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PHENOBARBITAL EFFECT ON GLIAL CELL RESPIRATION IN THE PRESENCE OF A HIGH CONCENTRATION OF POTASSIUM

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Abstract—Rates of oxygen uptake were measured micromanometrically in samples of about fifty five morphologically differentiated astrocytes obtained from primary cultures grown from the dissociated cerebral hemispheres of newborn DBA mice. With a low potassium concentration (5 mM) in the medium, the respiration was high and well maintained. Exposure to an increased potassium (K) concentration (55 mM) led to a rapid decline in the rate of oxygen uptake. This decline was prevented by 0.5–1.0 mM phenobarbital which had no effect on respiration in the medium containing 5 mM K.

Recent *in vivo* experiments using potassium-sensitive electrodes have convincingly demonstrated an increase of the extracellular K concentration in grey matter of the central nervous system (CNS) during excitation and seizures [1–3]. Normally, the extracellular K concentration does not reach levels above 10–12 mM but during spreading depression as high concentrations as 60–80 mM have been observed [4–6]. At the same time, there is an increase in oxidative metabolism which may be a metabolic manifestation of an active reaccumulation of extracellular potassium into adjacent cells [1, 3, 6, 7]. The concept of an active K uptake into cells is supported by the demonstration that the subsequent decrease of the extracellular K concentration may occur up to one hundred times as fast as can be accounted for by diffusion (G. Somjen and G. Cordingley, personal communication). The *in vivo* increase in both extracellular K concentration and metabolic response are maintained for a longer time in the presence of barbiturates [8].

The presence of such high extracellular K concentrations in the normal brain has attributed to a renewed interest in *in vitro* experiments in which brain tissue is exposed to elevated K concentrations in the incubation medium. In brain slices, these high K concentrations (e.g. about 50 mM) exert two effects on the rate of oxygen uptake: (1) an initial stimulation of up to 100 per cent (9–11) and (2) an acceleration of the rate at which the subsequent respiratory decline occurs [11, 12]. Both phenomena are specific for K (and certain other ions, e.g. rubidium) but are not due to the concomitant hypertonicity since they can not be evoked by equally

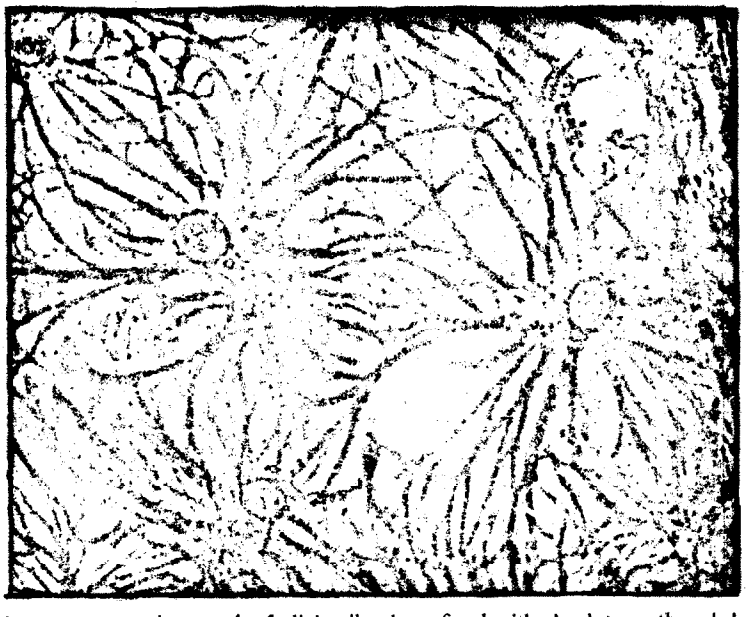

Fig. 1. Phase contrast micrograph of glial cell culture fixed with absolute methanol, but otherwise similar to those used for the metabolic experiments.

high concentrations of Na[11]. An elevated concentration (10 mM) of Mg, which has a depressant effect on the CNS[13], counteracts both the initial stimulation and the subsequent accelerated respiratory decline evoked by excess K[11], and the "K stimulated" respiration has been found more sensitive than the unstimulated respiration to relatively low concentrations of barbiturates[14, 15].

Experiments with bulk-prepared, microdissected or cultured glial cells have shown that glial cells react to K in the same way as brain slices, i.e. display both a shortlasting stimulation and a subsequent rapid decline (half-time about 30 min or even less, of the rate of oxygen uptake[16–19]. This decline is so fast that special precautions have to be taken (e.g. K addition during the metabolic experiments) in order to demonstrate the initial stimulation. Such experiments are not easily performed when micromethods are used, whereas the subsequent K-induced increase in rate of respiratory decline is easily established[17–19].

In order to study whether barbiturates may also specifically affect respiration in glial cells exposed to a high concentration of K oxygen uptake has in the present study been measured in microsamples of glial cells (astrocytes) from primary cultures. The cultures were obtained from the dissociated cerebral cortices of newborn DBA mice as previously described, and were grown in a modified Eagle's MEM with 20 per cent fetal calf serum for 3 weeks, during the last of which 0.25 mM dibutyryl cyclic AMP (dBcAMP) was added to the medium[20]. Such cultures are very much enriched in morphologically and biochemically[21] well differentiated astrocytes and most of the cells acquire the morphology shown in Fig. 1. Using a stainless steel insect pin, an area containing 50–60 of these cells was carefully cut out from the monolayer culture, loosened from the surface of the culturing flask and introduced together with about 1 μl of medium into a Cartesian diver. Rates of oxygen uptake were measured as previously described[17, 22].

RESULTS AND DISCUSSION

Figure 2 shows the average cumulative oxygen uptake (μl) per diver (50–60 cells) containing respectively, the modified Eagle's MEM with 20 per cent fetal calf serum, 20 mM hepes (pH 7.3) instead of bicarbonate, and a low (5 mM) concentration of K (upper solid line) and a corresponding medium to which 50 mM KCl had been added (middle solid line), as well as the apparent oxygen uptake in control divers containing no cells but the usual amount of medium (lower solid line). It can be seen that the apparent respiration in the control divers is not negligible. This is due to the presence of serum in the medium since proteins are susceptible to auto-oxidation. Exposure of the cells to a serum-free medium might, however, have affected their metabolic machinery as has previously been observed with neuroblastoma cells, and the relatively small S.E. for the controls allows a reliable correction of the experimental results by subtraction of the control values [23]. In the divers containing cells exposed to the medium with 5 mM K, the respiration

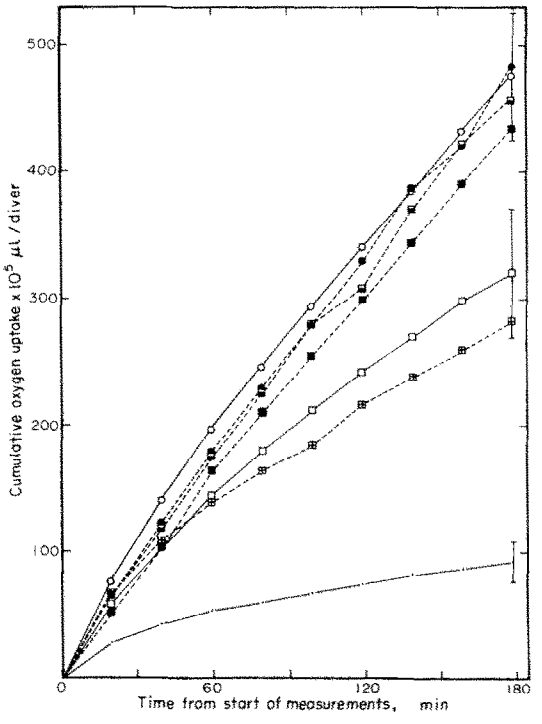

Fig. 2. Apparent respiration by control divers without cells (– · – · –) and cumulative oxygen uptakes by divers containing about 55 individual glial cells incubated in a hepes-buffered modified Eagle's MEM with 5 (○ and ●) or 55 mM (□, ⊞, ⬓, ■) mM K and zero (○ and □), 0.05 (⊞), 0.5 (⬓) or 1.0 (● and ■) mM phenobarbital. Standard errors are shown for the 180 min uptakes for (○, □ and – · –). Average number of cells per diver were 54 (⊞), 55 (○ and ⬓), 57 (□), 58 (●) or 62 (■) cells.

is well maintained and amounts (after correction for the control) to almost 400×10^{-5} μl per 3 hr or, with an average content of 55 cells per diver, 2.5×10^{-5} μl/hr per cell. This value is three times higher than that previously observed in rat astrocytes cultured in the absence of dBcAMP (0.75×10^{-5} μl/hr per cell or 130 μmole/hr per g wet wt.) and incubated in a K-buffered, glucose-containing saline[24]. Provided the cell size is the same in the present study, this suggests a respiratory rate of about 400 μmole/hr per g wet wt. under the present circumstances. However, an average protein content of 370 pg/cell indicates a somewhat larger size and, on the assumption of a protein content of about 100 mg/g wet wt.[21] (i.e. corresponding to that in grey matter of the brain), a respiratory rate of 300 μmole/hr per g wet wt. can be calculated. This high value supports the concept that glial cells account for at least one third of the respiration of the brain cortex[25]. In accordance with previous observations the respiration is less well maintained during incubation in the K-rich medium[17–19]. The decline in respiratory intensity occurs so rapidly that, after correction for the control, the oxygen consumption amounts to little more than 200×10^{-5} μl for the 3 hr period; this is significantly lower ($P < 0.05$) than the oxygen uptake of 400×10^{-5} μl per 3 hr in the presence of the low K concentration.

The stippled lines show the cumulative oxygen

uptakes with varying concentrations of phenobarbital added to normal or K-rich media. It can be seen that the oxygen uptakes are well maintained and amount to 350–400 $\times 10^{-5}$ μl per 3 hr during incubation in media with 0.5–1.0 mM phenobarbital regardless whether excess K is present or not, whereas the respiratory decline characteristic for the K-rich incubation media is observed when only 0.05 mM phenobarbital was added to the K-rich medium. Under the present experimental conditions pentobarbital is thus without any effect on the oxygen uptake during incubation in a medium with 5 mM K. This may seem at variance with the respiratory decrease of about 60 per cent observed by Roth–Schechter and Mandel when 1.0 mM pentobarbital was added to primary cultures of astrocytes or to cells from the NN glial cell line [26, 27]. This discrepancy might, however, be explained by the fact that two different barbiturates were studied and that the culturing conditions differed, since no dBcAMP had been added to the cells used in the experiments by Roth–Schechter and Mandel.

The complete prevention by 0.5–1.0 mM phenobarbital of the increased respiratory decline in astrocytes exposed to 50 mM K is compatible with the inhibition of K effects on brain slice respiration by barbiturates [14, 15]. Such a qualitative similarity between brain cortex respiration and glial respiration is easily understandable on the assumption that glial cells account for a major part of the oxygen uptake by the brain cortex. The effect by phenobarbital might be of pharmacological importance on account of the increase in the extracellular K concentration in the brain cortex under physiological and pathological conditions and in view of the indications that active uptake into glial cells may be involved in the primary removal of excess extracellular K [1–3]. Conceivably, a K-induced decline in glial energy metabolism might indicate a metabolic fatiguability of such a glial uptake system. 0.5 mM phenobarbital (116 mg/l) was found sufficient to prevent this decline. This concentration is only 2–3 times higher than the serum concentrations encountered in ambulatory epileptic patients under continuous treatment with phenobarbital, and the insufficient concentration of 0.05 mM is below that of about 15 mg/l often required to control seizures [28].

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