The estimation of protein was carried out according to the biuret methodof Gornall *et al.*[15] Ribonucleic acid (RNA) was estimated by the orcinol method of Mejbaum.[16] *Nucleotides*—The extracted RNA was hydrolysed by 0·3 M KOH solution at 37° for 18 hr. The nucleotides were separated by paper chromatography using the method of Magasanik *et al.*[17] The paper strips containing the nucleotides were scanned with an ultraviolet lamp to locate and identify the nucleotides spots. Each spot was cut and eluted over-night in M/15 phosphate buffer pH 7·0. The ultraviolet absorption spectrum of each nucleotide spot was obtained in the range of 245–290 mμ and the amounts of the respective nucleotides were calculated using the factors of Elson *et al.*[18] *Acid soluble nucleotides*—The concentration of acid soluble nucleotides was determined spectrophotometrically.

Enzymic estimations

Ribonuclease—The ribonuclease activity was measured by the principle of McDonall.[19] *Phosphomonoesterase*—The alkaline phosphomonoesterase activity was assayed by the method of Bessey *et al.*[20] *Phosphodiesterase*—The alkaline phosphodiesterase activity was measured by the method of Frisch-Niggemeyer and Reddi.[21]

Drugs

Isoniazid was a Squibb product and nialamide (niamid) was obtained from Dumex Private Ltd., Bombay, through Dr. B. B. Mazumdar, Drug Section, Govt. of West Bengal.

RESULTS

The chemical composition of ribosomes of drug-treated brain cortex slices

The present study was undertaken to find whether the drug-treatment of brain cortex slices caused any change in the chemical composition of the ribosomal particles.

Table 1 presents the chemical composition of the ribosomal particles isolated from the drug-treated brain cortex slices.

Results (Table 1) show that the drug-treatment of brain cortex slices did not cause significant changes in the chemical composition of the ribosomes. Figure 1 also shows that the ultraviolet absorption spectra of ribosomes of drug-treated slices under similar conditions exhibited no significant difference when compared to the absorption spectrum of ribosomes isolated from brain cortex slices, treated with glucose only.

TABLE 1. CHEMICAL COMPOSITION OF RIBOSOMES OF DRUG-TREATED BRAIN CORTEX SLICES

Ribosomes isolated from brain cortex slices previously treated with*	Nitrogen	Phosphorus	Protein	RNA
	mg/100 mg ribosomes†			
Glucose	14·7 ± 0·9	3·4 ± 0·2	70 ± 1·2	30 ± 1·0
Glucose + Isoniazid	13·8 ± 1·0	3·5 ± 0·2	69 ± 1·3	31 ± 0·9
Glucose + Nialamide	15·2 ± 1·0	3·0 ± 0·2	72 ± 1·3	28 ± 0·9

* Mean of seven determinations ± S.D.
† Calculated on the basis of weight per cent of RNA plus protein.

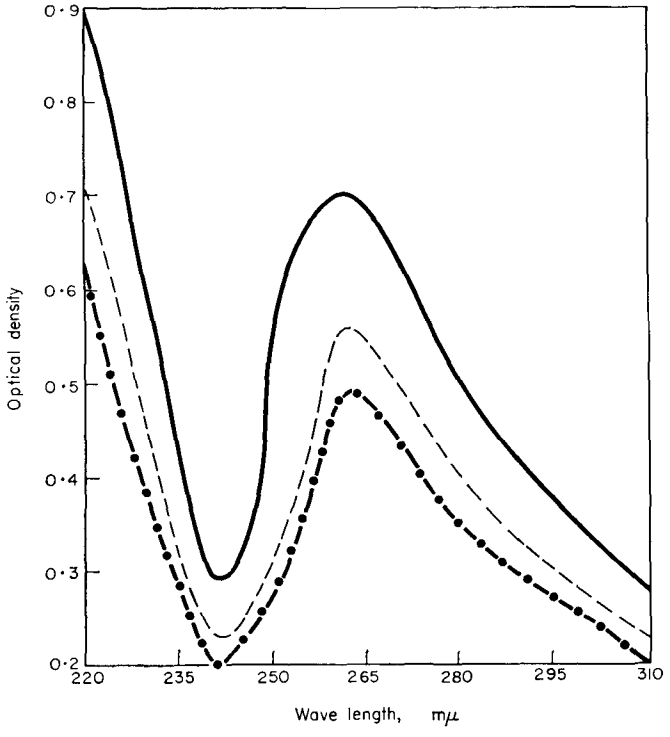

FIG. 1. Ultraviolet absorption spectra of ribosomes of drug-treated brain cortex slices. (The dilute solutions of ribosomes in 0·028 M acetate veronal buffer, pH 7·0 were used.)
———— Ribosomes of glucose-treated brain cortex slices,
– – – – – – – – Ribosomes of isoniazid-treated brain cortex slices,
–·–·–·–·– Ribosomes of nialamide-treated brain cortex slices.

The nucleotide composition of ribosomes of drug-treated brain cortex slices

Table 2 presents the nucleotide composition of the ribosomes of the drug-treated brain cortex slices.

Results (Table 2) show that the drug-treatment of cortex slices did not cause significant changes in the nucleotide composition of the ribosomes except that in the case of ribosomes of nialamide-treated slices the percentage of uridylic acid was relatively high.

TABLE 2. NUCLEOTIDE COMPOSITION OF RIBOSOMES OF DRUG-TREATED BRAIN CORTEX SLICES

Ribosomes isolated from brain cortex slices previously treated with	Adenylic acid	Cytidylic acid	Guanylic acid	Uridylic acid
	moles/100 moles of nucleotides*			
Glucose	22·6 ± 0·9	30·4 ± 1·1	32·6 ± 1·2	14·4 ± 0·6
Glucose + Isoniazid	21·4 ± 0·8	30·5 ± 1·2	32·8 ± 1·3	15·3 ± 0·6
Glucose + Nialamide	21·5 ± 0·9	28·6 ± 1·1	31·6 ± 1·3	18·3 ± 0·8

* Mean of seven determinations ± S.D.

The enzymic activities of ribosomes of drug-treated brain cortex slices

Datta and Ghosh[9,9a] reported that goat brain cortex ribosomes possess ribonuclease, alkaline phosphomonoesterase and alkaline phosphodiesterase activities to significant extents. The present study was undertaken to find whether the treatment of brain cortex slices with anti-depressant drugs has effect on the enzymic properties at the ribosomal level. The study was followed by isolating the ribosomes from the drug-treated slices and by determining the specific activities of acid ribonuclease, alkaline phosphomonoesterase and alkaline phosphodiesterase of the isolated ribosomes. Table 3 presents the per cent specific activities of the ribosomal enzymes.

Results (Table 3) show that the specific activities of ribonuclease, phosphomonoesterase and phosphodiesterase were generally reduced in the ribosomes of drug-

TABLE 3. SPECIFIC ACTIVITIES OF ENZYMES OF RIBOSOMES OF DRUG-TREATED BRAIN CORTEX SLICES

Ribosomes isolated from brain cortex slices previously treated with	% Specific activities		
	Ribonuclease at pH 5·4	Phosphomonoesterase at pH 8·1	Phosphodiesterase at pH 8·9
Glucose	100	100	100
Glucose + Isoniazid	68	36	64
Glucose + Nialamide	67	69	62

treated slices. The reduction of alkaline phosphomonoesterase activity of ribosome-of isoniazid-treated slices was noteworthy. The reduced specific activity of the ribosomal enzymes of drug-treated slices suggests that the drug-treatment of brain cortex slices left some permanent inhibitory effect on the enzymes of the ribosomes.

Effect of anti-depressant drugs on the enzymic activity of ribosomes of fresh brain cortex tissue

In the previous section it was observed that when brain cortex slices were pretreated with anti-depressant drugs, the ribosomes suffered significant loss in the specific activities of the enzymes present therein. The possibility of this inactivation of ribosomal enzymes due to the direct action of these drugs was further studied by measuring enzyme activities after incubation of fresh ribosomes in presence of anti-depressant drugs. The per cent activities of ribonuclease, phosphomonoesterase and phosphodiesterase in presence of isoniazid and nialamide (0·1 mg drug per mg ribosomal protein) are presented in Table 4.

It is evident from Table 4 that when the ribosomes of fresh brain cortex tissue were treated *in vitro* with the drugs, little inhibition in enzymic activities was observed,

TABLE 4. EFFECTS OF DRUGS ON ENZYMIC ACTIVITIES OF RIBOSOMES OF FRESH BRAIN CORTEX TISSUE

Ribosomes were isolated from fresh brain cortex tissue and the enzymic activities were estimated in presence of drugs (0·1 mg/mg ribosomal protein) at their respective optimum pH.

Conditions	% Activities		
	Ribonuclease at pH 5·4	Phosphomono-esterase at pH 8·1	Phosphodi-esterase at pH 8·9
Glucose*	100	100	100
Glucose + Isoniazid	97	94	96
Glucose + Nialamide	77	82	93

* The concentration of glucose was 0·05 M in all cases.

with nialamide however the inhibition was greater. When the per cent loss of specific activities of enzymes of the ribosomes of drug-treated slices (Table 3) is compared with those of ribosomes directly treated with drugs (Table 4), it appears that the lower specific activities of enzymes in the former case may not be entirely due to the direct effect of the drugs or ribosomes. Apparently these drugs have acted on ribosomal particles in an indirect manner i.e. by changing the natural environmental conditions like pH, ionic concentrations etc. surrounding the ribosomes. It is well-known that ribosomal particles are susceptible to degradation in presence of high sodium chloride or potassium chloride concentration[22] or in the absence of magnesium ions.[23, 24] It is possible that during the action of these drugs, instability is induced in ribosomes due to the changes in some of these factors. If such a view is correct, further investigation was thought desirable to examine the degree of instability induced in the ribosomal particles isolated from drug-treated brain cortex slices.

Studies on the stability of ribosomes of drug-treated brain cortex slices

The decreased stability of macromolecules is usually studied by measuring the release of components therefrom. Kihara *et al.*[25] studied the stability of 80S particles of yeast cells on the basis of the release of various components in different media. An approach was made in the present investigation to determine the state of stability of the ribosomes as induced by drug-treatment, by following the rate of degradation in some suitable suspension media. The choice of the different suspension media was

made on the basis that the stability of ribosomal particles are affected by high salt concentration,[22] urea,[26] phosphate,[25] EDTA[27–29] etc. In the present study the relative stability of the ribosomal particles from drug-treated slices was measured in terms of the release of protein, RNA and acid soluble nucleotides in the suspension media containing these different constituents. Gillchriest and Bock[30] observed that ribonucleoprotein particles remained more stable in 0·005 M magnesium–cacodylate buffer, pH 7·0. In the present study magnesium–cacodylate buffer of the same molarity and pH was used as a suitable control medium of suspension of ribosomes. Table 5 summarizes the results of the release of protein, RNA and acid soluble nucleotide components from the ribosomes of drug-treated slices into different media.

Results (Table 5) show that ribosomes isolated from drug-treated brain cortex slices became more susceptible to breakdown as revealed by the increased release of protein, RNA and acid soluble nucleotides in different media. A general idea about the degree of stability of the different ribosomal particles, may be formed from the results obtained after suspending in magnesium–cacodylate buffer, or in the presence of EDTA and urea. It is observed that in these suspension media greater release of proteins, total RNA and acid soluble nucleotides take place from the ribosomes of drug-treated tissue. In presence of phosphate there is a greater tendency for the release of protein component from the ribosomes of drug-treated tissue in comparison to those of the control. In presence of sodium chloride and magnesium chloride release of different components from ribosomes, obtained either from normal or drug-treated tissues, is not much different, except in the case of the release of RNA in magnesium chloride media from ribosomes of nialamide-treated cortex tissue. Whether nialamide has any specific effect on the release of ribosomal RNA in presence of Mg^{2+} is a matter for further investigation. These results further indicate that in intact tissue both isoniazid and nialamide act either directly or indirectly by affecting the different factors needed for the stability of ribosomal structure. The induction of instability in the ribosomal particles with consequent loss of enzymic activities may probably be a mechanism of action of these drugs at the subcellular level.

DISCUSSION

Earlier work from this laboratory[8] has shown that during the incubation of brain cortex slices with different convulsant and antidepressant drugs under *in vitro* conditions, degradation of nucleic acid constituents of brain cortex slices took place as revealed by the release of acid soluble nucleotides and inorganic phosphate in the medium. In view of the above observation it was of interest to study the effect of antidepressant drugs on the RNA-rich ribosome fraction of brain cortex slices, since this fraction is known to be affected during stress or under the influence of neurotropic drugs.[1–6]

Results of the present work indicate that in the drug-treated brain cortex slices the chemical and nucleotide compositions and the ultraviolet absorption characteristics of the ribosomes are not much affected (Tables 1, 2; Fig. 1) whereas the specific activities of three ribosomal enzymes viz., ribonuclease, alkaline phosphomonoesterase and alkaline phosphodiesterase are much reduced (Table 3). Results of the stability study of the ribosomes measured in terms of the release of proteins RNA and acid soluble nucleotides in different media, indicate that the ribosomal particles of drug-treated slices are more susceptible to breakdown than the ribosomal particles of

TABLE 5. STABILITY OF RIBOSOMES OF DRUG-TREATED BRAIN CORTEX SLICES

Ribosomes were isolated from drug-treated slices. 25 mg portions of ribosomes containing 17·5 mg protein and 7·5 mg RNA were suspended in 5 ml 0·005 M magnesium–cacodylate buffer, pH 7·0 in which the agents indicated in the table were dissolved with adjustment of pH were necessary. The suspensions of ribosomes were incubated at 37° for 3 hr and then centrigufed at 105,000 × *g* for 1 hr. The supernatants were carefully decanted and collected. The protein and nucleic acid contents of the supernatants were estimated spectrophotometrically by using the principle of Warburg and Christian.[31] The concentration of acid soluble nucleotides was determined on the basis of optical density value of 33 at 260 mμ of a solution containing 1 mg equivalent of nucleotide mixture obtained from complete hydrolysis of 1 mg yeast RNA per ml of 0·005 M magnesium–cacodylate buffer, pH 7·0.

Ribosomes isolated from brain cortex slices previously treated with	Components released mg/5 ml	Amounts of components released in suspension media of*					
		Controls (in 0·005 *M* Mg-cacodylate buffer, pH 7·0)	NaCl 0·5 M	MgCl 0·5 M	Phosphate 0·5 M	Urea 4 M	EDTA 0·1 M
Glucose	Protein	0·37 ± 0·03	2·31 ± 0·01	1·33 ± 0·14	1·63 ± 0·12	2·97 ± 0·16	0·74 ± 0·09
	Total RNA	0·11 ± 0·01	0·53 ± 0·04	0·42 ± 0·04	0·42 ± 0·03	0·56 ± 0·04	0·50 ± 0·04
	Acid soluble nucleotides	0·02 ± 0	0·23 ± 0·02	0·23 ± 0·03	0·09 ± 0·01	0·20 ± 0·02	0·24 ± 0·03
Glucose + Isoniazid	Protein	0·87 ± 0·11	2·17 ± 0·16	1·14 ± 0·10	2·45 ± 0·17	3·03 ± 0·21	1·19 ± 0·12
	Total RNA	0·35 ± 0·03	0·45 ± 0·03	0·31 ± 0·02	0·34 ± 0·04	1·13 ± 0·07	0·75 ± 0·07
	Acid soluble nucleotides	0·07 ± 0	0·22 ± 0·02	0·14 ± 0·03	0·23 ± 0·02	0·26 ± 0·03	0·38 ± 0·03
Glucose + Nialamide	Protein	1·01 ± 0·09	2·33 ± 0·17	1·56 ± 0·17	2·61 ± 0·23	3·66 ± 0·20	1·44 ± 0·18
	Total RNA	0·37 ± 0·03	0·48 ± 0·06	1·13 ± 0·09	0·40 ± 0·04	1·44 ± 0·03	0·80 ± 0·07
	Acid soluble nucleotides	0·12 ± 0·01	0·32 ± 0·04	0·24 ± 0·03	0·23 ± 0·03	0·26 ± 0·02	0·46 ± 0·04

* Mean of five different experiments ±S.D.

brain cortex slices treated without any drug (Table 5). The induced instability of the ribosomes due to drug-treatment may account for the loss of specific activities of the ribosomal enzymes, though the possibility that ribosomal enzyme proteins are released into the medium during the drug-action is not ruled out. Further study seems to be necessary in order to determine whether this drug-induced instability of the ribosomal particles is due to the removal of magnesium or other metals which normally stabilises the ribonucleoprotein structure[27] or due to the disruption of hydrogen bonds which are involved in the maintenance of the structures of ribosomes. The latter possibility is further supported by our observation of the loss of hydrogen bonded structure of the extracted RNA in the ribosomal particles from drug-treated brain cortex slices.[32]

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