Biochemical Pharmacology, Vol. 23, pp. 2005–2009. Pergamon Press, 1974. Printed in Great Britain.

INHIBITION OF GLUCOSE EFFLUX FROM HUMAN ERYTHROCYTES BY HASHISH COMPONENTS

AVITAL SCHURR, NINA SHEFFER, YOSEF GRAZIANI and AVINOAM LIVNE

Department of Biology and Research and Development Authority, University of the Negev, Beer Sheva, Israel

(*Received* 5 *September* 1973; *accepted* 5 *January* 1974)

Abstract—Glucose efflux from human erythrocytes under zero-*trans* conditions is inhibited by the hashish components Δ'-tetrahydrocannabinol and cannabidiol at concentrations above 5×10^{-7} M. The inhibitory effect is rapid and apparently readily reversible. Increasing concentrations of alcohols up to 20 mM do not change the rate of glucose efflux but they do amplify the extent of efflux inhibition by a given dose of hashish component. Methanol, ethanol and propanol show this amplifying effect in a decreasing order.

"MARIHUANA HUNGER", the special craving for sweets, is a common symptom of marihuana smoking. This symptom may indicate a lowered blood glucose level, however, on the basis of glucose tolerance test, Podolsky *et al.*[1] have shown no lowering of blood glucose level. Rather, these authors detected hyperglycemic changes attributable to the drug.

The effects of the hashish compounds, Δ^1-tetrahydrocannabinol (Δ^1-THC) and cannabidiol (CAN) on rat and human erythrocytes[2,3] and on rat liver mitochondria[4,5] and lysosomes[6] have been described recently. A paramount feature of the effects is an interaction between the hashish compounds and the membrane of these cells or organells. It seems therefore possible that the hyperglycemic changes might be related, among other factors, to an effect of the hashish compounds on glucose transport across cellular membranes.

As an approach to study such an effect in a defined system, we examined glucose transport in human erythrocytes. This paper illustrates a marked effect of the hashish compounds on glucose transport, an effect which is accentuated by added alcohols.

MATERIALS AND METHODS

Freshly drawn human blood in heparin or stored transfusion blood (up to 3 weeks of storage) were used in this study, showing essentially the same results. The procedure for washing the erythrocytes and for measuring glucose under zero-*trans* conditions was as described by Karlish *et al.*[7] Two modifications of this procedure were introduced. First, 0·4 ml of the cell suspension (60% hematocrit) preloaded with 80 mM glucose, was rapidly mixed with 100 ml of the zero-*trans* washout medium. Secondly, glucose analysis was performed in part of the experiments with glucose oxidase.[8] In these experiments, glucose was determined by the use of a colourimetric assay in which production of a coloured dye formed from *o*-dianizidine in a peroxide coupled reaction was measured. Parallel experiments verified that the results of this

analysis were identical to those obtained with radioactive glucose. The glucose oxidase reaction products and the hemoglobin content, using standard Drabkin procedure,[9] were determined colourimetrically, using Klett Summerson colorimeter with 42 and 54 filters, respectively.

All chemicals were of analytical grade. Δ^1-THC and CAN were kindly supplied by Prof. R. Mechoulam (School of Pharmacy, The Hebrew University, Jerusalem). The hashish compounds were introduced as alcoholic solutions and the control systems contained the same amount of alcohol.

RESULTS

Glucose transport across the human erythrocyte membrane has been studied by exchange[10,11] and by net flux measurements, either according to Sen-Widdas,[12] or according to the zero-*trans* procedure of Stein and co-workers.[7,13] The exchange and the Sen-Widdas net flux measurements require the presence of glucose on both sides of the membrane. Such requirement might complicate the interpretation of the effect of the hashish components. The zero-*trans* procedure, on the other hand, maintains the concentration of glucose at the *trans* (outer) face of the membrane at or near zero.[7] This procedure was therefore adopted for the present study.

Glucose efflux from human erythrocytes was inhibited by Δ'-THC and CAN at concentrations above 5×10^{-7} M. Representative data, depicting the effect of Δ'-THC and CAN on glucose efflux are shown in Fig. 1. The ordinate f values are the ratio of amount of glucose, present in the cells at any time, related to its amount in the cells at time zero.[7] CAN is significantly more effective than Δ'-THC in arresting glucose efflux. Table 1 presents the kinetic parameters[7] of the efflux data given in Fig. 1. While the K_m value increases significantly with increasing concentrations of the hashish components, V_{max} is only slightly affected by these components.

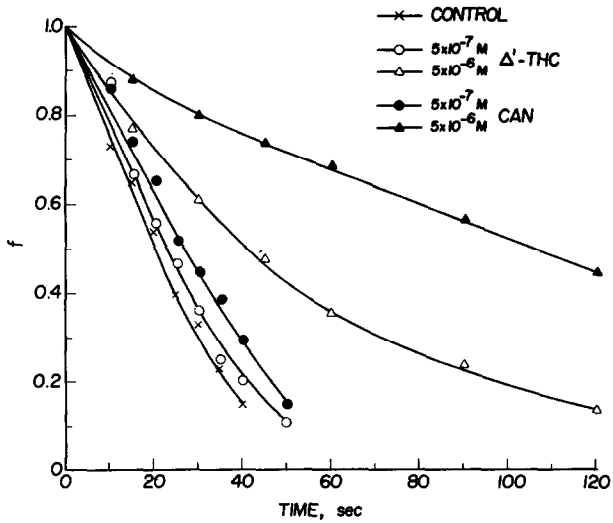

FIG. 1. Effect of Δ'-THC and CAN on glucose efflux from human erythrocytes. The ordinate f values are the ratio of amount of glucose, present in the cells at any time, related to its amount in the cells at time zero.[7]

TABLE 1. GLUCOSE EFFLUX FROM HUMAN ERYTHROCYTES UNDER ZERO-*trans* CONDITIONS AS AFFECTED BY HASHISH COMPONENTS

Addition (M)	n	K_m (mM)	V_{max} (μmoles/min per l. isotonic cell water)
Control	13	22 ± 2	189 ± 19
Δ'-THC			
5×10^{-7}	6	35 ± 3	198 ± 18
5×10^{-6}	6	63 ± 15	208 ± 29
CAN			
5×10^{-7}	6	43 ± 4	216 ± 21
5×10^{-6}	6	158 ± 30	217 ± 21

The cannabinoids were added from methanolic stock solutions; final methanol concentration in reaction mixtures: 32 mM. n = number of experiments. K_m and V_{max} were calculated according to Karlish *et al.*[7] Mean values ± S.E. are given.

Glucose loaded erythrocytes (60 per cent cell suspension) were incubated with 10^{-5} M CAN for 5 min at 37° and then tested for glucose efflux by mixing an aliquot with the washout medium (250 fold dilution). Under these conditions CAN caused no change in glucose efflux, in contrast to its profound inhibitory effect when added directly to the efflux medium, as shown in Fig. 1. Apparently the effect of the hashish compounds is readily reversible, as already shown with respect to erythrocyte stabilizing effect of these compounds.[3]

Since Δ^1-THC and CAN are not water soluble, they were added from methanolic or ethanolic stock solutions. While the alcohols, at a concentration range of

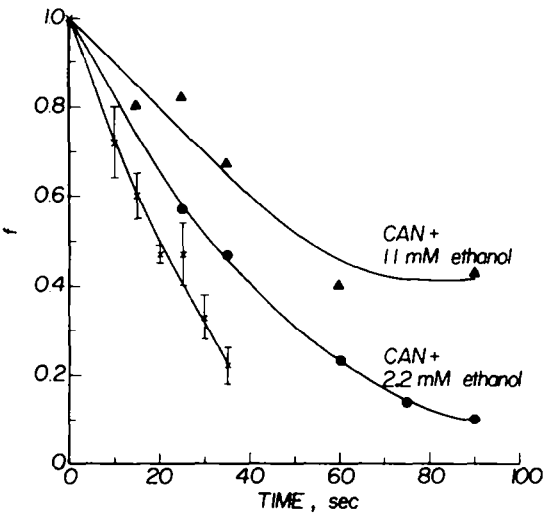

FIG. 2. Effect of ethanol concentration on the inhibition of glucose efflux by 5×10^{-6} M CAN. Standard errors for the control, including the ethanol treatments without CAN, are given as vertical bars. The ordinate *f* values are as in Fig. 1.

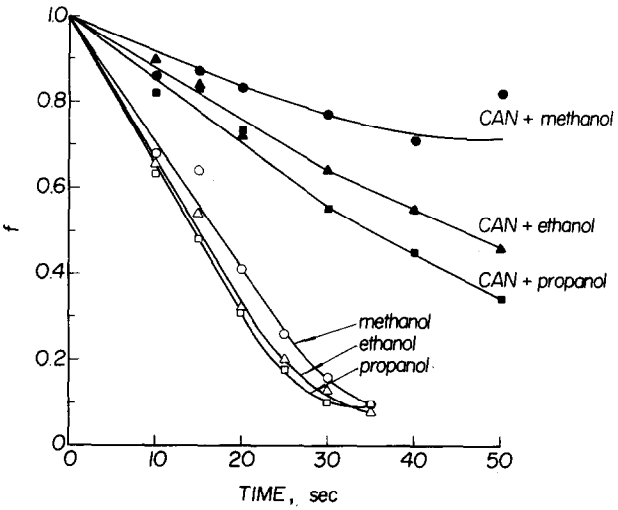

FIG. 3. Effect of 12·5 mM alcohol on the inhibition of glucose efflux by 5×10^{-6} M CAN. The ordinate f values are as in Fig. 1.

2×10^{-3} to 2×10^{-2} M barley changed glucose efflux, they did, however, amplify the inhibition of glucose efflux by the hashish components. Figure 2 shows that the extent of efflux inhibition by a given dose of CAN is increased with increasing alcohol concentration. The increased inhibition due to alcohol is significant. Furthermore, Fig. 3 shows that this amplifying effect depends on the length of the alcohol aliphatic chain: methanol, ethanol and propanol are effective in a decreasing order.[4] This trend appeared in all three experiments.

DISCUSSION

Various membrane systems are affected by Δ^1-THC and CAN.[2–6] These reports, along with the present study, indicate that cellular membranes are indeed a major site of interaction with the hashish components. The effect of Δ^1-THC and CAN on both osmotic fragility[3] and glucose efflux are very rapid and readily reversible, but while Δ^1-THC is more effective than CAN with respect to conferal of osmotic stability,[3] the opposite is true with regards to inhibition of glucose efflux (Fig. 1). This disparity and the multiplicity of membrane systems and functions affected by hashish components exclude the possibility of a single site and mechanism for the various effects of the hashish components.

The kinetic properties of the drug-affected efflux (Table 1) show features of competitive inhibition, as already demonstrated for phloretin.[14] If the inhibition is indeed competitive, it is expected that it will be dependent on the erythrocyte glucose concentration. However, the degree of inhibition of glucose efflux by 5×10^{-6} M CAN was independent of glucose concentration at a range of 30–120 mM. In view of the interaction of the cannabinoids with phospholipids[4] and with lipoproteins,[15] it is possible that these drugs modify the lipophylic environment of the glucose carrier[11,16] and thus change the apparent affinity of the carrier to glucose.

The physiological significance of the inhibition of glucose transport by Δ'-THC and CAN should be considered. For hashish smokers, temporary halt in glucose

transport from the plasma into the erythrocytes is possible, particularly since the effective concentration of the drugs in inhibiting glucose transport (Fig. 1) correlates with the doses leading to physiological reactions in hashish smokers.[17,18] It is possible that the hashish components affect glucose transport not only in erythrocytes but in other cells as well, thus leading eventually to side-effects and symptoms of hashish smoking, such as "marihuana hunger", hyperglycemia and glucosuria. It is noteworthy that several lines of evidence indicate that glucose transport into adipose cells[19] and across the muscle cell membrane[20] show distinct properties of facilitated diffusion which are also typical for human erythrocytes.

The interacting effects of alcohols and the hashish components in inhibiting glucose transport (Figs. 2 and 3) may be due to an increase of the effective drug concentration in the membrane by the alcohol. Interestingly, hashish smokers have disclosed to us a custom of drinking alcoholic beverages while smoking hashish in order to attain a more pronounced "high". This claim of an *in vivo* interaction of alcohol and hashish should be rigorously tested.

Acknowledgements—This work was supported in part by a grant from the Chief Scientist, Ministry of Health, Israel.

REFERENCES

1. S. Podolsky, C. G. Pattavina and M. A. Amaral, *Ann. N.Y. Acad. Sci.* **191,** 54 (1971).
2. A. Chari-Bitron, *Life Sci.* **10,** 1273 (1971).
3. A. Raz, A. Schurr and A. Livne, *Biochim biophys. Acta* **274,** 269 (1972).
4. J. M. Mahoney and R. A. Harris, *Biochem. Pharmac.* **21,** 1217 (1972).
5. T. Bino, A. Chari-Bitron and A. Shahar, *Biochim. biophys. Acta.* **288,** 195 (1972).
6. A. Raz, A. Schurr, A. Livne and R. Goldman, *Biochem. Pharmac.* **22,** 3129 (1973).
7. S. J. D. Karlish, W. R. Lieb, D. Ram and W. D. Stein, *Biochim. biophys. Acta.* **255,** 126 (1972).
8. O. Wieland, in *Methods of Enzyme Analysis* (Ed. H. U. Bergmeyer) p. 271. Academic Press, New York (1965).
9. M. M. Wintrobe, *Clinical Hematology*, 5th Edn. Lea & Febiger, Philadelphia (1961).
10. R. C. Mawe and H. G. Hempling, *J. Cell Comp. Physiol.* **66,** 95 (1965).
11. H. Zipper and R. C. Mawe, *Biochim. biophys. Acta.* **282,** 311 (1972).
12. A. K. Sen and W. F. Widdas, *J. Physiol., Lond.* **160,** 393 (1962).
13. W. R. Lieb and W. D. Stein, *Biophys. J.* **10,** 585 (1970).
14. P. G. LeFerve, *Symposia Soc. exp. Biol.* **8,** 118 (1954).
15. M. Wahlqvist, I. M. Nilsson, F. Sandberg, S. Agurell and B. Granstrand, *Biochem. Pharmac.* **19,** 2579 (1970).
16. A. Kahlenberg and B. Banjo, *J. biol. Chem.* **247,** 1157 (1972).
17. H. Isbell, C. W. Gordetsky, D. Taninsky, U. Claussen, F. V. Spalak and F. Korte, *Psychopharmacologia* **11,** 184 (1967).
18. L. E. Hollister, R. K. Richard and H. K. Gillespie, *Clin. Pharmac. Ther.* **9,** 183 (1968).
19. M. Blecher, *Biochim. biophys. Acta.* **137,** 557 (1967).
20. H. E. Morgan, D. M. Regen and C. R. Park, *J. biol. Chem.* **239,** 369 (1964).
21. A. B. King and D. L. Cowen, *J. Am. Med. Assoc.* **210,** 724 (1969).