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SOME OBSERVATIONS ON THE *IN VIVO* AND *IN VITRO* EFFECT OF (−)-EMETINE ON THE SUCCINIC DEHYDROGENASE OF GUINEA PIG HEART*

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Abstract—(−)-Emetine induced significant fall in SDH activity of guinea-pig heart homogenates when administered intraperitoneally to the animals. Under similar experimental conditions lysozyme counteracted this effect when given together with emetine, while by itself the enzyme was unable to affect the level of the SDH activity. Emetine added to mitochondrial suspensions of guinea pig heart homogenates at $6{\cdot}25 \times 10^{-4}$ M concentration caused 80 per cent activation of the SDH activity. Sodium lauryl sulphate had a marked inhibitory effect on the SDH activity of the mitochondrial suspension and together with emetine it counteracted the activation caused by emetine alone. Lysozyme had little effect in such experiments.

THE CYSTICIDAL action of emetine in conjunction with surfactants and enzymes on the cysts of amoebae has been reported earlier.[1–5] The persistence of cysts is presumably the cause of human carrier cases of amoebiasis. There is, so far, no drug which can kill cysts of amoebae without being toxic to the host of the amoebic parasite. Intestinal amoebiasis in rats produced by caecal infection by *Entamoeba histolytica* can be cured by emetine and surfactant, or enzymes fed together in lesser number of doses than would be required to cure the same infection if emetine is given alone.† Before exploring the cysticidal action of the above combinations of emetine in human amoebic infections, Mukherjee *et al.*,[6] on the basis of pharmacological experiments, have reported that the emetine induced cardiac toxicity in dogs could be reduced considerably by surfactants and enzymes. In view of the above findings it was thought of interest to study the action of emetine at an enzymatic level. For this purpose succinic dehydrogenase (SDH) activity estimation of the guinea pig heart was selected, since this well established, key respiratory enzyme is linked to the cytochrome system and could well serve as a "marker" in determining the emetine poisoning of the heart muscle. In this paper are presented the results of *in vivo* and *in vitro* action of emetine on the SDH activity of guinea pig heart in the presence or absence of a surfactant and an enzyme.

MATERIALS AND METHODS

Animals. Healthy guinea pigs (300–600 g), of either sex, maintained on standard animal house diet in the Central Drug Research Institute colony, were taken in batches of four, in which one was kept as a control and the other three were injected

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† Imam, unpublished data.

intraperitoneally with (−)-emetine dihydrochloride (10 mg/kg), lysozyme (10 mg/kg) and (−)-emetine followed by lysozyme in identical concentrations individually. All the solutions were prepared in bidistilled water.

After a lapse of 2 hr the animals were killed, their hearts excised, perfused with cold normal saline to remove traces of blood and blotted, following which a 10% homogenate was made in cold 1·15% KCl with the help of a Potter–Elvehjem homogenizer. The homogenate was spun in a refrigerated centrifuge at 700 *g* for 20 min to remove unbroken cells and debris. In another set of experiments animals were starved to deplete hepatic glycogen levels. This crude cell-free extract was used immediately after its preparation as an enzyme source.

For the preparation of mitochondrial homogenate, the guinea pig was invariably starved over-night, the heart was quickly excised, blotted and homogenized with 9 vol. of cold 0·25 M sucrose. The homogenate was then subjected to the usual procedure of differential centrifugation and the mitochondrial pellet was obtained by centrifuging, in the cold at 12,000 *g* for 15 min, the crude cell-free homogenate. The pellet was washed once again with 0·25 M sucrose then finally suspended in a suitable volume of cold 0·25 M sucrose and homogenized for 1 min in a Potter–Elvehjem homogenizer to bring the mitochondria into a fine opalescent suspension with a final protein concentration of 200–250 μg per 3 ml. This suspension was immediately used as an enzyme source for the *in vitro* studies.

Chemicals. (−)-Emetine dihydrochloride was a gift from Burroughs Wellcome Private India Ltd., lyophilized crystalline lysozyme (15,000 enzyme units/mg) was purchased from Merck, Sharp and Dohme and sodium lauryl sulphate from Sigma Chemical Co., was used.

Assay of succinic dehydrogenase activity. The enzyme activity was assayed spectrophotometrically according to the method of Slater and Bonner,[7] using potassium ferricyanide as the artificial electron acceptor.

The assay system, in a final volume of 1·6 ml contained 50 μmoles of phosphate buffer, pH 7·2, 20 μmoles of neutralized potassium cyanide, 5 μmoles of potassium ferricyanide, 10 μmoles of succinate and 0·5 ml of enzyme suspension. The reaction was run for 10 min at 37°, stopped by the addition of 1·5 ml of 5% TCA, and the precipitated proteins were centrifuged off after 1 hr. The extinction of the supernatant was measured at 400 nm in a Beckman model DU Spectrophotometer using cuvettes of 1 cm light path. Controls were run for enzyme by adding the enzyme after the addition of TCA and for substrate by substituting water for it in the reaction mixture. The enzyme activity is expressed as the number of μmoles of potassium ferricyanide reduced under the experimental conditions. The specific activity denotes the number of enzyme units present per milligram of protein.

The protein content of the enzyme preparation was determined colorimetrically according to Lowry *et al.*[7] using bovine serum albumin as standard.

RESULTS

Without starvation. Calculations to determine the significance of differences between the four treatments were carried out on logarithms of the specific activity of SDH instead of actual values, because this tended to eliminate the differences in variance. Two-way analysis of variance was done to remove the effect of differences between experiments either due to differences in the animals used or due to variations in the

estimation procedures from one experiment to another. For example, if the values are overestimated or underestimated by some factor on a particular day, this would not affect the comparisons between treatments.

From Table 1 it may be observed that a significant fall in SDH activity was produced by administering emetine alone, i.e. 84·4 per cent of the control value, with fiducial limits 74·6–95·5 per cent. Lysozyme treatment was able to counteract the *in vivo* inhibitory effect of the emetine to an appreciable extent, but this effect was not found to be statistically significant. Lysozyme treated animals showed almost the same SDH activity as that of the control.

TABLE 1. SUCCINIC DEHYDROGENASE ACTIVITY IN GUINEA PIG HEART HOMOGENATES (UNSTARVED GUINEA PIG)

No starvation—8 Experiments

Treatment	Mean specific activity of succinic dehydrogenase (enzyme units/mg protein)
I Control	0·76
II Emetine	0·64
III Lysozyme	0·75
IV Emetine + lysozyme	0·68

S.E. log ± 0·0182

Difference between	Significance
I and II	P < 0·01 highly significant
I and IV	Not significant P < 0·05
II and IV	Not significant P < 0·05
I and III	Not significant P < 0·05

No starvation—log values

	Sum of squares	d.f.*	Mean square	Significance
Between treatment	0·029744	3	0·00991	P < 0·05
Between experiments	7·905935	7	1·13 P < 0·01	Highly significant
Residual	0·005733	21	0·00265	
Total	7·991412	31		

* Degrees of freedom.
The enzyme was assayed as described in Materials and Methods.

With starvation. From Table 2 it is evident that the fall in SDH activity due to emetine treatment was highly significant, i.e. 76·6 per cent of the control value with fiducial limits 50·2–87 per cent. Emetine plus lysozyme treatment significantly enhanced this level (significant at 2 per cent level), the elevated value being 92·8 per cent of the control value with fiducial limits 71·1–121·3 per cent.

Lysozyme, when administered alone had a slight inhibitory, though statistically insignificant effect.

It was noticed that with starvation the relative variations in SDH activities of nine sets of experiments was negligible whereas in eight sets of experiments without starvation the relative levels of SDH activities was highly statistically significant.

TABLE 2. SUCCINIC DEHYDROGENASE ACTIVITY IN (STARVED) GUINEA PIG HEART HOMOGENATES

Starvation (overnight)—9 Experiments

Treatment	Mean specific activity of succinic dehydrogenase (Enzyme units/mg protein)
I Control	1·22
II Emetine	0·83
III Lysozyme	1·04
IV Emetine + lysozyme	1·13

S.E. log ± 0·0397

Difference between	Significance
I and II	Significant $P < 0.01$
II and IV	Significant $P < 0.05$
I and IV	Not significant
I and III	Not significant

	Sum of squares	d.f.*	Mean square	Significance
Between treatment	0·156394	3	0·0521	$P < 0.05$
Between experiment	0·052531	8	0·0066	Not significant
Residual	0·339738	24	0·0142	
Total	0·548663	35		

* Degrees of freedom.
The enzyme was assayed as described in Materials and Methods.

Mitochondrial homogenates. Experiments *in vitro* with emetine at $6{\cdot}25 \times 10^{-4}$ M concentration stimulated the SDH activity by 80 per cent. At a lower concentration of emetine this activity was less and it levelled off at $2{\cdot}06 \times 10^{-4}$ M (Table 3).

Lysozyme alone did not affect the SDH activity, when added direct to the reaction mixture (Table 3). If at all, it had a slight inhibitory effect when present alone or in combination with emetine. Sodium lauryl sulphate (SLS) had a marked inhibitory effect on the SDH activity at a higher concentration and the degree of inhibition diminished by lowering the concentration. At $2{\cdot}08 \times 10^{-5}$ M, SLS had almost no effect on this enzyme. As expected, the addition of this surfactant to emetine at different concentration levels counteracted the stimulating effect of emetine on the SDH activity.

DISCUSSION

The SDH activity of heart homogenates of guinea pig pretreated with emetine showed a significant fall as compared to the control, whether the animals were starved or not and this inhibitory effect was counteracted substantially if lysozyme was also administered along with emetine. This finding compares favourably with the results obtained with dogs *in situ*, where emetine induced inhibition of cardiac activity was counteracted by giving lysozyme and emetine successively by the intravenous route.[6] The present study perhaps leads us to believe that there is some sort of correlation between the pharmacological findings and the results obtained at the enzyme level.

TABLE 3. SUCCINIC DEHYDROGENASE ACTIVITY OF MITOCHONDRIAL HOMOGENATES OF GUINEA PIG HEARTS

Treatment	Molarity	Specific activity (Enzyme units/mg protein)	Inhibition activation (%)*
Control		2·506	
Emetine HCl	$6{\cdot}25 \times 10^{-4}$ M	4·639	+125
	$2{\cdot}06 \times 10^{-4}$ M	3·214	+28
	$1{\cdot}03 \times 10^{-4}$ M	2·964	+18
	$6{\cdot}25 \times 10^{-5}$ M	3·234	+25
	$0{\cdot}69 \times 10^{-5}$ M	3·128	+25
Sodium lauryl sulphate	$2{\cdot}06 \times 10^{-4}$ M	0·382	−75
	$1{\cdot}03 \times 10^{-4}$ M	0·592	−77
	$6{\cdot}25 \times 10^{-5}$ M	1·350	−47
	$4{\cdot}12 \times 10^{-5}$ M	1·644	−35
	$2{\cdot}08 \times 10^{-5}$ M	2·457	−2
	$1{\cdot}00 \times 10^{-5}$ M	2·457	−2
Lysozyme	$6{\cdot}25 \times 10^{-4}$ M	2·282	−5
	$2{\cdot}06 \times 10^{-4}$ M	2·399	−5
	$1{\cdot}03 \times 10^{-4}$ M	2·253	−11
	$6{\cdot}25 \times 10^{-5}$ M	2·117	−14
	$2{\cdot}08 \times 10^{-5}$ M	2·546	0
	$1{\cdot}00 \times 10^{-5}$ M	2·500	0
Emetine + S.L.S.	$6{\cdot}25 \times 10^{-4}$ M + $2{\cdot}06 \times 10^{-4}$ M	1·542	−39
	$2{\cdot}06 \times 10^{-4}$ M + $2{\cdot}06 \times 10^{-4}$ M	1·271	−50
	$6{\cdot}25 \times 10^{-5}$ M + $6{\cdot}25 \times 10^{-5}$ M	1·059	−58
Emetine + lysozyme	$6{\cdot}25 \times 10^{-4}$ M + $2{\cdot}06 \times 10^{-4}$ M	4·871	+94
	$2{\cdot}06 \times 10^{-4}$ M + $2{\cdot}06 \times 10^{-4}$ M	2·922	−12
	$6{\cdot}25 \times 10^{-5}$ M + $6{\cdot}25 \times 10^{-5}$ M	2·758	+10

* (−) Inhibition, (+) activation. For studying the effect of activators and inhibitors on the mitochondrial SDH, to 0·3 ml of enzyme suspension was added 0·2 ml of suitable concentration of the compound(s) to be tested (0·2 ml of water in the case of controls), the tube was gently shaken and incubated for 15 min at 37°. The assay for SDH activity was run, after adding the other components of the reaction mixture, for 10 min, as described in Materials and Methods. Figures of specific activity cited in Table 3 are net average values of three experiments carried out in duplicate each time.

Emetine acted in different ways on the SDH activity of heart of guinea pigs, depending upon whether emetine was administered *in vivo* or added directly *in vitro*. In this context it may be mentioned that emetine does not effect the succinic dehydrogenase activity of the cell free extracts of *Entamoeba histolytica*,[7] whereas some workers[10,11] have shown that this drug stimulates the oxygen uptake of myocardial homogenates of guinea pig, rabbit and rat in the presence of succinate. Without investigating further, it is difficult to speculate about the mechanism of emetine action *in vitro*. It may, however, be presumed that the lowering of the SDH activity after the direct addition

of SLS *in vitro* is due to the disruption of the integrity of the SDH system by the detergent.

The SDH activity in the mitochondria would depend to a great extent on the colloidal structure and integrity of components of this system, which can ensure mutual accessibility.[12] It seems that emetine, which activates the SDH activity in mitochondrial preparation, does not affect greatly the mutual accessibility of the components during *in vitro* experiments. The attachment of emetine to the bioreceptor at the outer membrane surface seems to be mediated by weak chemical forces as envisaged by Dhar[13] and Beller.[14] Whether the inhibition brought about by emetine *in vivo* is due to some secondary effect or due to lack of transport to the site of action through the membrane is not clear.

The counteraction of emetine induced inhibition of SDH activity *in vivo* experiments by lysozyme, which by itself produces little effect on the enzyme activity, occurs by some mechanism of unknown nature.

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