Biochemical Pharmacology, Vol. 16, pp. 2221–2222. Pergamon Press Ltd. 1967. Printed in Great Britain

SHORT COMMUNICATIONS

Decamethonium uptake by slices of mouse liver

(*Received* 15 *May* 1967; *accepted* 7 *July* 1967)

FOLLOWING i.v. injection of ^{14}C-decamethonium into mice Waser[1] has observed a rapid fall of radioactivity in the blood. This is obviously to be explained by a specific uptake of decamethonium in the striated muscles as shown by Waser and Lüthi[2] in the diaphragm of mice with an autoradiographic technique. In an attempt to account quantitatively for the distribution of decamethonium in mice we, however, found a hepatic uptake which considerably exceeded the muscular uptake. In an unpublished series of experiments we found 50–60 per cent of the dose in the liver 20 min after i.v. injection of 190 ng/g ^{14}C-decamethonium. Radioactivity extracted from liver tissue with 80% methanol corresponded chromatographically to decamethonium. The present investigation shows that decamethonium also accumulates in liver tissue of mice in vitro and suggests an active transport mechanism for this accumulation.

Male albino mice weighing 25–28 g were decapitated and bled. The liver was sliced by hand with a razor blade and 4 slices weighing in total about 300 mg were placed in 30 ml Krebs–Ringer bicarbonate solution containing 1 g of glucose/l. A carbon dioxide-oxygen mixture (5:95) was bubbled through the medium in order to adjust the final medium to pH 7·3. ^{14}C-decamethonium bromide (The Radiochemical Centre, Amersham) was added and the mixture was shaken at 37°. After the incubation period the slices were separated from the medium by filtration on cotton and weighed. The radioactivity of slices and medium was measured by means of a Packard Tri-Carb liquid scintillation spectrometer, model 314 EX (conf.[3]).

The results of slice-uptake experiments were expressed as a slice-to-medium (S/M) concentration ratio of decamethonium, the concentration of decamethonium in slices being expressed in terms of the wet weight.

After 1 hr was found an S/M concentration ratio of 3·0 (mean of 12 experiments) and after 2 hr the S/M ratio had increased to 4·6 (mean of 6 experiments, range 3·7–5·9). In a nitrogen atmosphere the 1-hr S/M concentration ratio of decamethonium averaged 1·6 (range 1·5–1·9 in 6 experiments). At a temperature of 22° the 1-hr S/M ratio was 0·7–1·1. The influence of concentration of decamethonium on uptake is shown in Table 1. The uptake is not proportional to the concentration

TABLE 1. UPTAKE OF DECAMETHONIUM BY LIVER SLICES IN ABSENCE AND PRESENCE OF HEXAMETHONIUM (INCUBATION PERIOD 1 HR)

Compound	Initial concentration in medium, M		No. of exp.	Slice-medium concn. ratio*	
	Decamethonium	Hexamethonium		mean	range
Decamethonium alone	$1·4 \times 10^{-6}$	0	12	3·0	2·5–3·5
	$1·4 \times 10^{-4}$	0	6	2·0	1·6–2·5
	$1·4 \times 10^{-3}$	0	6	1·6	1·3–1·7
Decamethonium in presence of hexamethonium	$1·4 \times 10^{-6}$	$1·4 \times 10^{-4}$	6	2·6	2·0–3·2
	$1·4 \times 10^{-6}$	$1·4 \times 10^{-3}$	6	1·9	1·4–2·4

* Slice-medium concentration ratio is calculated as radioactivity per g slices (wet wt.)/radioactivity/ml medium.

of decamethonium in the medium, indicating that decamethonium is taken up by a concentrating mechanism that can be saturated. Table 1 further shows that the S/M concentration ratio of decamethonium decreases when nonlabelled hexamethonium chloride (May & Baker Ltd.) is added to the medium.

The results suggest that decamethonium is taken up by liver slices of mice by a process showing several of the characteristics of active transport. There is an uptake against a concentration gradient, the process is saturable, it is inhibited by low temperature and under anaerobic conditions. Further, hexamethonium acts as an inhibitor of decamethonium uptake, suggesting that the two methonium compounds share a common process involved in the transport.

The fate of decamethonium taken up in the liver of mice *in vivo* is not known. As mentioned we found no evidence of biotransformation in the liver within the first 20 min after i.v. injection. It is possible that decamethonium is either redistributed to plasma or excreted into bile. Biliary excretion does not represent a major pathway of elimination in rat, rabbit and cat[3–5]. In these species, however, the hepatic uptake of decamethonium is significantly lower than in the mouse.

Further studies of the hepatic uptake of decamethonium in various species are in progress.

Department of Pharmacology,
University of Copenhagen,
Denmark.

C. BROEN CHRISTENSEN

REFERENCES

1. P. G. WASER, *Proc. 2nd Int. Pharmac. Meet.*, Prague **3**, 131 (1963).
2. P. G. WASER and URSULA LÜTHI, *Archs Int. Pharmacodyn. Thér.* **112**, 272 (1957).
3. C. BROEN CHRISTENSEN, *Acta pharmac. tox.* **23**, 275 (1965).
4. L. S. SCHANKER, *Biochem. Pharmac.* **11**, 253 (1962).
5. URSULA LÜTHI and P. G. WASER, *Archs Int. Pharmacodyn. Thér.* **156**, 319 (1965).

Biochemical Pharmacology, Vol. 16, pp. 2222–2227. Pergamon Press Ltd. 1967. Printed in Great Britain

An hypothesis on the mechanism of action of 6-thioguanine

(*Received* 21 *March* 1967; *accepted* 5 *May* 1967)

THE GUANINE analog, 6-thioguanine (TG), is one of the purine antimetabolites that possess potent growth-inhibitory activity against both transplanted tumors in animals and neoplasms in man. For this reason, the biochemical mode of action of this compound has been the subject of many investigations, and a considerable number of metabolic alterations have been ascribed to this agent; these findings have been adequately summarized in recent reviews.[1–6] Several hypotheses have been suggested to account for the carcinolytic potency of TG, and these include: (a) incorporation of the analog into the nucleic acids; (b) feedback inhibition of the *de novo* biosynthetic pathway for purine nucleotides; and (c) interference with the interconversion of purine nucleotides. Recent findings in our two laboratories now make it possible to propose a unifying concept. Inhibitions of the enzymes, phosphoribosylpyrophosphate (PRPP) amidotransferase, inosine 5′-phosphate dehydrogenase, and ATP:GMP phosphotransferase by 6-thioguanosine 5′-phosphate (6-thioGMP) have been shown. These demonstrated inhibitions are proposed to act in concert with competitive substrate interaction between guanine and TG with guanylic pyrophosphorylase as well as with product inhibition of this enzyme by 6-thioGMP to diminish the rate of biosynthesis of guanine nucleotides. Such metabolic blockade would be expected to limit markedly the availability of guanine nucleotides for both coenzyme function and nucleic acid synthesis, thereby resulting in inhibition of growth and death of neoplastic cells.