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EFFECTS OF BARBITURATES ON ENERGY AND INTERMEDIARY METABOLISM IN CULTURED ASTROCYTES

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Abstract

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Effects of barbiturates on utilization of the two substrates, glucose and glutamate, were studied in astrocytes in primary cultures. Carbon dioxide formation from glucose was under ordinary conditions not affected by barbiturates but in the presence of 10 μM malate there was a potassium-induced stimulation (20-25%) which was significantly ($P < 0.001$) inhibited (30-35%) by pentobarbital (0.5 mM). Glutamate oxidation was not enhanced by excess potassium but there was a distinct dose-dependent reduction in the presence of pentobarbital. In contrast, pentobarbital or phenobarbital had no effect on the formation of glutamine from glutamate.

Key words: astrocytes, barbiturates, carbon dioxide, energy metabolism, glucose, glutamate, pentobarbital

Abbreviations: carbon dioxide (CO_2), potassium (K^+)

Introduction

Barbiturates undoubtedly act on the central nervous system by several different mechanisms of action. One of these is that they reduce the metabolic activity of the brain in vivo in parallel with the depression of cortical activity (Kety, 1957; Lassen, 1959; Sokoloff et al., 1977). Approximately identical observations have been made in the perfused cat brain under similar conditions (Geiger and Magnes, 1947). An effect of barbiturates on oxygen consumption by brain slices has also been observed by several authors but it seems that it is mainly the stimulation evoked by, e.g., high potassium (K^+) concentrations which is sensitive to the barbiturates. This can be seen from Table 1 which shows that the stimulation of oxygen uptake evoked by excess potassium (respiration in high K^+ medium minus respiration in low K^+ medium) is inhibited by 50% in the presence of 1.0 mM pentobarbital whereas in the absence of an elevated potassium concentration there is only a relatively slight, statistically non-significant inhibition. A similar potassium induced stimulation of oxygen uptake has been observed in microdissected glial cells (Hertz, 1966; Aleksidze and Blomstrand, 1969) and in primary cultures of astrocytes (Hertz et al., 1973; Hertz and Hertz, 1979; Hertz, 1981). Again,barbiturates inhibit the respiration only in the presence of excess potassium whereas in the presence of a normal potassium concentration, they have little effect (Table 1, right part). In neurons the situation is quite different. Most authors agree that potassium does not cause any stimulation of oxygen uptake (for a review see, e.g., Hertz, 1978). This has also been found to be the case in cortical

Table 1

Rate of oxygen uptake (μmol/min per 100 mg protein) by brain slices or primary cultures of astrocytes

	Brain Slices[1]		Primary Cultures[2]	
	5 mM K^+	55 mM K^+	5 mM K^+	55 mM K^+
Control	1.42±0.10	1.74±0.08*	5.3±0.91	7.9±0.58**
Pentobarbital (1 mM)	1.19±0.16	1.35±0.07	6.0±1.05	4.5±0.68

[1] From Weiss et al., 1972; [2] from Hertz, 1981; * Significantly different from 1.42 ($P < 0.05$) and from 1.35 ($P < 0.01$); ** Significantly different from 5.3 ($P < 0.05$) and from 4.5 ($P < 0.01$).

neurons in primary culture where there was no indication of a potassium induced stimulation (Hertz, 1981). Both in the presence and absence of excess potassium, pentobarbital seemed to cause a slight inhibition, which, however, was not statistically significant (Hertz, 1981).

Since the oxygen consumption in astrocytes is affected by barbiturates, these drugs must in all likelihood also affect substrate utilization and in the present work we have studied the CO_2 production from the two substrates, glucose and glutamate, both of which are known to be metabolized by astrocytes in primary cultures (Yu and Hertz, 1983). In addition to being oxidatively metabolized, glutamate can be converted to glutamine in astrocytes (Yu et al., 1982), and also this process was followed.

Methods

Cell Cultures: Primary cultures of astrocytes were prepared as described by Hertz et al. (1982). The cerebral cortex of newborn Swiss mice was freed of meninges and dissociated in a modified Eagle's minimum essential tissue culture medium (MEM) with 20% horse serum. The cell suspension was passed twice through sterile nylon Nitex® sieves (80 μm and 10 μm pore size respectively) and introduced into 60-mm Falcon petri dishes. The cultures were incubated at 37°C in a 95%/5% (vol/vol) mixture of atmospheric air and CO_2. After 2 weeks they reached confluency and were grown for another 1-2 weeks in the additional presence of 0.25 mM dibutyryl cyclic AMP which induced a morphological differentiation. Such cultures constitute an excellent model for astrocytes *in situ*, 95% of the cells in the cultures are astrocytes, and neurons are absent (Hertz et al., 1982).

CO_2 Production: $^{14}CO_2$ production rates from either L-[1-^{14}C]-glutamate or [U-^{14}C]-glucose were measured as described by Yu et al. (1982). Individual culture dishes without lids were placed in a gas tight chamber equilibrated with a CO_2/air atmosphere. At the beginning of the experimental period, the radioisotope, diluted in medium, was added to the cultures by injection (0.1 μCi/ml for glutamate and 13 μCi/ml for glucose). At its end 0.4 M perchloric acid (500 μl) was injected into the culture dishes to acidify the medium, and hyamine hydroxide (2 ml) was injected into a suspended beaker. The radioactivity in the hyamine hydroxide was determined after quantitative trapping of CO_2 by heating of the chamber for 30 min from underneath in a water bath at 60°C. The CO_2 production per milligram of protein was calculated from the counts in the hyamine hydroxide, the specific activity of glutamate or glucose in the medium, and the protein content in the cultures.

Glutamine Production from [U-^{14}C]-glutamate: The specific activity of glutamine was determined as described by Potter et al. (1982) and Yu et al. (1982), i.e., by allowing [^{14}C]-glutamine to react with [^{3}H]-dansyl chloride and use the $^{14}C/^3H$ ratio as an indication of specific activity. From the alteration in specific activity of glutamine

with time, the specific activity of the precursor, glutamate, and the pool size of glutamine, the actual glutamine production rates can calculated (for details see Potter et al., 1982; Yu et al., 1982). Since short term exposure to barbiturates does not seem to affect the glutamine pool size or the specific activity of glutamate, the specific activities after 15 min of exposure to labelled glutamate were used as an indication of glutamine production rate.

Results

Carbon dioxide production from ^{14}C-glucose: Table 2 (left part) shows the CO_2 formation rate from [^{14}C]-glucose in cultured astrocytes incubated in the normal tissue culture medium in the absence or presence of 0.5 mM pentobarbital and/or 55 mM potassium. All values are shown as percentages of the CO_2 production in the control medium (with normal K^+ concentration and no pentobarbital) which amounted to 0.56±0.04 µmol/min per 100 mg protein and it can be seen that the formation of CO_2 was not significantly altered by pentobarbital, by potassium, or by the two compounds in combination.

Low concentrations of malate are known to affect glucose metabolism in astrocytes (Yu and Hertz, 1983). The effect of pentobarbital was therefore also studied in the presence of 10 µM of this compound which by itself had no effect on CO_2 production from glucose (0.52±0.02 µmol/min per 100 mg protein in the presence of malate). From Table 2 (right part) it can be seen that under these conditions a high concentration of potassium does stimulate glucose metabolism and that this stimulation is abolished in the presence of 0.5 mM pentobarbital. In the absence of excess potassium, pentobarbital had no significant effect.

Table 2

Effects of potassium (55 mM) and/or pentobarbital (0.5 mM) on $^{14}CO_2$ production from [U-^{14}C]-glucose in primary cultures of astrocytes in the absence or presence of 10 µM malate

	No Malate		10 µM Malate	
	5 mM K^+	55 mM K^+	5 mM K^+	55 mM K^+
Control	100.0±3.3	102.1±6.6	100.0±3.2	120.6±2.6*
Pentobarbital (0.5 mM)	108.9±11.6	111.2±20.3	90.0±5.8	82.1±2.5**

* $P < 0.01$ for difference from 100%; ** $P < 0.001$ for difference from 120.6%

Carbon dioxide production from glutamate: Glutamate is known to be metabolized by an oxidative deamination, catalyzed by glutamate dehydrogenase, in astrocytes in primary cultures (Yu et al., 1982; Yu and Hertz, 1983). The effect of barbiturates on CO_2 formation from L-[1-^{14}C]-glutamate was therefore also studied, both during incubation in media with normal (5 mM) and elevated (55 mM) potassium concentrations. In the control medium the CO_2 production from [1-^{14}C]-labelled glutamate amounted to 0.31±0.03 µmol/min per 100 mg protein. From Fig. 1 it can be seen that potassium alone had no effect on the metabolic degradation of glutamate. One mM pentobarbital inhibited the $^{14}CO_2$ production to a considerable extent (more than 50%) at both high and low potassium concentrations. At a lower concentration of pentobarbital (0.25 and 0.5 mM) the CO_2 production was, however, only inhibited by the barbiturate in the presence of excess potassium. A still lower concentration of pentobarbital (0.1 mM) caused a statistically significant ($P < 0.005$) enhancement of the $^{14}CO_2$ production (35%) in the presence of a normal concentration of potassium, but had less effect in the presence of the elevated potassium concentration. This enhancement at low concentrations may well correspond to other indications that low concentrations of barbiturates have an excitatory effect whereas higher concentration have a depressant effect (Roth et al., this volume).

Glutamine formation from glutamate: In astrocytes glutamate is not only degraded to carbon dioxide but also converted to glutamine, a process catalyzed by glutamine synthetase. Fig. 2 shows the specific activity of glutamine after 15 min of incubation in a medium containing L-[U-^{14}C]-glutamate. As described in "Methods" this is, under certain conditions, an indication of the rate with which glutamine becomes radioactively labelled, i.e., of its rate of synthesis. From Fig. 2 it can be seen that the specific activity of glutamine is the same regardless of the addition of different concentrations of pentobarbital and that phenobarbital also has no effect.

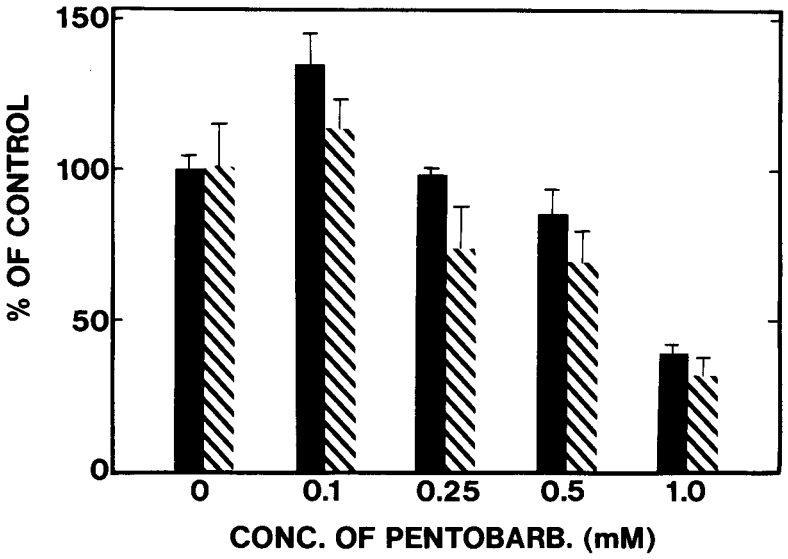

Fig. 1. Rates of CO_2 formation from L-[1-^{14}C]-glutamate, as percentages of control values, in primary cultures of astrocytes in the absence (■) and presence (▨) of excess potassium (55 mM) and during exposure to different concentrations of pentobarbital.

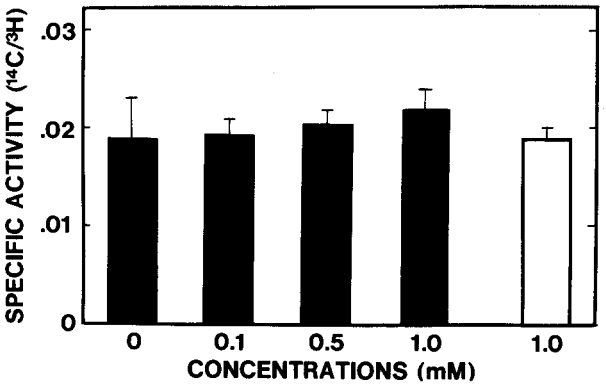

Fig. 2. Specific activity of glutamine after 15 min of exposure to [U-^{14}C]-glutamate and different concentrations of pentobarbital (■) or 1.0 mM phenobarbital (□).

Discussion

In agreement with the effect of barbiturates on oxygen uptake shown in Table 1 the barbiturate effects on CO_2 production in astrocytes from either glucose or glutamate were more pronounced in the presence of an elevated potassium concentration. This may be of major functional importance since CNS activity leads to an increase in the extracellular potassium concentration (e.g., Walz and Hertz, 1983).

The effect on glucose oxidation may be the easiest to interpret and it seems logical that a decreased oxygen uptake in the presence of a barbiturate is paralleled by a decreased CO_2 production from glucose. The effect on CO_2 production from glutamate may be more difficult to interpret. This CO_2 production is known to reflect a conversion of glutamate by an oxidative deamination to -ketoglutarate and a subsequent decarboxylation of this compound to succinate (Yu et al., 1982). The inhibitory effect of barbiturates on carbon dioxide production from glutamate therefore suggests that the activity of glutamate dehydrogenase, the enzyme which catalyzes the oxidative deamination, is inhibited by barbiturates. This is in good agreement with the finding by Roth-Schechter et al. (1979) that chronic exposure to barbiturates, which induces a tolerance in cultured astrocytes, leads to a very marked increase in the activity of the glutamate dehydrogenase in the cells. This effect seems to be quite specific since it is not reflected by a barbiturate induced effect on glutamine synthesis (Fig. 2), another metabolic route from glutamate.

It is unknown whether there is any common denominator for the barbiturate effects on glucose metabolism and on glutamate metabolism. One possible interaction would be if glutamate could replace malate, which under our experimental conditions was essential for the potassium-induced enhancement of CO_2 production from glucose and for the effect of barbiturates on this process (Table 2). If this is indeed the case, then a decreased formation of -ketoglutarate from glutamate in the presence of barbiturates might possibly cause a lack of tricarboxcylic acid cycle constituents which are essential for the responsiveness of glucose metabolism to excess potassium.

A different question is the lack of quantitative agreement between the rate of oxygen consumption and that of CO_2 production from glutamate plus that from glucose. One explanation for this discrepancy is that glutamate is further metabolized from succinate and that the use of [1-^{14}C]-glutamate fails to include this additional metabolic degradation and another is that also other substrates, e.g., glutamine and lactate, may be metabolized by astrocytes (Yu and Hertz, 1983).

Conclusion

The present work has demonstrated certain effects of barbiturates on energy metabolism in astrocytes. Mainly the effects of relatively high concentrations were discussed, but the biphasic character of the effect of pentobarbital on carbon dioxide formation from glutamate should be kept in mind. It should also be remembered that barbiturates exert several other actions on astrocytes, e.g., a decrease in uptake rate for the inhibitory transmitter GABA and for potassium (Hertz et al., 1980) and a displacement of benzodiazepines from astrocytic binding sites (Hertz et al., this volume). The effects of barbiturates on astrocytes do not preclude that barbiturates also affect neurons. The neuronal effects of barbiturates are probably distinctly different from those exerted on astrocytes as, e.g., reflected by different effects on benzodiazepine binding in the two cell types (Hertz et al., this volume). An understanding of the effects of these drugs on the function of the central nervous system thus requires information of both neuronal and astrocytic effects.

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