Observations on the Mechanism of Emetine Poisoning of Myocardial Tissue

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ABSTRACT

The effect of emetine hydrochloride on protein synthesis in rat myocardium was studied. Emetine inhibited incorporation of tritiated leucine into soluble proteins and actomyosin in an in vitro system using minced rat myocardium in a supporting medium. Fifty percent inhibition of incorporation of the isotope into soluble proteins and actomyosin occurred at an emetine concentration of $5 \times 10^{-7} \text{M}$. Incorporation was also significantly inhibited when animals were treated with emetine for three days. The data suggest that emetine toxicity in the myocardium may be mediated through its effects on protein biosynthesis.

ADDITIONAL KEY WORDS

cycloheximide  protein synthesis  emetine  actomyosin  cardiac toxicity  rat

The value of emetine as a therapeutic agent has been appreciated since the seventeenth century, but its recent use has been curtailed by increased awareness of its toxic properties. The most serious manifestations of toxicity induced by emetine have been cardiac, and death due to "myocarditis" has occasionally been reported in association with emetine therapy [1]. The reports of toxic effects of this alkaloid in man have been mainly concerned with a description of the electrocardiographic and physiologic alterations induced by its administration. In the study of Klatskin and Friedman [2], 83% of 93 patients receiving emetine for amebiasis displayed some abnormality of cardiovascular function. In the few adequate autopsy studies [1, 3] of patients dying with emetine toxicity, little has been described that would suggest an acute cytotoxic effect of the drug. The histologic descriptions have been those of chronic inflammation, and the authors have not noted significant polymorphonuclear infiltration.

Experimental studies in mammals [4] have determined the relative concentrations of emetine in various organs. Because liver, kidney, spleen, lung, and brain concentrate the drug more than does heart muscle, it has been suggested that the heart is especially susceptible to its toxic action. Pathologic descriptions of the heart in experimental emetine intoxication [5] have furnished little information about the mode of its toxicity. Previous biochemical studies of emetine intoxication [6, 7] have been concerned with intermediary cardiac metabolism and have suggested that toxic effects may be mediated via interference with substrate oxidation or enzyme activity.

Grollman [8] has recently shown that emetine is a potent inhibitor of protein synthesis in mammalian cells, plants, and yeast. The following studies were undertaken to determine whether emetine inhibition of protein synthesis might be demonstrated in the intact rat myocardium and if this could be related to the production of cardiac toxicity observed with the clinical use of this alkaloid.

Methods and Materials

In vitro experiments were carried out with a preparation of uniform particles prepared from the myocardium of Sprague-Dawley rats weighing 100 to 300 g. The hearts were removed from the animals after brief ether anesthesia and were chilled and rapidly minced with a scalpel or microtome blade. The mince was carried out in an ice bath, and the tissue was kept moist at...
0°C with 0.1 M KCl buffered to pH 7.4 with .01 M phosphate. In a typical experiment, the hearts of six animals were pooled in this buffer and thoroughly mixed after mincing. Microscopic examination of tissue preparations prepared by this technique revealed that most of the cardiac cells were intact. Equal portions of the pooled tissue were then added to reaction flasks containing 9.8 ml of Eagle's minimal essential tissue culture medium (9) prepared without leucine and 0.1 ml of an emetine hydrochloride solution, or in the case of control flasks, 0.1 ml of normal saline. After incubation of 15 minutes, 50 μc of 3H-L-leucine (specific activity, 5 c/mm) in 0.1 ml of 0.025 N HCl was added with the same pipette to each flask. Incubation at 39°C was initially carried out for 4 hours, but when incorporation into the controls was shown to be linear for 3 hours a shorter incubation time, usually 2 hours, was selected.

After incubation the tissue was washed in buffer and homogenized in a Virtis homogenizer at medium speed for 1 minute. Proteins soluble in low ionic strength were extracted in 25 ml of 0.1 M KCl, pH 7.4, for 30 minutes at 0°C. This extract was then treated with trichloroacetic acid (TCA) at 0°C at a final concentration of 5%. The acid-insoluble material was washed three times with 5% TCA at 0°C to remove unincorporated radioactivity and finally washed by the method of Siekieritz (10). TCA-precipitable material was then dissolved in 1 M NaOH, and an aliquot was taken for determination of protein content by the method of Lowry (11). Another aliquot was added to 17 ml of a liquid scintillation counting medium made up of 700 mg p-bis [2-(5-phenyl-oxazolyl)] benzene (POPOP), 7 g 2,5-diphenyl-oxazole (PPO), 1400 ml toluene, 1200 ml ethanol and 100 g Cab-O-Sil (12). Scintillation counting was carried out in a Packard Tri-Carb liquid counter. Appropriate corrections for counting efficiency were made using an internal standard. The specific activity of the protein extracted from each incubation flask (counts/min per mg) was calculated as an index of protein synthesis.

A modification of the method of Szent-Gyorgyi (13) was used to extract actomyosin. The tissue remaining after soluble protein extraction was stirred for 18 hours at 0°C in 0.6 M KCl, pH 7.0, after the addition of 1 mg ATP. This method is known to yield a mixture of actin, myosin, and actomyosin. After extraction of these fibrous proteins from the tissue, purification was accomplished by precipitating twice in 0.06 M KCl. This also served to remove unincorporated radioactive material as demonstrated by absence of counts in the supernatant. The isolated actomyosin was...
Specific activity of soluble proteins (SP) and actomyosin (AM) isolated from rat heart incubated in the presence of added saline (control) and $10^{-8}$ M emetine. Note that the incorporation of $^3$H-leucine is linear with increasing incubation time and that inhibition of both fractions by emetine is also linear.

Specific activity of soluble myocardial proteins (top) and actomyosin (bottom) after incubation with $10^{-8}$ to $10^{-9}$ M emetine. Fifty percent inhibition of protein synthesis occurs in each at approximately $5 \times 10^{-7}$ M concentration.
on the concentration of emetine added. Log-probit (14) plots of the data shown in Figure 3 show the 50% inhibitory concentration to be approximately $5 \times 10^{-7}$ M for both soluble proteins and actomyosin.

When animals were given emetine at a dosage of 1.3 mg/kg for 3 days (total dose for a 150-g animal equal to 0.6 mg emetine hydrochloride) and killed on the fourth day, significant inhibition of leucine incorporation into soluble proteins and actomyosin was noted consistently in protein extracts prepared after incubation as described with the above in vitro experiments. Inhibition of leucine incorporation was also noted after 3 days of in vivo treatment of rats with cycloheximide at a dosage of 2 mg/kg. The results of one such experiment are shown in Table 1. Treatment of such animals for 10 days with the same dose of both compounds resulted in the death of the animals that were given emetine and marked diminution of weight gain in those receiving cycloheximide.

**Discussion**

The experiments described above demonstrate that incorporation of isotopically labeled amino acids linear with time into cardiac muscle proteins may be accomplished in an in vitro system utilizing intact myocardial tissue. The proteins extracted from tissue incubated in this system have been isolated on the basis of their solubility in buffers of differing ionic strengths. Although these experiments have not characterized the protein content of the fractions, the extract obtained in 0.1 M KCl undoubtedly consists of a mixture of many proteins, whereas the fraction isolated from 0.6 M KCl is made up mainly of actin, myosin, and actomyosin (10). It is apparent from Figure 1 that the turnover rate of the actomyosin isolated from this system is considerably less than that of the soluble proteins, or at least of a component of that extract. These experiments also demonstrate that emetine, a drug known to be cardiotoxic, is an inhibitor of the incorporation of $^3$H-leucine into both of these isolated protein fractions at very low concentration. In vitro, a sigmoidal relationship is demonstrated between the concentration of inhibitor added and the degree of inhibition of leucine incorporation. The potency of emetine in this inhibition is demonstrated by the finding that 50% inhibition occurs at only $5 \times 10^{-7}$ M concentration and significant inhibition occurs even at lower concentrations.

Siegel and Sisler (15) have reported that cycloheximide inhibits the transfer of activated aminoacyl compounds from sRNA to ribosomes. Grollman (8), has shown that emetine has a similar site and mode of action for inhibition of protein synthesis and his studies of the conformational, configurational and electrostatic properties of the emetine molecule suggest that both emetine and cycloheximide share certain structural properties around two nitrogen atoms that are essential for their activity. It is clear from our in vivo experiments (Table 1) that treatment of rats with sublethal doses of emetine administered intraperitoneally results in significant inhibition of leucine incorporation into both the rapidly incorporating soluble proteins and the slowly incorporating actomyosin.
emetine is known to have cardiac toxicity, and since our in vivo experiments demonstrate that emetine is as potent an inhibitor of leucine incorporation into cardiac proteins as cycloheximide, it is likely that the production of toxicity depends on interference with myocardial protein synthesis.

Myocardial toxicity induced by emetine may be mediated through its effects on protein synthesis in several ways. It may be related to interference with the synthesis of a rapidly turning over protein constituent of the cardiac cell membrane which functions as a carrier in membrane transport. Evidence for the existence of such substances in cell membranes has been presented by Elsas and Rosenberg (16) and by Kennedy and co-workers (17). In addition, emetine might also act by inhibiting cellular oxidation via interference with synthesis of respiratory enzymes. Although interference with substrate oxidation by low concentrations of emetine cannot be demonstrated in vitro, it has been shown by Appelt and Heim (6, 7) that treatment of animals with low doses of emetine causes significant inhibition of myocardial respiration. Their in vivo observations suggest that the inhibition (which could only be achieved at high concentration in vitro) may have been due to diminished activity of the respiratory enzymes involved and that such reduction in enzyme levels may have taken place during the time emetine was being administered. Alternatively, emetine intoxication via inhibition of protein synthesis might occur on a chronic basis by interference with slowly synthesized contractile protein. These experiments suggest that actomyosin synthesis, although slow, is significantly impaired by low concentrations of emetine.

References
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