Biochemical Pharmacology, Vol. 18, pp. 1239–1241. Pergamon Press. 1969. Printed in Great Britain

Effect of halothane and ether on glutamic-oxalacetic efflux from brain slices*

(*Received* 22 *August* 1968; *accepted* 4 *October* 1968)

THE PERMEABILITY of membranes to glutamic-oxalacetic transaminase (GOT) is normally so low that the enzyme exists primarily intracellularly. Neuropathologic as well as pharmacologic increases in central nervous system permeability to GOT have, however, been reported.[1–3] Since anesthetics affect membrane permeability in a direction and manner which depend upon the type of anesthetic and its concentration, as well as upon the nature of the compound passing through the membrane (and the means by which it passes through), and since the effects of anesthetics on central nervous system GOT levels have not been reported, we have done a preliminary study on the effect of anesthetics on central nervous system permeability to GOT by measuring the effect of halothane (Fluothane;® $CF_3CHBrCl$) and diethyl ether on the rate of loss (efflux) of GOT from rat brain slices.

Adult female (200 g) Sprague–Dawley rats were studied. Rats from which slices of brain were prepared were fed *ad libitum* until time of sacrifice. Rats from which heart and liver slices were obtained were fasted for 24 hr. Each animal was sacrificed by exsanguination during light methoxyflurane-induced analgesia. The brain was immediately removed and placed in ice-cold Krebs–Ringer phosphate buffer (pH 7·4) solution (KR). Simultaneous specimens were obtained from the right and left sides of the cortex and midbrain, one side being exposed to the anesthetic, the other serving as a simultaneous control. Cortical slices consisted of grey matter from the temporoparietal region. Midbrain slices were obtained from the region of the reticular formation by using the method of Schmidt.[4] Slices of cortex and midbrain were prepared freehand with a razor blade and glass guide according to the method described by McIlwain.[5] A number of cortical slices were homogenized with a Teflon pestle in KR rather than being studied as slices; the homogenate was diluted to a concentration of 1·25 mg of cortical tissue per ml suspension. Heart slices were prepared from the left ventricle with the serosal surface still intact. Liver slices were prepared free of capsule after immediate perfusion with ice-cold KR through the portal vein. Heart and liver slices were cut with a Stadie–Riggs hand microtome, each slice being halved so one would serve as a control while the other was exposed to the anesthetic. The thickness of all slices was 0·4–0·6 mm. Each slice was blotted, weighed and placed in 5 ml KR solution in a test tube and incubated at 37°. When cortical homogenates were studied, 10-ml aliquots of the suspension were incubated at 37°. During incubation, anesthetic gases were gently bubbled through the medium surrounding the tissue slices or from the bottom of the homogenate. Two concentrations of each anesthetic were studied. Halothane was vaporized by flowing 4 l./min of a mixture of 95% oxygen and 5% carbon dioxide through a temperature compensated vaporizer (Fluotec). Concentrations of halothane delivered as determined by an infrared gas analyzer were 1·02 and 2·20%. Ether was vaporized by flowing the same carrier gas mixture through a constant temperature vaporizer (Kopper Kettle®) and diluting the effluent with 95% oxygen and 5% carbon dioxide so that 2·0 and 4·0% ether was delivered to the incubation medium. Control samples were simultaneously exposed to the same oxygen–carbon dioxide without anesthetics.

After incubation for either 30 or 60 min, slices were washed twice with KR solution, the washings were added to the incubation medium and the total volume was adjusted to 10 ml. GOT activity in the 10 ml of washings plus incubation medium was determined colorimetrically by modifying the technic of Reitman and Frankel[6] as follows: to 1·0 ml substrate (standardized solution of aspartic and α-ketoglutaric acids) 0·2 ml of washings was added. After 60 min in a 37° bath, 1·0 ml of color reagent (standardized 2,4-dinitrophenylhydrazine and HCl) was added and after 20 min at room temperature an additional 10 ml of 0·4 N NaOH was added and mixed by shaking. Five min later, optical density was read at 505 mμ in a Coleman Junior colorimeter with a 19 mm tube. One Reitman–Frankel unit of GOT per ml at 37° formed $4{\cdot}82 \times 10^{-4}$ μM glutamate/min at pH 7·5. Two measurements were made on each sample. Efflux of GOT was expressed as Q_{60} (or Q_{30}), i.e. units of GOT activity appearing in the incubating medium per milligram wet weight of tissue slice per 60 (or 30) min.

Both concentrations of halothane were associated with an efflux of GOT from cortical slices that

* Supported by a research grant from the Josiah Macy, Jr. Foundation.

TABLE 1. EFFECT OF HALOTHANE AND ETHER ON GOT EFFLUX FROM RAT BRAIN SLICES*

Anesthetic (concn)	Control	Exper.	% Change	Significance
Cortex				
Halothane (1·02%)	2·9 ± 0·40	4·1 ± 0·49	+41·3	$P < 0·01$
Halothane (2·20%)	2·8 ± 0·42	4·5 ± 0·72	+60·7	$P < 0·01$
Ether (2·0%)	2·6 ± 0·25	2·2 ± 0·19	−15·4	n.s.
Ether (4·0%)	2·9 ± 0·39	3·0 ± 0·43	+3·4	n.s.
Brain stem				
Halothane (1·02%)	3·9 ± 0·58	4·0 ± 0·40	+2·5	n.s.
Halothane (2·20%)	4·7 ± 0·68	4·4 ± 0·49	−6·4	n.s.
Ether (2·0%)	4·3 ± 0·45	4·1 ± 0·38	−4·6	n.s.
Ether (4·0%)	5·3 ± 0·76	5·0 ± 0·65	−5·6	n.s.

* Mean Q_{60} ± S.E. Ten experiments in each group; n.s., not significant.

was statistically significantly greater than the efflux which occurred in the same period of time in paired controls simultaneously exposed to the carrier gas without anesthetic (Table 1). The increase in efflux was greater with 2·20 than with 1·02% halothane. The difference between the two concentrations, though not significant, suggested a dose-related response. The effect was time related, though not linearly so. Q_{30} and Q_{60} for cortical slices exposed to 2·20% halothane averaged 3·1 and 5·0 respectively. The effect of halothane on cerebral cortical slices appeared quite tissue specific. Neither 1·02 nor 2·20% halothane significantly affected GOT efflux from midbrain slices (Table 1) and 2·20% halothane had no effect on loss of GOT from heart or liver slices (Table 2). Neither 2·0 nor

TABLE 2. EFFECT OF HALOTHANE ON GOT EFFLUX FROM HEART AND LIVER SLICES*

Tissue	Control	Halothane (2·20%)
Heart	26·7 ± 1·18	26·5 ± 1·69
Liver	6·2 ± 0·94	6·0 ± 0·84

* Mean Q_{60} ± S.E. Ten experiments in each group.

4·0% ether affected the rate at which GOT effluxed from cortex or midbrain slices. Neither anesthetic significantly affected GOT activity in cerebral homogenates. GOT activity per ml of cerebral cortical homogenate exposed to 95% oxygen and 5% carbon dioxide at 37° for 60 min averaged 88 Reitman–Frankel units; after similar exposure to 2·20% halothane and 4·0% ether, GOT activity in cerebral homogenates averaged 84 and 80 respectively.

The minimum alveolar anesthetic concentration in man (i.e. the tension of anesthetic to which the brain is exposed[7]) is 0·76% for halothane and 1·92% for ether.[7] The tension to which the brain slices in the present experiments were exposed corresponds, therefore, in the case of the lower of the concentrations, to concentrations adequate to provide moderately deep surgical levels of anesthesia. At these concentrations halothane increased GOT efflux from cortical slices while ether did not. At the higher concentrations, i.e. those corresponding to clinically toxic concentrations, the effect of halothane became more evident, but ether still had no effect. Since the two anesthetics had dissimilar effects at essentially equi-anesthetic concentrations, the results with halothane cannot be ascribed to an effect on membrane permeability which might be a characteristic common to inhalation anesthetics. The data also suggest that the effect of halothane on GOT efflux from brain slices may not even be related to the anesthetic effects of halothane. GOT efflux from midbrain slices was unaffected while efflux from cortical slices was increased. Since the midbrain slices contained significant amounts of reticular formation, the area upon which halothane has its primary anesthetic effect, changes in GOT

efflux should have been more prominent in midbrain slices than in cortical slices if the effect of halothane on GOT efflux is related to its anesthetic action.

The observed changes in GOT efflux in cerebral cortical slices exposed to halothane are best explained on the basis of increased cell membrane permeability. Which nerve cells are affected, how they are affected and the significance of the effect warrant further investigation.

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Biochemical Pharmacology, Vol. 18, pp. 1241–1243. Pergamon Press. 1969. Printed in Great Britain

Indole-2-carboxylic acids, a new class of hypoglycemic compounds

(*Received* 16 *August* 1968; *accepted* 1 *November* 1968)

IN THE COURSE of screening for hypoglycemic agents, 5-methoxyindole-2-carboxylic acid (MICA) was found to consistently lower blood sugar in the fasted rat. Many similar compounds were tested and the results of these experiments are herein reported.

Alloxan monohydrate was obtained from Eastman Organic Chemicals; sodium tolbutamide was a gift of the Upjohn Company. Test compounds were obtained from commercial sources or were synthesized in these laboratories, as indicated in the tables. Commercially obtained compounds were used without further purification. High viscosity sodium carboxymethylcellulose (7HF) was obtained from the Hercules Powder Company and dissolved in water by prolonged boiling. Other chemicals were Merck, reagent grade. Rats were of the Sherman (Wistar) strain, raised in these laboratories; mice were either of the ICR or MF-1 strain from Manor Farms, New York.

Test compounds were homogenized in 0·5% sodium carboxy-methylcellulose at an appropriate concentration so that the desired dose could be administered in 1 ml/100 g of body weight. They were injected i.p. into unanesthetized animals or injected intragastrically by stomach tube under light ether anesthesia. Blood samples (0·1 ml) were obtained from rats from the tail, diluted 10-fold in 0·25% sodium oxalate, and analyzed for reducing sugar content by the alkaline ferricyanide method[1] as modified for the Technicon autoanalyzer.[2] Mice were alloxanized by the rapid i.v. injection of 0·1 ml of 2% alloxan monohydrate. Mice were tested for glycosuria by use of a glucose oxidase paper.* The occasional mice which did not develop glycosuria were discarded, and the remaining diabetic mice were used within 1–3 weeks of alloxanization. Blood was obtained from decapitated mice from the cervical stump, diluted 20-fold and analyzed for reducing sugar as above.

Results of testing in the rat are given in the tables. Compounds in the indole series are shown in Table 1. The following structurally related non-indoles were tested at 200 mg/kg and were not hypoglycemic: benzofuran-2-carboxylic acid, benzoxazole-2-carboxylic acid, benzimidazole-2-

* Available under the trademark Tes-Tape (Eli Lilly & Co.).