Biochemical Pharmacology, Vol. 22, pp. 1524–1526. Pergamon Press, 1973. Printed in Great Britain.

Evidence of multiple and independent mechanisms for the action of hypoglycin *in vivo*

(*Received* 22 *June* 1972; *accepted* 26 *December* 1972)

THE TOXIC amino acid hypoglycin, α-amino-β-methylene-cyclopropanepropionic acid, occurs in the ackee, is a hypoglycaemic agent, and is implicated in Jamaican vomiting sickness.[1,2] It has been shown that the oxidation of isovaleryl CoA arising from leucine metabolism is blocked by a metabolite of hypoglycin;[3,4] the resultant isovalericacidaemia has been proposed as a major factor in the development of extreme symptoms such as prostration and coma seen in man and experimental animals. On the other hand, the hypoglycaemic effect has been attributed to inhibition of long-chain fatty acid oxidation by a hypoglycin metabolite, accompanied by increased utilization of carbohydrate and inhibition of gluconeogenesis.[5–7]

It remained to be established whether inhibition of isovaleryl CoA dehydrogenase is specifically related to the hypoglycaemic effect. We, therefore, investigated the extent to which blood glucose levels of rats decreased after administration of hypoglycin, with or without additional doses of isovaleric acid, and also monitored the excretion into urine of *N*-isovalerylglycine, a characteristic end-product resulting from inhibition of isovaleryl CoA dehydrogenase.[3,4]

Male albino rats weighing 150–200 g were fasted 48 hr. Animals thus depleted of liver glycogen are more susceptible to the toxin.[8,9] Each group of three animals was given by intramuscular injection either 15 or 25 mg of hypoglycin/100 g body wt, and 20 min later either 5 or 15 mg of neutralized isovaleric acid/100 g body wt. Control groups received either hypoglycin alone, isovaleric acid alone, or neither. Water but no food was allowed. Blood samples were taken from the cut ends of the animals' tail once before injections and thereafter at 2, 4 and 5 hr. Glucose was determined in 50 μl of blood by the glucose oxidase method using a Glucostat (Worthington Biochemical Corp.). It was determined in these experiments that rats evinced varying degrees of hyperglycaemia, lasting up to 2 hr, which apparently resulted from the trauma associated with injections and blood-letting. Also any hypoglycaemia which did occur was only evident about 4 hr after injection of hypoglycin.

The findings for changes in blood glucose concentrations at 4 and 5 hr are shown in Table 1, expressed as percentage change from levels at zero time.

TABLE 1. RESPONSE OF BLOOD SUGAR LEVELS TO HYPOGLYCIN AND ISOVALERIC ACID IN VARIOUS COMBINATIONS

Hypoglycin (mg/100 g body wt)	Isovaleric acid (mg/100 g body wt)	Per cent change in blood glucose concn (initial value = 100) At 4 hr	At 5 hr
0	0	+ 12·8 ± 3·2*	+ 7·3 ± 3·5
0	5	+ 9·3 ± 0·7	+ 18·2 ± 1·9
0	15	+ 3·0 ± 1·0	+ 19·9 ± 0·7
15	0	− 13·2 ± 1·6	− 6·2 ± 2·3
15	5	− 12·2 ± 2·0	+ 1·1 ± 1·0
15	15	− 17·6 ± 4·3	+ 12·8 ± 4·3
25	0	− 47·4 ± 7·6	− 45·4 ± 1·3
25	5	− 21·5 ± 6·0	− 23·3 ± 6·4
25	15	− 40·7 ± 1·4	− 31·1 ± 2·3

* Mean ± S.E.M. for each group of three rats.

It is evident that isovaleric acid alone is not hypoglycaemic. Hypoglycin induced more profound hypoglycaemia at 25 than at 15 mg/100 g body wt; with neither dose level did a subsequent dose of isovaleric acid significantly increase the hypoglycaemia or prolong its duration.

To obtain evidence of inhibition of isovaleric acid oxidation, 24-hr urine was collected from one animal of each group. The collection vessel contained 2 ml of 2·5 N H_2SO_4. The urine was made up with water to a known volume, usually 10 ml; then one-fifth of the total was extracted with chloroform-*n*-butanol mixture, 5:1. Thin-layer chromatography of extracts on Silica gel (Eastman Chromagram sheets) was performed by a method designed to detect *N*-isovalerylglycine.[10] Graded amounts (5–25 μl) of solutions derived from urine were chromatographed.

After visualization of the compounds on the strips by spraying with alkaline alcoholic bromcresol purple, comparisons of excretion levels in the various samples could be made with fair accuracy on the basis of areas and intensity of the spots. Typical chromatograms developed in two solvent systems are shown in Fig. 1. Only urine from rats which received hypoglycin, with or without isovaleric acid,

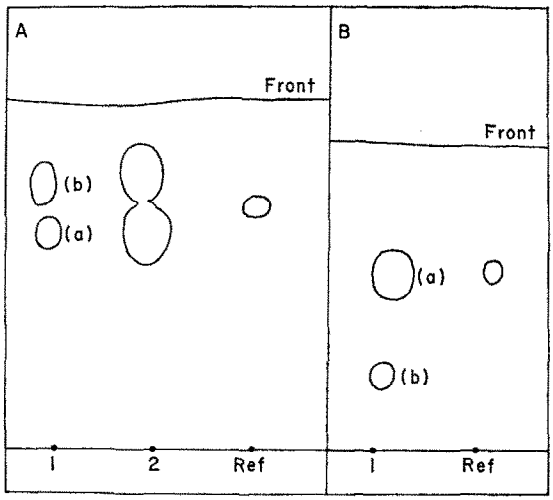

FIG. 1. *N*-isovalerylglycine (a) and *N*-methylenecyclopropaneacetylglycine (b) in extract of urine from hypoglycin-treated rat, detected on Silica gel thin-layer chromatograms. Spray reagent, alkaline alcoholic bromcresol purple. A = solvent system, benzene–isoamyl alcohol–formic acid, 14:5:1. Code figures 1 and 2 indicate different amounts of the same extract. Ref. indicates synthetic hippuric acid. B = solvent system, *n*-butanol–acetic acid–H_2O, 5:1:1.

gave the two characteristic spots shown. Of these, one was evidently *N*-isovalerylglycine; supporting evidence was obtained by using [1-^{14}C]isovalerate. Rats were given, per 100 g body wt, 15 mg of hypoglycin followed by 15 mg of isovalerate containing 5 μCi radioactivity. Chromatograms were prepared from extracts of 24-hr urine as described above. Only a single, well-defined area of radioactivity was found by scanning with a gas-flow detector. This area coincided with the spot identified as *N*-isovalerylglycine. The R_f in solvent system 1 (benzene–isoamyl alcohol–formic acid, 14:5:1) was 0·63; in solvent system 2 (*n*-butanol–acetic acid–H_2O, 5:1:1), R_f was 0·61, identical with synthetic hippuric acid.[10]

A second spot which appeared on the chromatograms of urines from hypoglycin-treated rats had R_f 0·76 in solvent 1, and R_f 0·23 in solvent 2. This compound contained no radioactivity from [1-^{14}C]isovalerate. It was most probably *N*-methylenecyclopropaneacetylglycine, derived from hypoglycin through formation of methylenecyclopropaneacetyl CoA followed by conjugation with glycine.

Of the metabolic pathways open to exogenous isovalerate, the major one would normally be conversion to the CoA ester. Oxidation would then be initiated by a dehydrogenase enzyme which is probably specific for isovaleryl CoA.[11,12] In the event of inhibited or deficient dehydrogenase function, isovaleryl CoA would either undergo conjugation with glycine, or be hydrolyzed to regenerate free isovalerate.

The 24-hr output of *N*-isovalerylglycine increased with the dose level of hypoglycin, but was unaffected by administered isovaleric acid. It is, therefore, likely that, in animals given both hypoglycin and isovaleric acid, the capacity to form the conjugate was exceeded and isovalericacidaemia

resulted. In these circumstances, however, the hypoglycaemic effect of hypoglycin was no greater, so it may be concluded that the mechanism of the effect is quite unrelated to inhibition of isovaleryl CoA dehydrogenase. A variety of other mechanisms have been proposed for hypoglycaemic action of hypoglycin and related compounds[6,13,14] and these remain for further investigation.

Acknowledgement—This work was supported by a grant from the Wellcome Trust.

Biochemistry Department,
University of the West Indies,
Kingston 6, Jamaica

ECCLESTON A. KEAN
ISMAY J. RAINFORD

REFERENCES

1. H. S. A. SHERRATT, *Br. med. Bull.* **25**, 250 (1969).
2. R. BRESSLER, C. CORREDOR and K. BRENDEL, *Pharmac. Rev.* **21**, 105 (1969).
3. K. TANAKA, E. M. MILLER and K. J. ISSELBACHER, *Proc. natn. Acad. Sci., U.S.A.* **68**, 20 (1971).
4. K. TANAKA, K. J. ISSELBACHER and V. SHIH, *Science* **175**, 69 (1972).
5. C. VON HOLT, M. VON HOLT and H. BOHM, *Biochim. biophys. Acta* **125**, 11 (1966).
6. C. CORREDOR, K. BRENDEL and R. BRESSLER, *Proc. natn. Acad. Sci. U.S.A.* **58**, 2299 (1967).
7. A. E. SENIOR and H. S. A. SHERRATT, *Biochem. J.* **104**, 56 (1967).
8. P. C. FENG and S. J. PATRICK, *Br. J. Pharmac. Chemother.* **13**, 125 (1958).
9. K. K. CHEN, R. C. ANDERSON, M. C. MCKOWEN and P. N. HARRIS, *J. Pharmac. exp. Ther.* **121**, 272 (1957).
10. J. ANDO and W. L. NYHAN, *Clin. Chem.* **16**, 420 (1970).
11. K. TANAKA, M. A. BUDD, M. L. EFRON and K. J. ISSELBACHER, *Proc. natn. Acad. Sci., U.S.A.* **56**, 236 (1966).
12. K. TANAKA and K. J. ISSELBACHER, *J. biol. Chem.* **242**, 2966 (1967).
13. C. J. TOEWS, C. LOWY and N. B. RUDERMAN, *J. biol. Chem.* **245**, 818 (1970).
14. W. G. DUNCOMBE and J. J. RISING, *Biochem. Pharmac.* **21**, 1089 (1972).

Biochemical Pharmacology, Vol. 22, pp. 1526–1529. Pergamon Press, 1973. Printed in Great Britain.

Effect of olivomycin on the induced synthesis of tyrosine aminotransferase and tryptophan oxygenase in rat liver

(*Received* 10 *October* 1972; *accepted* 6 *December* 1972)

WHILE some inhibitors of RNA synthesis have been used to study the regulation of protein synthesis antibiotics of the olivomycin–mythramycin–chromomycin group have not been examined in this respect. The present paper shows that olivomycin inhibits almost completely the hydrocortisone induction of tyrosine aminotransferase (EC 2.6.1.5) and tryptophan oxygenase (EC 1.13.1.12), exerting only a slight effect on the substrate induction of tryptophan oxygenase. Part of these results has been reported previously.[1]

Female albino rats, weighing 150–180 g, were used throughout the experiments.

Olivomycin (a product of the Moscow plant for medical preparations No. 1) was dissolved in saline *ex tempore* and injected intraperitoneally (30 mg/kg body wt) 30 min prior to the injection of the inducers.

Hydrocortisone acetate, microcrystalline suspension (G. Richter, Budapest) was administered intraperitoneally, the dose being 100 mg/kg.

L-Tryptophan ("Serva") was dissolved in saline by the addition of 6 N NaOH. The solution was adjusted to pH 7·2–7·5 with hydrochloric acid and injected intraperitoneally at a dose of 1·0 g/kg body wt.

Abbreviations used: TAT, tyrosine aminotransferase; TO, tryptophan oxygenase; HC, hydrocortisone.