together with the products of cecal microbial digestion during the period of low Na-transport for a second passage through the gastrointestinal tract.

We thank Mrs J. De Beir Simaels for excellent technical assistance, and Prof. Dr. P. Vescei for the radioimmunoassay of aldosterone. D-aldosterone (Aldocorten) was kindly supplied by Ciba-Geigy, and spironolactone was a kind gift of Boehringer Mannheim AG. This study was supported by grants from the Deutsche Forschungsgemeinschaft (Cl 63/1-2 and Cl 63/4-1), and a travelling award from the H. Wilhelm Schaumann Stiftung.

1 Schultz, S. G., A. Rev. Physiol. *46* (1984) 435.
2 Clauss, W., in: Intestinal Absorption and Secretion, p. 273. MTP Press, Lancaster 1984.
3 Hörnicke, H., Livestock Prod. Sci. *8* (1981) 361.
4 Clauss, W., and Dürr, J. E., Pflügers Arch. *403* (1985) R11.
5 Clauss, W., Schäfer, H., Horch, I., and Hörnicke, H., Pflügers Arch. *403* (1985) 278.
6 Van Driessche, W., J. Physiol. *356* (1984) 79.
7 Clauss, W., Dürr, J., Skadhauge, E., and Hörnicke, H., Pflügers Arch. *403* (1985) 186.
8 Gögelein, H., and van Driessche, W., Pflügers Arch. *389* (1981) 105.
9 Zeiske, W., Wills, N. K., and van Driessche, W., Biochim. biophys. Acta *688* (1982) 201.
10 Lindemann, B., A. Rev. Physiol. *46* (1984) 497.
11 Crabbé, J., in: Modern Pharmacology-Toxicology Series, vol. 8, p. 513. Marcel Dekker, New York 1977.
12 Frizzell, R. A., and Schultz, S. G., J. Membr. Biol. *39* (1978) 1.
13 Garty, H., and Edelman, I. S., J. gen. Physiol. *81* (1983) 785.
14 Palmer, L. G., Li, J. H.-Y., Lindemann, B., and Edelman, I. S., J. Membr. Biol. *64* (1982) 91.
15 Rossier, B. C., J. Membr. Biol., Special Issue (1978) 187.

0014-4754/88/070608-03$1.50 + 0.20/0

Stimulation by D-glucose of mitochondrial respiration

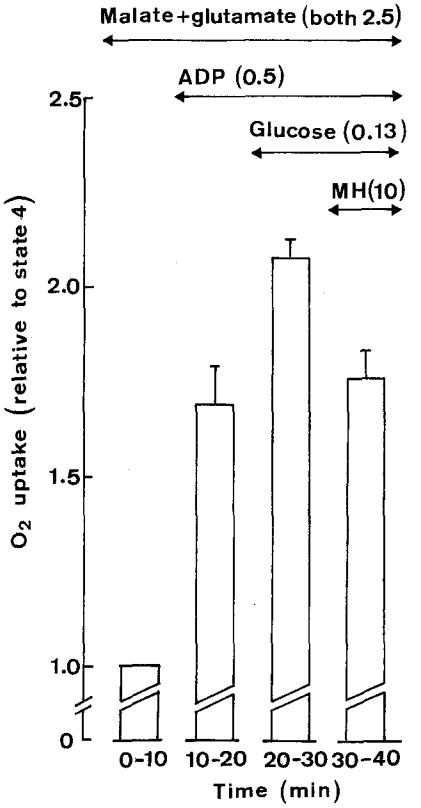
W. J. Malaisse and A. Sener

Laboratory of Experimental Medicine, Brussels Free University, Boulevard de Waterloo 115, B-1000 Brussels (Belgium)
Received 11 January 1988; accepted 21 March 1988

Summary. D-glucose increases O_2 uptake by cerebellum mitochondria. This effect is abolished by D-glucose-6-phosphate and D-mannoheptulose. It is proposed that the phosphorylation of D-glucose as catalyzed by bound hexokinase directly affects mitochondrial respiration.
Key words. Mitochondria; respiration; D-glucose.

In several cell types, e.g., in brain cells, a large fraction of hexokinase is bound to mitochondrial porin[1-3]. Bound hexokinase may use mitochondrial rather than cytosolic ATP as a substrate for D-glucose phosphorylation[4,5]. It was recently proposed that such a process may represent a novel pathway for the direct coupling of hexose phosphorylation to mitochondrial respiration[6]. In the present study, the validity of the latter proposal was assessed by examining the effect of D-glucose on O_2 uptake by isolated brain mitochondria.

Groups of two cerebella removed from fed albino rats were weighed (400–500 mg wet weight), minced and homogenized in Potter-Elvehjem tubes (20 strokes) with 2.2 ml of a Hepes-KOH buffer (10 mM, pH 7.4) containing sucrose (250 mM), KCl (20 mM) and EDTA (0.2 mM). After a first centrifugation for 5 min at 1000 × g and 4 °C, an aliquot (2.0 ml) of the supernatant was removed and centrifuged for 20 min at 4300 × g and 4 °C[7]. After removal of the supernatant, the pellet was washed twice and eventually resuspended in 0.5 ml of the same buffer. Aliquots (usually 0.1 ml each) were placed in the incubation chamber of a Gilson oxygraph in 1.6 ml of a Hepes-KOH buffer (40 mM, pH 7.4) containing KCl (100 mM), $MgCl_2$ (5 mM) and KH_2PO_4 (10 mM). The oxygraph was equipped with a YSI Clark oxygen electrode (Gilson Medical Electronics, Middleton, WI). Respiratory activity in the mitochondrial suspension was measured in the closed and magnetically stirred chamber at 37 °C, and was found to be proportional to the volume of mitochondrial suspension with a reproducibility of 4.6% (n = 12). Metabolites were added by introducing small volumes (20–40 µl) of appropriately concentrated solutions and the O_2 uptake measured over the ensuing 10 min. Calibration was performed over 30–60 s at 37 °C using freshly dissolved β-D-glucose in the presence of glucose oxidase, as described elsewhere[8]. All results are expressed as the mean (± SEM) together with the number of individual observations (n). The



Time course for O_2 uptake by rat cerebellum mitochondria. The effect of ADP, D-glucose and mannoheptulose (MH) were judged from paired comparison (n = 3 or more) of measurements performed over 10-min incubation in each case. The concentration of each metabolite is expressed as mM (in parentheses).

statistical significance of differences was assessed by the use of Student's t-test.

Mitochondria were incubated in the presence of malate and glutamate (2.5 mM each). The addition of ADP (0.5 mM) increased O_2 uptake by 68.9 ± 10.1% (paired comparison; $n = 22$; $p < 0.001$). In the presence of ADP, the respiratory rate averaged 19.1 ± 1.9 pmol of O_2 per min and per mg wet weight of cerebellum tissue ($n = 15$). In the presence of ADP, the addition of D-glucose (0.13 mM or more) further increased O_2 uptake by 22.8 ± 3.1% (paired comparison; $n = 59$; $p < 0.001$). The magnitude of the latter increase was little affected by the concentration of D-glucose in the 0.13–8.0 mM range, which is in large excess of the K_m for the hexose of mitochondrial brain hexokinase[6]. The relative magnitude of the hexose-induced increase in O_2 uptake was inversely related, however, to the ADP concentration (0.1 to 1.0 mM), decreasing ($p < 0.01$) from 35.2 ± 2.4% ($n = 3$) at low ADP concentration (0.1 mM) to 12.2 ± 4.9% ($n = 6$) in the presence of 1.0 mM ADP. When D-mannoheptulose (10.0 mM) was added to mitochondria already exposed to both ADP (0.5 mM) and D-glucose (0.13 mM), the rate of O_2 uptake was decreased by 15.3 ± 3.5% (paired comparison; $n = 3$; $p < 0.05$) and, hence, became close to that recorded prior to the introduction of D-glucose (fig.). Glucose 6-phosphate (3.0 mM) exerted an inhibitory effect comparable to that of D-mannoheptulose (data not shown). Moreover, when either mannoheptulose or D-glucose 6-phosphate were present in the medium together with ADP prior to the addition of D-glucose, the hexose failed to stimulate respiration (paired change: + 0.3 ± 1.5%; $n = 6$; $p > 0.8$). In the presence of ADP but absence of D-glucose, neither mannoheptulose nor glucose 6-phosphate inhibited O_2 uptake ($n = 6$).

The present results document that D-glucose increases state 3 respiration in brain mitochondria. In a prior study[9], an effect of glucose upon O_2 uptake by brain mitochondria had been observed after but not during ADP stimulation. In the present work, the effect of D-glucose appeared to be related to the capacity of the hexose to act as a substrate for mitochondria-bound hexokinase since it was suppressed by D-mannoheptulose or D-glucose 6-phosphate[6,10]. Incidentally, the fact that the response to D-glucose was inhibited by D-glucose 6-phosphate and modulated by the concentration of ADP strongly suggests that it indeed results from the stimulation of mitochondria rather than contaminating synaptosomes. Further work is required to assess such items as the concentration-relationship of the response to D-glucose; its stoichiometry relative to both ADP and hexose phosphorylation as well as substrate oxidation; its anomeric specificity; its possible simulation by other substrates (e.g. glycerol) susceptible to be phosphorylated by porin-bound kinases such as glycerol kinase[11], and its physiological relevance.

Meanwhile, our findings are compatible with the view that the phosphorylation of glucose as catalyzed by bound hexokinase may directly affect mitochondrial respiration through the consumption of ATP and concomitant generation of ADP[6].

Acknowledgments. This study was supported by a grant from the Belgian Foundation for Scientific Medical Research. The authors wish to thank G. Vandenbroeck for technical assistance and C. Demesmaeker for secretarial help.

1 Fegner, P. L., and Wilson, J. E., Archs Biochem. Biophys. *182* (1977) 282.
2 Panfili, E., and Sandri, G., Biochem. biophys. Res. Commun. *131* (1985) 6.
3 Malaisse-Lagae, F., and Malaisse, W. J., Biochem. Med. *39* (1987) 80.
4 Bustamente, E., and Pedersen, P. L., Proc. natl Acad. Sci. USA *74* (1977) 3735.
5 Nelson, B. D., and Kabir, F., Biochim. biophys. Acta *841* (1985) 195.
6 Malaisse, W. J., and Malaisse-Lagae, F., Brain Res. *419* (1987) 147.
7 Gellerfors, P., and Nelson, B. D., Analyt. Biochem. *93* (1979) 200.
8 Blachier, F., Sener, A., and Malaisse, W. J., Biochim. biophys. Acta *921* (1987) 494.
9 Land, J. M., Booth, R. F. G., Berger, R., and Clark, J. B., Biochem. J. *164* (1977) 339.
10 Malaisse, W. J., Lea, M. A., and Malaisse-Lagae, F., Metabolism *17* (1968) 126.
11 Kaneko, M., Kurukawa, M., and Ishibashi, S., Archs Biochem. Biophys. *237* (1985) 135.

0014-4754/88/070610-02$1.50 + 0.20/0

Enhanced toxicity of the immunosuppressant ovalicin upon application to the skin

H. Stähelin

Preclinical Research, Sandoz Ltd., CH-4002 Basel (Switzerland)
Received 19 February 1988; accepted 10 March 1988

Summary. The non-myelotoxic immunosuppressive sesquiterpene ovalicin, of fungal origin, is much more toxic when applied to the skin of animals than when injected i.v., the LD-50 in guinea pigs being 0.2 in the first case and 7 mg/kg in the second. It elicits aphagia and adipsia. It is assumed that ovalicin effects are due to slow, tissue-specific, metabolic toxification.
Key words. Ovalicin; immunosuppression; toxicity; metabolic toxification; skin.

Screening fungal broths for cytostatic activity, using mouse mastocytoma P-815 cells, had already led to the finding of a number of interesting compounds like phomin (cytochalasin B), verrucarins and anguidine (see Stähelin[1]) when, in 1962, culture filtrates of the fungus *Pseudeurotium ovalis* Stolk were found to inhibit proliferation of mastocytoma cells. The metabolite responsible for this effect was then isolated[2,3] and turned out to be a sesquiterpene with two epoxy groups. The compound, which was given the name ovalicin, exhibited potent cytostatic activity against P-815 cells in vitro with an ID-50 below 1 ng/ml. Despite its comparatively low toxicity it did not inhibit the growth of P-815 tumor cells in mice, but reduced the spleen weight of the treated animals. For this and other reasons, the compound was tested for immunosuppression and turned out to considerably decrease the formation of hemagglutinins in mice immunized with sheep erythrocytes. Further evaluation showed ovalicin to be a quite effective immunosuppressant in a number of tests (skin transplantation, experimental allergic encephalomyelitis and others) without reducing the number of granulocytes in the blood of the treated mice and rats[4–7]. In other studies, a high in vitro potency of ovalicin as inhibitor of