J. Pharm. Pharmac., 1970, **22**, 659–663 *Received September* 18, 1969

The subcellular distribution of (±)-2,3-dehydroemetine and (−)- emetine in rat liver and changes in hepatic lipid content after treatment of rats with (±)-2,3-dehydroemetine

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Treatment of female rats with (±)-2,3-dehydroemetine leads to increases in the total lipid content of their livers, without affecting phospholipid content appreciably. The subcellular distribution of (±)-2,3-dehydroemetine or (−)-emetine in the livers of rats pretreated with these drugs, shows that each compound is associated with the mitochondria rather than the microsomal fraction or the cell sap.

We have previously examined the action of emetine (Jondorf & Szapary, 1968) and related compounds (Jondorf, Drassner & others, 1969) on protein biosynthesis in female rats of the Sprague-Dawley strain and found them to vary according to whether the experiments were conducted *in vivo* or *in vitro*. Thus, in rats killed 24 h after injection with (−)-emetine (dihydrochloride) or with (±)-2,3-dehydroemetine (dihydrochloride) at 18 μmol/kg, a non-toxic dose in this strain, there is an increase in liver size (25–30%), and increased L-amino-acid incorporation into protein *in vitro* (about 100%) by liver microsomal preparations from pretreated animals (Jondorf & others, 1969). However, short-term (2 h) pretreatment of rats with either drug inhibits the uptake of labelled amino-acid into hepatic protein *in vivo*. We have also examined the inhibition by emetine and 2,3-dehydroemetine of drug metabolism by liver microsomes in rats (Jondorf, Johnson & Donahue, 1969; Jondorf, Johnson & Drassner, 1969).

Emetine (Gimble, Davison & Smith, 1948; Davis, Dodds & Tomich, 1962) and 2,3-dehydroemetine (Schwartz & Rieder, 1961a; Schwartz & Herrero, 1965) do not appear to be metabolized and persist unchanged in various tissues such as the liver.

We have now investigated the subcellular distribution of emetine and 2,3-dehydroemetine in rat liver and have also studied changes in lipid composition of rat liver after pretreatment of 2,3-dehydroemetine.

EXPERIMENTAL

Animal treatment. Female Sprague-Dawley rats, of the same age and each weighing 160 g, had access to Purina rat chow and water in stress-free and insecticide-free standard conditions. Groups of two or more animals were pretreated with freshly made aqueous solutions of the dihydrochlorides of (±)-2,3-dehydroemetine or

* Taken in part from a thesis presented by H. H. Miller to the Department of Pharmacology, The Graduate School of Arts and Sciences, The George Washington University, in accordance with the requirements for the M.S. degree.

(−)-emetine at 18 μmol/kg (equivalent to 8·6 mg/kg free base), or with water, by intraperitoneal injection (injection volume 0·8 ml/160 g rat). To lower glycogen levels, food was withheld for the 24 h (9 a.m.–9 a.m.) between pretreatment and death. In some experiments the time between pre-injection and death was 1 h. Animals were killed by stunning and decapitation in the cold room (0–4°). Livers were immediately excised and treated in one of two ways after rinsing in ice-cold 0·25M sucrose and subsequent blotting.

Procedure for total lipid extraction from liver. Samples of livers were minced with scissors and were subjected to the procedure of Folch, Lees & Sloane-Stanley (1957) for the extraction of total lipids. Aliquots of the extracts (in hexane) so obtained were analysed for total lipid content by the method of Bragdon (1951) using stearic acid as the standard. Total cholesterol estimation of the total lipid extract was according to Zlatkis, Zak & Boyle (1953). Further aliquots of the total lipid extracts were analysed for phospholipid content by the method of Fiske & Subbarow (1925). Results of the phosphate analyses were compared with a disodium hydrogen phosphate standard and were then multiplied by 25, the factor for converting inorganic phosphorus to organic phosphorus (Vahouny, Moede & others, 1963). Neutral fat content could then be calculated by difference from the values obtained for total lipid and the combined values for total cholesterol and phospholipid.

Preparation of subcellular fractions from liver for spectrofluorimetric assay of emetine or 2,3-dehydroemetine. Portions (1·5 g) of freshly excised livers were set aside for the determination of alkaloid in whole liver. These portions were homogenized with five volumes of ice-cold citric acid in disodium hydrogen phosphate buffer pH 3 (Schwartz & Rieder, 1961b) in a motor-driven coaxial Teflon-glass homogenizer with a clearance of 0·84 mm, rotating at 4000 rev/min for ten complete vertical passes. The homogenates were then deep-frozen to await analysis.

The remaining portions of the liver were homogenized with five volumes of ice-cold 0·25M sucrose under the same standardized conditions. The homogenates were subjected to centrifugation at 600 *g* for 10 min to obtain the cell debris pellet. The supernatant portion from this was spun at 4°, at 15 000 *g* for 15 min to obtain the mitochondrial fraction as a pellet. The post-mitochondrial fraction after 60 min at 105 000 *g* (4°) yielded a microsomal pellet and a supernatant fraction. All the pelleted subcellular fractions were carefully surface-rinsed with standard amounts (2 ml, three times) of citric acid in disodium hydrogen phosphate buffer and were then dispersed in this buffer. All samples including the buffer rinses were then analysed for emetine or 2,3-dehydroemetine according to Schwartz & Rieder (1961b). Recoveries of known amounts of alkaloid added to samples were 91·3 (±1·8) %. All values quoted under Results are corrected for recoveries. Protein in the mitochondrial, microsomal and the 105 000 *g* supernatant fractions was measured by the method of Lowry, Rosebrough & others (1951).

RESULTS

Changes in hepatic lipid content. After injection with 2,3-dehydroemetine, there is an increase in total lipid content of livers from pretreated rats (Table 1). There is also a significant increase in total cholesterol (free and esterified) and neutral fat (triglycerides). Whilst other categories of lipids increased in quantity after pre-

treatment with 2,3-dehydroemetine, the phospholipid values remained almost at control levels.

Table 1. *Effect of pretreatment with* 2,3-*dehydroemetine* (18 μmol/kg, 24 *h*) *on the lipid composition of female rat liver*

Treatment	No. of livers	Total lipid	Cholesterol (total)[2]	Phospho-lipid	Neutral fat[3]
Control	4	45·8 ± 1·4[1]	2·1 ± 0·20	17·0 ± 3·9	26·7 ± 4·2
2,3-Dehydroemetine ..	3	71·3 ± 3·8*	5·4 ± 0·54*	19·2 ± 0·7	46·7 ± 3·9*

[1] All values are expressed as mg/g of liver (wet weight) ± standard error of the mean.
[2] Total cholesterol refers to free + esterified cholesterol.
[3] Neutral fat or triglyceride was calculated as the difference between the combined values of total cholesterol and phospholipid, and total lipid.
* Indicates a significant difference ($P < 0{\cdot}05$).

The subcellular distribution of 2,3-*dehydroemetine and emetine in the liver.* 2,3-Dehydroemetine and emetine are present in the liver at 1 h after a single injection of either drug at a dose level of 18 μmol/kg (Table 2). After 24 h, the concentration of emetine in the whole liver is more than twice that found for 2,3-dehydroemetine.

Table 2. *The concentration of either* 2,3-*dehydroemetine or emetine in whole liver and in various subcellular fractions following pretreatment of female rats with the respective compound for one or for* 24 *h*

Time of pre-treatment	Whole liver μg/g	Cell debris μg/g liver	Mitochondria		Micro-somes[1]	105 000 × *g* supernatant[2]
			μg/g liver	μg/mg protein	μg/mg protein	μg/mg protein
			2,3-*Dehydroemetine*			
1 h	63·2 ± 3·7[3] (5)[5]	22·0 ± 0·8[3] (3)	13·7 ± 0·4[3] (3)	1·1 ± 0·1[4] (3)	0·41 ± 0·01[4] (3)	0·27 ± 0·10[4] (6)
24 h	17·0 ± 1·8 (8)	8·0 ± 0·9 (3)	5·9 ± 0·5 (4)	0·40 ± 0·02 (4)	0·06 ± 0·01 (4)	0·11 ± 0·01 (6)
			Emetine			
1 h	79·2 ± 12·4 (3)		26·5 ± 2·6 (5)	1·7 ± 0·2 (5)	0·3 ± 0·05 (3)	0·18 ± 0·03 (3)
24 h	39·9 ± 7·1 (3)		12·6 ± 1·2 (6)	0·8 ± 0·1 (6)	0·06 ± 0·002 (3)	0·08 ± 0·01 (3)

[1] Yield, 9 mg microsomal protein per g liver. (Combined washings from surface rinsing of microsomal pellets had negligible alkaloid content.)
[2] Yield, 50 mg 105 000 × *g* supernatant protein per gram liver.
[3] All values expressed as μg free base of alkaloid ± standard error of the mean per gram of liver.
[4] All values expressed as μg free base ± standard error of the mean per mg protein.
[5] Figures in parentheses refer to number of animals.

The amount of 2,3-dehydroemetine in the cell debris (600 *g* pellet) at 1 h after pretreatment is about one third of that found in the whole liver. At 24 h after pretreatment the concentration is lower, but is half that found in the whole liver at that time. The amounts of emetine localized in the liver mitochondria at 1 h and 24 h after injecting rats (18 μmol/kg) are about double the amounts after corresponding pretreatment with 2,3-dehydroemetine.

2,3-Dehydroemetine and emetine are found only in small quantities in the 105 000 *g* supernatant fraction and in the microsomal fraction (Table 2). The differential centrifugation procedure for obtaining microsomal and 105 000 *g* supernatant fractions is essentially similar to that used for the preparation of these fractions in experiments on protein synthesis *in vitro* (Jondorf & others, 1969). The amounts of emetine or 2,3-dehydroemetine detected in the liver microsomal preparations at 24 h would be insufficient to inhibit protein synthesis *in vitro* (Jondorf & Szapary, 1968; Jondorf, Drassner & others, 1969).

We conclude, therefore, that both 2,3-dehydroemetine and emetine have a preferential affinity for liver mitochondria.

DISCUSSION

At 24 h after pretreatment of rats with 2,3-dehydroemetine, there is an increase in liver size, and an increase in liver microsomal protein synthesizing activity *in vitro* (Jondorf & others, 1969). We now find that livers obtained from 2,3-dehydroemetine-treated rats have a measurably higher total lipid content than the corresponding controls but the amount of phospholipid does not increase significantly (Table 1).

In agreement with Schwartz & Rieder (1961) the concentration of emetine found in the liver after 24 h was more than twice that found for 2,3-dehydroemetine; both are found in relatively high concentrations in the mitochondrial fraction of the liver (Table 2). This latter finding lends support to those observations (Appelt & Heim, 1964, 1965; Chang & others, 1966; Watkins & Guess, 1968) where emetine has been implicated in decreasing oxygen consumption and in inhibiting oxidative phosphorylation in a number of different tissue preparations. That pretreatment of rats with 2,3-dehydroemetine was found not to significantly affect the amount of phospholipid phosphorus in the liver, whereas other categories of lipid did increase, implies that the availability of ATP may be impaired.

It is also possible that pretreatment with 2,3-dehydroemetine (and by analogy, with emetine) might inhibit the utilization of lipid in the liver. The increased hepatic lipid content after 24 h pretreatment with 2,3-dehydroemetine may be due in part to a decrease in production of the necessary carrier proteins (Robinson & Seakins, 1962; Sabesin & Isselbacher, 1965), because of a transient inhibition of protein synthesis *in vivo* (Jondorf & Szapary, 1968; Jondorf & others, 1969), and may also be dependent on faulty utilization of the lipid material transported to the liver. If the latter is the case, the lack of availability of DPNH and TPNH generated from fatty acid oxidations would greatly impair the hepatic drug metabolism dependent on TPNH (Brodie, Maickel & Jondorf, 1958) and help to explain the observed inhibitory effects exerted by 2,3-dehydroemetine (Jondorf, Johnson & Donahue, 1969; Jondorf, Johnson & Drassner, 1969) on drug metabolizing enzyme activity in the liver.

Acknowledgements

This work was supported financially by the U.S. Public Health Service (Grants GM-13749 and 5-T01-GM26), by Burroughs Wellcome (U.S.A.) Ltd. and by Hoffmann-La Roche. We are grateful to Drs. W. E. Scott and H. T. Openshaw for gifts of material and to Dr. G. V. Vahouny for advice in techniques of lipid analysis.

REFERENCES

APPELT, G. D. & HEIM, H. C. (1964). *J. pharm. Sci.*, **53**, 1080–1083.

APPELT, G. D. & HEIM, H. C. (1965). *Ibid.*, **54**, 1621–1625.

BRAGDON, J. H. (1951). *J. biol. Chem.*, **190**, 513–517.

BRODIE, B. B., MAICKEL, R. P. & JONDORF, W. R. (1958). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **17**, 1163–1174.

CHANG, H.-Y., YUAN, C.-T., MO, P.-S. & CHUNG, H.-L. (1966). *Acta biochim. Biophys. sin.*, **6**, 103–109.

DAVIS, B., DODDS, M. G. & TOMICH, E. G. (1962). *J. Pharm. Pharmac.*, **14**, 249–252.

FISKE, C. H. & SUBBAROW, Y. (1925). *J. biol. Chem.*, **66**, 375–400.

FOLCH, J., LEES, M. & SLOANE-STANLEY, D. H. (1957). *Ibid.*, **226**, 497–509.

GIMBLE, A. I., DAVISON, C. & SMITH, P. K. (1948). *J. Pharmac. exp. Ther.*, **94**, 431–438.

JONDORF, W. R., DRASSNER, J. D., JOHNSON, R. K. & MILLER, H. H. (1969). *Arch. Biochem. Biophys.*, **131**, 163–169.

JONDORF, W. R., JOHNSON, R. K. & DONAHUE, J. D. (1969a). *Biochem. J.*, **113**, 10P–11P.

JONDORF, W. R., JOHNSON, R. K. & DRASSNER, J. D. (1969b). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **28**, 418.

JONDORF, W. R. & SZAPARY, D. (1968). *Archs Biochem. Biophys.*, **126**, 892–904.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.

ROBINSON, D. & SEAKINS, A. (1962). *Biochim. Biophys. Acta*, **62**, 163–165.

SABESIN, S. M. & ISSELBACHER, K. J. (1965). *Science, N.Y.*, **147**, 1149–1151.

SCHWARTZ, D. E. & HERRERO, J. (1965). *Am. J. trop. Med.*, **14**, 73–83.

SCHWARTZ, D. E. & RIEDER, J. (1961a). *Bull. Soc. Path. exot.*, **54**, 38–48.

SCHWARTZ, D. E. & RIEDER, J. (1961b). *Clinica. chim. Acta*, **6**, 453–463.

VAHOUNY, G. V., MOEDE, A., SILVER, B. & TREADWELL, C. R. (1963). *J. Nutr.*, **79**, 45–52.

WATKINS, W. D. & GUESS, W. L. (1968). *J. pharm. Sci.*, **57**, 1968–1974.

ZLATKIS, A., ZAK, B. & BOYLE, A. (1953). *J. Lab. clin. Med.*, **41**, 486–492.