ELSEVIER

European Journal of Pharmacology 446 (2002) 53–61

www.elsevier.com/locate/ejphar

Amobarbital inhibits K^+-stimulated glucose oxidation in cerebellar granule neurons by two mechanisms

Liang Peng [a], Leif Hertz [a,b,*]

[a] *Hong Kong DNA Chips, Limited, Kowloon, Hong Kong, China*
[b] *Department of Pharmacology, University of Saskatchewan, Saskatoon, Canada*

Received 29 November 2001; received in revised form 1 May 2002; accepted 6 May 2002

Abstract

The study aimed at determining mechanism(s) by which amobarbital (amytal) suppresses glucose oxidation in cerebellar granule neurons in primary cultures, a glutamatergic preparation. When challenged with a depolarizing K^+ concentration (55 mM), the cells doubled their rate of glucose oxidation (production of $^{14}CO_2$ from U-[^{14}C]glucose) and glycolysis (lactate accumulation). At normal K^+ concentration, amobarbital reduced $^{14}CO_2$ production with half-maximum effect at 0.5–1 mM; at 55 mM K^+, the inhibition was more potent, with more than half of the K^+-induced stimulation abolished at 50 μM. Dixon plot analysis showed a single inhibitory mechanism at 5.4 mM K^+, but at 55 mM K^+, two kinetically different mechanisms could be distinguished. A more pronounced compensatory amobarbital-induced increase in glycolysis at 5.4 than at 55 mM K^+ suggested that amobarbital in addition to its inhibition of mitochondrial respiration inhibited K^+-induced increase in energy demand, probably by its known suppression of stimulated glutamate release.

Keywords: Anesthesia; Barbiturates; Cytoprotection; Glucose phosphorylation; Glutamate; Oxidative metabolism

1. Introduction

Barbiturates decrease oxygen and glucose consumption in intact brain (Carlsson and Siesjo, 1975; Nilsson and Siesjo, 1975; McQueen et al., 1984; Hodes et al., 1985; Blacklock et al., 1987; Otsuka et al., 1991; Khan et al., 1997). They have a similar effect in brain slices, where the increased respiration caused by electrical stimulation or exposure to a depolarizing K^+ concentration is more sensitive to the inhibition than the unstimulated oxygen uptake (McIlwain, 1953; Ghosh and Quastel, 1954). The barbiturates inhibit energy metabolism by interference with NADH oxidation, and amobarbital (amytal) is the prototype of a barbiturate inhibiting mitochondrial respiration (Aldrich and Parker, 1960; Gutman et al., 1970). This is the reason that amobarbital was chosen for the present study in spite of its limited clinical use. Regulatory mechanisms triggered by impairment of ATP re-synthesis by oxidative phosphorylation (including energy charge, NAD^+/NADH ratio and concentrations of glycolytic and oxidative intermediates [reviewed by Hertz and Dienel, in press]) lead in synaptosomes and in the perfused heart to an increase in glycolysis, which compensates for a considerable part of the reduction in oxidative production of ATP (Nishiki et al., 1979; Dagani and Erecinska, 1987).

Barbiturates have hypnotic-sedative and anesthetic effects (Fischer and von Mering, 1903; Bolliger and Maddox, 1931), and they may enhance neuronal survival following cerebral energy failure (Belopavlovic and Buchthal, 1980; Spetzler and Hadley, 1989; Mortier et al., 2000). It has been disputed whether the anesthetic effect is secondary to barbiturate-induced inhibition of energy metabolism (Quastel and Wheatley, 1932) or to inhibition of energy-requiring processes (Larrabee, 1965; Michenfelder and Theye, 1970; Michenfelder, 1974). In support of the latter concept, it was recently found that relatively low concentrations of barbiturates inhibit evoked release of glutamate from cultured cerebellar granule cells and brain slices (Miao et al., 1998; Qu et al., 2000; Buggy et al., 2000). This observation raises the question whether inhibition of glutamate release by barbiturates may contribute to their spe-

* Corresponding author. RR 2 Box 245, Gilmour, Ontario, Canada K0L 1W0. Tel.: +1-613-474-0537; fax: +1-613-474-0538.
E-mail address: lhertz@northcom.net (L. Hertz).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01794-6

cially marked effect on stimulated respiration in brain tissue, that is, whether evoked release of glutamate normally increases energy metabolism.

Primary cultures of cerebellar granule cells release large amounts of glutamate in a Ca^{2+}-dependent manner in response to K^+-induced depolarization (Drejer et al., 1982; Levi et al., 1984; Peng et al., 1991; Varming et al., 1997). Exposure to high K^+ concentrations also increases the rate of oxidative metabolism (Peng et al., 1994), although this response is virtually abolished in cultures grown in such a manner that the K^+-induced glutamate release is impaired and the cultures show dendritic degeneration (Peng and Hertz, 1993). This finding suggests that the metabolic response to depolarizing K^+ concentrations mainly may be exerted by release of glutamate, whereas the direct stimulatory effect of K^+ on glucose metabolism is modest, possibly due to rapid inactivation of Na^+ channels during maintained depolarization. Cultured cerebellar granule cells express glutamate receptors together with high-affinity GABA (γ-aminobutyric acid) receptors of both the $GABA_A$ and the $GABA_B$ subtype (Meier and Schousboe, 1982; Drejer et al., 1986; Schousboe, 1999), and synaptic profiles are abundant (Peng et al., 1991). However, no information seems to exist whether exposure to glutamate can increase the metabolic activity in cerebellar granule neurons.

The first aim of the present study was to investigate whether glutamate does, indeed, stimulate energy metabolism in cerebellar granule cells. This was the case, supporting the hypothesis that amobarbital may exert part of its metabolic inhibition of K^+-stimulated glucose metabolism in cerebellar granule neurons by inhibiting glutamate-mediated stimulation of both oxidative metabolism and glycolysis. The second goal was to compare the effect on K^+-stimulated and non-stimulated glucose oxidation by a wide range of amobarbital concentrations to investigate potential differences between amobarbital's effect on stimulated and non-stimulated metabolism as well as differences between different concentration ranges of amobarbital (i.e., whether evidence could be obtained for a distinction between more and less potent effects). A kinetic analysis revealed that amobarbital inhibits only one parameter during exposure to a normal K^+ concentration, but two different parameters, one more potently than the other, during exposure to a depolarizing K^+ concentration. The third goal was to distinguish between the possibilities that (1) amobarbital inhibits K^+-stimulated metabolism by two different mechanisms, which both suppress energy metabolism directly, or (2) that amobarbital in addition to its well-established inhibition of one energy-yielding mechanism (NADH oxidation) also inhibits an energy-requiring mechanism (such as glutamate-mediated stimulation of energy metabolism). In support of the second interpretation, it was found that amobarbital inhibits glycolysis to a larger extent during incubation in medium with a normal K^+ concentration than in K^+-rich medium. This observation suggests that during exposure to a depolarizing K^+ concentration, amobarbital increases glycolysis (due to inhibition of oxidative metabolism) and suppresses an energy-requiring process.

2. Methods and materials

2.1. Cell cultures

Cerebellar granule cells were cultured as previously described (Schousboe et al., 1989; Peng et al., 1991), with minor modifications, and following procedures approved by the Animal Care Committee of University of Saskatchewan. Briefly, 7-day-old mouse pups were rapidly decapitated and the brains removed. After removal of the meninges, the tissue was cut into cubes of ~0.4 mm side dimensions, exposed to trypsin in a calcium–magnesium-free salt solution, reintroduced into tissue culture medium, passed through nylon sieves (80 μm pore width) and seeded into polylysine-coated standard 35-mm-Falcon tissue culture dishes, using one cerebellum per culture dish. The cultures were grown in a slightly modified Dulbecco's medium (Juurlink and Hertz, 1992), in which the glucose concentration was increased to 30 mM and the K^+ concentration to 24.5 mM, the glutamine concentration was decreased to 0.8 mM, and 100 μU/l insulin and 7% horse serum were added. The elevated K^+ concentration is necessary for normal development of the cells (mimicking incoming neuronal activity during development in vivo), the glucose concentration was increased to eliminate medium change, which neuronal cultures tolerate poorly, and the reduced glutamine concentration enhances the survival of these cells. After 2 days, cytosine arabinoside was added to the medium to a final concentration of 40 μM to curtail the number of astrocytes that develop in the cultures (to <10% of total cell volume in mature cultures). Since this compound degrades rapidly at 37 °C and the cells do not tolerate change of medium well, no subsequent medium change was made. The cells were used at the age of 10 days, when they have developed characteristics of mature cerebellar granule neurons in vivo (Schousboe and Hertz, 1987; Peng et al., 1991).

2.2. Measurement of glucose oxidation

Rates of oxidative metabolism were determined by measuring production of labeled CO_2 from U-[^{14}C]glucose (Yu et al., 1982), and they were expressed as nanomoles glucose metabolized per minute per milligram protein. This value is nominal, because the intracellular specific activity of glucose is unknown, and because label is diverted by retention of glucose in intermediates, inclusive released lactate. Tissue culture medium with [^{14}C]-labeled glucose and either 5.4 or 55 mM K^+ was added to intact cultures after aspiration of culturing medium and washing with fresh, serum-free medium. Immediately thereafter, each dish was

introduced into its own airtight chamber, which was closed with a rubber stopper. At the end of the 60-min incubation period at 37 °C in an atmosphere of air with 5% CO_2, acetic acid was injected through the stopper into the culture dishes to acidify the medium to expel all CO_2. At the same time, a NaOH solution was injected into plastic beakers presuspended in the chambers to trap released CO_2. After 30 min, the chambers were opened, and the beakers with trapped radioactivity and samples of incubation medium were transferred separately into scintillation counting vials for determination of radioactivity. The amount of protein per culture was determined by the Lowry method (Lowry et al., 1951), using serum albumin as the standard.

2.3. Measurement of lactate production

Glycolysis was measured by determination of lactate in the medium and in the cells at the end of 1 h of incubation in tissue culture medium in an air/CO_2 atmosphere by the aid of an Amersham kit, in which lactate was oxidized to pyruvate and concomitant reduction of NAD^+ was recorded. Each vial of 10 mg NAD^+ was reconstituted with 2 ml glycine buffer, double-distilled water and 0.1 ml of the supplied lactate dehydrogenase preparation. One milliliter of the reaction mixture was added to each medium sample (0.1 ml), to an appropriate amount of cell extract, and to blanks (fresh medium alone). The total reaction volume was increased to 3 ml by addition of double-distilled water, mixed by vortexing, and incubated for 30 min at 25 °C. At the end of the incubation period, absorbance values of NADH were read at 340 nm, using a water blank as the reference. The lactate contents were calculated from a standard calibration curve, and expressed per milligram protein, determined as described above. These values represent the balance between production of lactate from glucose and utilization of lactate and its precursor pyruvate by oxidative metabolism, and >80% of the total amount of lactate was found in the incubation media. From previous experiments, it is known that the lactate concentration in the medium increases almost rectilinearly during the first few hours of incubation, although lactate oxidation increases with the increase in its concentration, suggesting that the rate of lactate accumulation provided an only slightly underestimated measurement of the rate of glycolysis.

2.4. Measurement of glucose phosphorylation

Determination of $^{14}CO_2$ formation from labeled glucose or of lactate accumulation in the medium was unsuited for determination of the effect of glutamate on energy metabolism, because the amount of protein per culture became moderately decreased, compared to control cultures incubated in normal medium. Instead, the rate of glucose phosphorylation was measured using U-[^{14}C]deoxy-D-glucose, a glucose analog that is phosphorylated but not further degraded and accumulates in intact cells as [^{14}C]deoxy-D-glucose phosphate. After an initial wash, the cells were incubated at 37 °C for 30 min in tissue culture medium in an air/CO_2 atmosphere in the presence of U-[^{14}C]deoxy-D-glucose. After the experiments, the cultures were rinsed by three thorough washes (~ 5 s each) with tissue culture medium before cell lysis in a solution of NaOH. This method has previously been found appropriate to remove non-phosphorylated deoxyglucose (Hertz et al., 1998). Protein content and radioactivity were measured in aliquots of the NaOH solution, and from these values, combined with the measured radioactivity in the incubation media, relative rates of glucose phosphorylation were calculated and expressed both per culture (there was only a slight variability in the protein contents per culture within each batch of cultures) and per milligram protein.

2.5. Statistical analysis

Statistical analysis was performed by ANOVA (analysis of variance) except for Fig. 2, where 95% confidence limits for both the experimental value observed at 1 mM amobarbital and the value at this concentration, which could be extrapolated from the correlation between metabolism and amobarbital concentration in the concentration interval 0–0.5 mM, were determined as described by Zar (1984).

3. Results

3.1. Effect of glutamate on glucose phosphorylation

Phosphorylation of deoxy-D-glucose per culture during 30 min of incubation was significantly increased by 50 μM glutamate, and 500 μM had a larger stimulatory effect (Table 1). This was in spite of the fact that the protein content in the glutamate-treated cultures had declined by 15–20%. Accordingly, expression of glutamate stimulation per milligram protein (which is justifiable, because only deoxy-D-glucose phosphate remaining in the cells is measured) showed a stimulation of >40% by 500 μM glutamate. However, if the incubation period was extended to 60 min, there was a distinct reduction of deoxy-D-glucose phosphorylation per culture (due to cell death during the incubation), and there was no longer any stimulation of deoxy-D-glucose phosphorylation per milligram protein (results not presented).

3.2. Amobarbital effect on glucose oxidation

In the medium with normal K^+ concentration (5.4 mM) and no amobarbital (control conditions), $^{14}CO_2$ production by intact primary cultures of mouse cerebellar granule neurons amounted to 0.79 ± 0.05 nmol/min per mg protein ($n = 20$). Elevation of the concentration of K^+ to 55 mM almost doubled $^{14}CO_2$ production rate. There was only little variability between individual cultures from the same cul-

Table 1
Effects of extracellular L-glutamate on rate of glucose phosphorylation, measured as phosphorylation of [^{14}C]deoxyglucose (deoxy-D-glucose-P), during 30 min of incubation in tissue culture medium

Parameter	0 Glutamate	50 μM Glutamate	500 μM Glutamate
Deoxy-D-glucose-P per culture, %	100 ± 3.5[a]	115.6 ± 3.9	124.5 + 1.8
Protein per culture, mg	337.6 ± 6.4	300.5 ± 3.6	295.1 + 1.3
Deoxy-D-glucose-P per milligram protein, %	100 ± 2.5	130.7 ± 3.9	142.2 + 1.2

All results in the presence of glutamate are statistically significantly different ($P < 0.05$ or better) from controls (0 glutamate), and the effect of 500 μM glutamate on deoxy-D-glucose phosphorylation, expressed per milligram protein, is statistically different from that of 50 μM glutamate.

[a] All rates of deoxy-D-glucose phosphorylation (dpm/min) are expressed as percentages ± S.E.M. values of that in 'sister' cultures, which were not exposed to glutamate. Results are averages of three individual experiments.

ture batch but more pronounced batch-to-batch variation. To compensate for the variability in rates of CO_2 production between cultures from different batches in the examination of the correlation between amobarbital concentration and rates of glucose oxidation, all values were expressed relative to those observed in the same batch of cultures ('sister' cultures) during incubation under control conditions.

Fig. 1 shows the effect of amobarbital concentrations between 50 μM and 2.5 mM on rate of $^{14}CO_2$ formation from U-[^{14}C]glucose during incubation in media containing either 5.4 or 55 mM K^+. In the medium with 5.4 mM K^+, $^{14}CO_2$ production was only little affected by amobarbital concentrations up to 0.25 mM, but at amobarbital concentrations above 0.25 mM, it decreased gradually to one fifth of its value in the absence of amobarbital. EC_{50} was between 0.5 and 1.0 mM. The 'stimulated' respiration during exposure to 55 mM K^+ was much more potently inhibited by amobarbital, with more than 50% reduction of the stimulatory effect (but not of total respiration) at 50 μM. At higher amobarbital concentrations, there were only minor differences between the respiratory rates in the presence and absence of excess K^+, although rates of $^{14}CO_2$ production were consistently slightly lower in the K^+-rich medium at amobarbital concentrations at or above 1 mM (Fig. 1).

A Dixon-type plot of the same results is shown in Fig. 2. It can be seen that the reciprocal values of the relative rates of CO_2 production during incubation in the low-K^+ medium followed a straight line (correlation coefficient 0.98), when expressed as a function of the concentration of amobarbital, except at very low and very high amobarbital concentrations. The adherence to a straight line suggests that amobarbital perturbed only a single parameter (Dixon, 1953; Nishiki et al., 1979), which is likely to be its well-established direct suppression of energy metabolism. The deviation at the low concentrations can be explained by a relatively low potency,

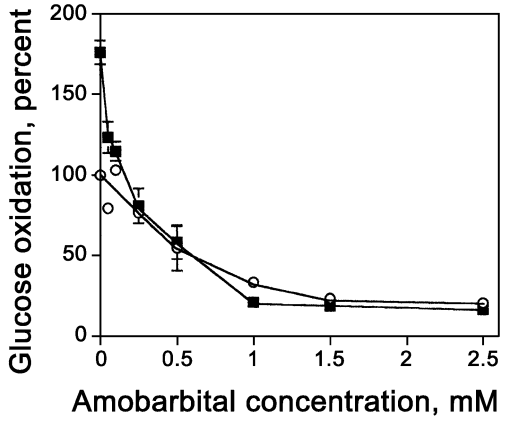

Fig. 1. Effects of amobarbital concentrations between 50 μM and 2.5 mM on rates of conversion of U-[^{14}C]glucose to $^{14}CO_2$ by primary cultures of mouse cerebellar granule neurons incubated in tissue culture medium containing either 5.4 (open circles) or 55 mM K^+ (filled squares) for 1 h. All rates are expressed relative to those observed in same-day experiments in cultures from the same batches incubated at 5.4 mM K^+ in the absence of amobarbital (shown as 100%). Results are mean ± S.E.M. of 4–9 individual experiments from at least two culture batches in the presence of amobarbital and 20 and 21 individual experiments without amobarbital in the presence and absence of elevated K^+, respectively. S.E.M. values are shown by vertical bars, when reaching beyond the symbols. Control rates of U-[^{14}C]glucose oxidation without amobarbital in the low-K^+ medium were 0.79 ± 0.05 nmol/min per mg protein ($n = 20$). The rates of glucose oxidation at 5.4 and 55 mM K^+ differed significantly from each other ($P < 0.05$ or less) at amobarbital concentrations of 0, 0.05 and 1.0 mM.

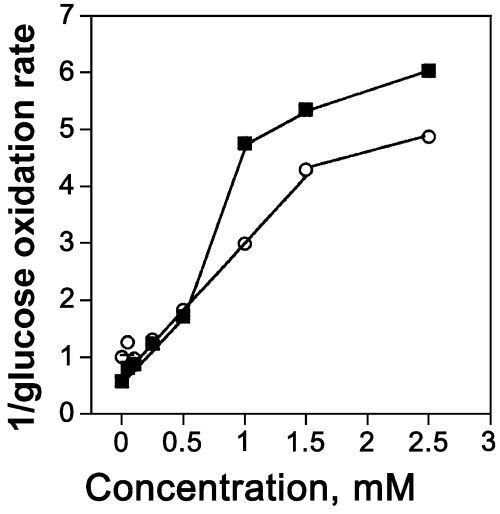

Fig. 2. Dixon-type plot, showing the reciprocal values of the respiratory rates from Fig. 1 as a function of the concentration of amobarbital. In the medium with 5.4 mM K^+ (open circles), a straight line is followed, except at the extremes of amobarbital concentrations (correlation coefficient 0.98), suggesting that only a single mechanism is perturbed by the drug. In the medium with 55 mM K^+ (filled squares), a straight line is followed from 0 to 0.5 mM amobarbital (correlation coefficient 0.99), reflecting the higher potency, but subsequently, the reciprocal values of the metabolic rates deviate from the straight line toward a higher value, that is, a more pronounced inhibition. This behavior is not consistent with impairment of only one mechanism, but indicates the recruitment of a second inhibitory factor when the concentration of amobarbital exceeded 0.5 mM. For statistical analysis of this effect, see Results.

and the deviation at the high concentration of amobarbital reflects that maximum inhibition had been approached or reached. In the K^+-rich medium, the correlation between the reciprocal value of the respiratory intensity and the concentration of amobarbital was different (Fig. 2). There was no deviation at low barbiturate concentrations, reflecting the high potency of the inhibition, and a straight line (correlation coefficient 0.99; *y*-axis intercept at 0 mM 0.64 percentage^{-1}; slope 2.22 percentage^{-1}/mM) was followed only up to an amobarbital concentration of 0.5 mM. When the concentration of amobarbital was further increased to 1 mM, a deviation from the straight line occurred toward a much higher reciprocal value of the rate of glucose oxidation, that is, a lower than expected glucose oxidation rate.

The measured value at 1 mM amobarbital was 4.76 percentage^{-1}, corresponding to a CO_2 production rate of 21.0% of control value, with 95% confidence limits of 3.45 and 6.07 percentage^{-1} (29.0% and 16.5% of control value). This degree of inhibition is significantly higher than the extrapolated value to 1 mM of the straight line delineating the effect of amobarbital concentrations between 0.05 and 0.5 mM, which was 2.86 percentage^{-1} (0.64 + 2.22 percentage^{-1}), corresponding to a CO_2 production rate of 35.0% of control value, with 95% confidence limits of 3.20 and 2.52 percentage^{-1} (31.3% and 39.7%). The statistically significant difference between the extrapolated and the measured values conclusively shows that the increase of amobarbital-mediated respiratory inhibition in the range 0.5–1.0 mM is larger than expected from an isolated effect of the inhibitory mechanism at work in the range 0.05–0.5 mM amobarbital (Dixon, 1953). Since the suppressing effect of amobarbital is more potent during exposure to a depolarizing K^+ concentration than in its absence, it is reasonable to assume that the inhibition within the lower concentration range of amobarbital represents inhibition of K^+-induced glutamate release, and that the additional effect unmasked at higher concentrations represents the conventional barbiturate-mediated inhibition of NADH oxidation, which is approximately equipotent at high and low K^+ concentrations.

3.3. Amobarbital effect on lactate formation

To corroborate the conclusion that amobarbital during exposure to a high K^+ concentration acts both by inhibiting an energy-requiring process (glutamate-mediated stimulation) and by directly suppressing an energy-yielding process (NADH oxidation), the effect of a maximally effective concentration of amobarbital (2.5 mM) on lactate production rates was studied. Under control conditions, the accumulation of lactate in the cells and the incubation medium amounted to ~ 10 nmol/min per mg protein during the first hours of incubation (Fig. 3). This value is in agreement with previously published results (Dienel and Hertz, 2001), and it corresponds to utilization of 5 nmol glucose/min per mg protein. The reason that the rate of lactate release is much

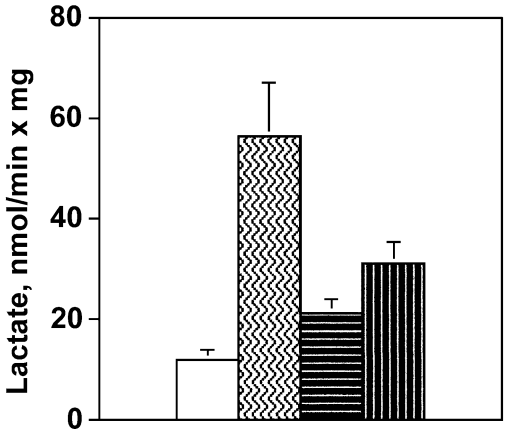

Fig. 3. Effects of 2.5 mM amobarbital on rates of lactate production by primary cultures of mouse cerebellar granule neurons. The cultures were incubated in tissue culture medium containing either 5.4 mM K^+ and no drug (open column), 5.4 mM K^+ and 2.5 mM amobarbital (column indicated by wavelines), 55 mM K^+ and no drug (horizontally striped column), or 55 mM K^+ and 2.5 mM amobarbital (vertically striped column) for 1 h. Results are means ± S.E.M. of three to five individual experiments, using cultures from one to two batches. All values are statistically significantly different ($P < 0.05$ or less) from that at 5.4 mM K^+ in the absence of amobarbital, and the values at 55 mM K^+ with and without amobarbital are significantly different from that at 5.4 mM K^+ plus amobarbital, but not from each other.

higher than the measured rate of glucose oxidation is twofold: (1) the measured rate of glucose oxidation is a nominal value, which represents an underestimate, because isotope equilibration has not taken place; and (2) a large volume of incubation medium compared to a small amount of cells allows lactate efflux to continue at the rate determined by its rate of formation and the activity of the monocarboxylate transporter, a carrier facilitating carrier-mediated transport, which results in net release as long as a concentration gradient exists between intracellular and extracellular lactate concentrations (for further discussion, see Dienel and Hertz, 2001).

During exposure to 2.5 mM amobarbital, lactate production in the medium containing 5.4 mM K^+ increased by a factor of 5, reflecting activation of regulatory mechanisms when ATP production by oxidative phosphorylation is reduced (Fig. 3); in the absence of amobarbital, an increase of the concentration of K^+ to 55 mM approximately doubled the rate of lactate accumulation, a relative increase corresponding to that of glucose oxidation; however, exposure to 2.5 mM amobarbital in the presence of excess K^+ had no additive effect, but increased lactate production significantly less than in the presence of a normal K^+, suggesting that joint exposure to amobarbital and a high K^+ concentration affected glycolysis in two opposite directions: as in the medium with normal K^+ concentration, glycolysis was stimulated due to decreased oxidative phosphorylation, but at the same time, glucose metabolism was decreased by an amobarbital-induced inhibition of energy-requiring processes normally caused by elevated K^+.

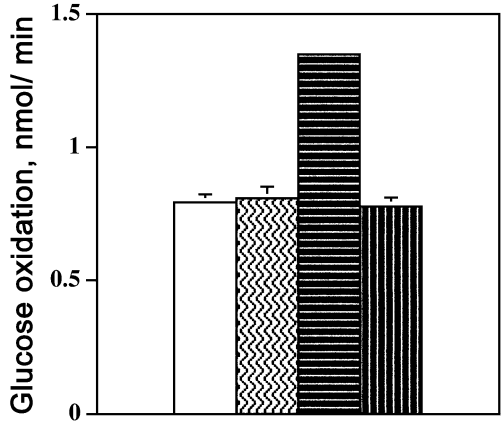

Fig. 4. Effects of omission of Ca^{2+} in the incubation medium, combined with an increase of the concentration of Mg^{2+} to 10 mM, on rates of glucose oxidation by primary cultures of mouse cerebellar granule neurons. The cultures were incubated in tissue culture medium containing either 5.4 mM K^+ and 1.5 mM Ca^{2+} (open column), 5.4 mM K^+, zero Ca^{2+} and 10 mM Mg^2 (column indicated by wavelines), 55 mM K^+ and 1.5 mM Ca^2 (horizontally striped column), or 55 mM K^+, zero Ca^{2+} and 10 mM Mg^2 (vertically striped column) for 1 h. Results are means ± S.E.M. of three individual experiments using cultures from the same batch. The value at 55 mM K^+ and 1.5 mM Ca^{2+} is statistically significantly ($P<0.05$ or less) different from all other values.

3.4. Effect of Ca^{2+} depletion on glucose oxidation

Since K^+-evoked release of glutamate from cerebellar granule neurons, including the presently used preparation (Peng et al., 1991) is Ca^{2+}-dependent, and since the accumulated evidence suggests that the effect of amobarbital on K^+-stimulated glucose metabolism is secondary to the barbiturate-induced inhibition of glutamate release, the effect of Ca^{2+} depletion on glucose oxidation was tested. This was done by omitting Ca^{2+} from the medium and adding 10 mM Mg^{2+} (as the chloride) to displace traces of Ca^{2+} from the tissue. Under control conditions, the rate of glucose oxidation was similar to that illustrated in Fig. 1 (see legend of the figure), and it was stimulated to the same extent by elevated K^+ (Fig. 4); depletion of Ca^{2+} had no effect in the presence of 5.4 mM K^+, but it abolished the K^+-induced stimulation of glucose oxidation.

4. Discussion

4.1. Linkage between K^+ stimulation of glucose oxidation and glutamate

Two lines of evidence suggest that the K^+-induced stimulation of glucose oxidation and phosphorylation in cerebellar granule neurons is mediated by K^+-induced release of glutamate: (1) at normal K^+ concentration, glutamate stimulated glucose phosphorylation; and (2) inhibition of glutamate release by Ca^{2+} depletion abolished the stimulatory effect of elevated K^+. In addition to a direct effect of the exogenously added glutamate, it is likely that a glutamate effect on presynaptic receptors may have led to synaptic release of endogenous glutamate, since stimulation of glutamate receptors leads to glutamate release in cultured cerebellar granule neurons (Schousboe et al., 1985).

The conclusion that depolarizing concentrations of K^+ mainly exert a long-term (60 min) stimulation of neuronal energy metabolism by the evoked release of glutamate is consistent with previous observations that cerebellar granule cells show very little K^+-stimulated glucose oxidation, when they are cultured in such a manner that they release no glutamate when exposed to a depolarizing K^+ concentration (Peng and Hertz, 1993), and that primary cultures of cerebrocortical interneurons, a GABAergic preparation, likewise show only little response to excess K^+ (Peng et al., 1994). These findings should not be interpreted as indicating that depolarization and ensuing Na^+ uptake (Rose and Ransom, 1996; Takahashi et al., 1997) exert no metabolic effects on neurons, but rather that such effects may be short-lasting (compared to an incubation period of 1 h), due to inactivation of Na^+ channels. That activation of glutamatergic receptors also stimulates energy metabolism in intact brain tissue is indicated by the observation that NMDA (*N*-methyl-D-aspartate) causes an increase in glycolysis in hippocampal slices (Zhan et al., 1997, 1998).

4.2. Metabolic effects of amobarbital at 5.4 mM K^+

A suppression of glucose oxidation was the only inhibitory effect seen in cerebellar granule neurons incubated at 5.4 mM K^+, as indicated by a straight line on the Dixon plot. It had a similar dose dependence as amobarbital's inhibition of oxidative metabolism in the perfused heart (Nishiki et al., 1979). Moreover, as in the heart and in isolated synaptosomes (Dagani and Erecinska, 1987), the inhibition of oxidative metabolism was accompanied by a compensatory increase in glycolysis. It is not possible to quantitate the degree to which the increase in glycolysis energetically compensated for the decrease in oxidative metabolism, because of the lack of precise quantitative information about rate of glucose oxidation and possible oxidation of other substrates.

4.3. Metabolic effects of amobarbital at 55 mM K^+

During incubation of cerebellar granule cells at a depolarizing K^+ concentration, there was, in addition, a second effect of amobarbital, indicated by the enhanced respiratory depression in the concentration range 0.5–1.0 mM demonstrated in the Dixon-type plot. This effect by amobarbital is likely to reflect an inhibition of an energy-requiring process, since it was accompanied by a decrease, not an increase, in glycolysis. The greater potency of amobarbital's effect on K^+-stimulated respiration, than on the non-stimulated respiration together with a similar, high potency of thiopental in inhibition of K^+-stimulated glutamate release from cerebellar

granule neurons (Miao et al., 1998) suggest that the inhibition of K^+-stimulated respiration at low concentrations of amobarbital primarily is evoked by inhibition of glutamate release from the cells. The additional inhibitory effect of amobarbital on metabolism in cerebellar granule cells called into action at amobarbital concentrations above 0.5 mM may accordingly be due to its inhibition of the respiratory chain, an effect which is likely to have approximately similar concentration dependence in media with normal and excess K^+ concentration. This conclusion provides an explanation why barbiturates have been found to affect stimulated respiration more potently than unstimulated metabolism in incubated brain tissues (McIlwain, 1953; Ghosh and Quastel, 1954): this is not due to special metabolic characteristics of the stimulated respiration, but because barbiturates abolish the stimulus as such and does this more potently than it suppresses energy metabolism directly.

The conclusion that amobarbital exerts the potent part of its inhibition of K^+-stimulated respiration by abolishing evoked release of glutamate is based upon consistent observations by other authors that barbiturates inhibit glutamate release from cultured cerebellar granule neurons (Miao et al., 1998; Qu et al., 2000) and from rat brain cortex slices (Buggy et al., 2000). In the latter preparation, the reduction of glutamate release was mediated by stimulation of $GABA_A$ receptors, which hyperpolarized the cells. In contrast to observations by Damgaard et al. (1996), using slightly younger mouse cultures of cerebellar granule cells, and Pearce et al. (1981), using rat cultures, there is no K^+-induced GABA release in the present cultures, which are highly enriched in cerebellar granule cells of typical morphology (Peng et al., 1991) and show no K^+-induced release of GABA (L. Peng and L. Hertz, unpublished observation). However, cerebellar granule cells do express GABA receptors (Meier and Schousboe, 1982; Schousboe, 1999), and barbiturates activate receptor channels even in the absence of GABA (Mathers and Barker, 1980; Nicoll and Wojtowicz, 1980; Wong et al., 1984; Amin and Weiss, 1995; Sanna et al., 1995; Rho et al., 1996; Thompson et al., 1996). The concept that barbiturates exert two different effects on K^+-stimulated energy metabolism in glutamatergic neurons, that is, a direct inhibitory effect of oxidative phosphorylation at relatively high concentrations and a GABA receptor channel-mediated inhibition of glutamate release and ensuing reduction of glutamate-stimulated glucose metabolism at much lower concentrations, is thus consistent with well-established properties of barbiturates.

4.4. Correlation with barbiturate anesthesia and cytoprotection

The primary goal of the present study was to understand the mechanism(s) by which a barbiturate known to greatly suppress the respiratory chain affects glucose metabolism under stimulated and non-stimulated conditions. To obtain this information, relatively high concentrations of amobarbital had to be employed. However, without doing so, it would not have been possible to reach the conclusion that amobarbital at low, pharmacologically relevant concentrations appears to inhibit glucose oxidation primarily as a result of the abolishment of the stimulatory effect of K^+-mediated glutamate release. This is a novel conclusion, which may be highly relevant for the understanding of barbiturate-mediated anesthesia and cytoprotection. It is consistent with observations by others that also non-barbiturate anesthetics interfere with glutamatergic function (Larsen et al., 1994; Miao et al., 1995; MacIver et al., 1996; Miyazaki et al., 1997; Larsen and Langmoen, 1998; Huang and Hertz, 2000; Zuo, 2001). Thus, the present study supports the concept that during anesthesia, 'the decline in oxygen uptake was a result rather than a cause of the altered neuronal response' (Larrabee, 1965). It tentatively identifies inhibition of stimulated release of glutamate as a major reason for the altered neuronal activity and the subsequent reduction in energy metabolism.

The cytoprotective action of barbiturates may also be correlated with inhibition of evoked release of glutamate rather that with a direct effect on energy metabolism. During anoxia and hypoxia, only the initial part of the release of glutamate occurs by exocytosis (Pocock and Nicholls, 1998; Kawakami et al., 2001), whereas the remainder originates from non-synaptic parts of neurons and from glial cells by reversal of active uptake, by swelling-induced opening of stretch-sensitive anion channels or by damage of cell membranes secondary to generation of free radicals and fatty acids. Reduction of stimulated release of glutamate may, nevertheless, contribute to the neuroprotective effect of barbiturates, since prevention of the initial exocytotic release of glutamate during anoxia reduces subsequent glutamate release from cultured cerebellar granule cells by other mechanisms, and accordingly enhances cell survival (Huang and Hertz, 1995). Moreover, the concept that the neuroprotective effect of barbiturates is not predominantly due to their inhibition of mitochondrial respiration is in agreement with the observation by Warner et al. (1996) and Schmid-Elsaesser et al. (1999) that concentrations of barbiturates with sub-maximum effect on electroencephalogram (EEG) activity and brain metabolism are equally effective in neuroprotection as are higher concentrations. So are emerging concepts (Nava-Ocampo et al., 2000; Huang et al., 2000; Koinig et al., 2001; Kawakami et al., 2001; De Cristobal et al., 2002) that suppression of glutamate release by direct or indirect mechanisms may have neuroprotective effects.

References

Aldrich, W.N., Parker, V.H., 1960. Barbiturates and oxidative phosphorylation. Biochem. J. 76, 47–56.

Amin, J., Weiss, D.S., 1995. $GABA_A$ receptor needs two homologous domains of the beta-subunit for activation by GABA but not by pentobarbital. Nature 366, 510–511.

Belopavlovic, M., Buchthal, A., 1980. Barbiturate therapy in cerebral ischemia. Anaesthesia 35, 235–245.

Blacklock, J.B., Oldfield, E.H., Di Chiro, G., Tran, D., Theodore, W., Wright, D.C., Larson, S.M., 1987. Effect of barbiturate coma on glucose utilization in normal brain versus gliomas. Positron emission tomography studies. J. Neurosurg. 67, 71–75.

Bolliger, B., Maddox, K., 1931. Experimental anesthesia with tri-bromethyl alcohol (Avertin) and sodium *iso*-amyl-ethyl-barbiturate (Amytal). Curr. Res. Anest. Anal. 10, 112–115.

Buggy, D.J., Nicol, B., Rowbotham, D.J., Lambert, D.G., 2000. Effects of intravenous anesthetic agents on glutamate release: a role for $GABA_A$ receptor-mediated inhibition. Anesthesiology 92, 1067–1073.

Carlsson, C., Siesjo, B.K., 1975. Metabolic changes in the cerebral cortex of the rat induced by intravenous pentothalsodium. Acta Anaesthesiol. Scand., Suppl. 57, 1–17.

Dagani, F., Erecinska, M., 1987. Relationships among ATP synthesis, K^+ gradients, and neurotransmitter amino acid levels in isolated rat brain synaptosomes. J. Neurochem. 49, 1229–1240.

Damgaard, I., Trenkner, E., Sturman, J.A., Schousboe, A., 1996. Effect of K^+- and kainate-mediated depolarization on survival and functional maturation of GABAergic and glutamatergic neurons in cultures of dissociated mouse cerebellum. Neurochem. Res. 21, 267–275.

De Cristobal, J., Cardenas, A., Lizasoain, I., Leza, J.C., Fernandez-Tome, C., Lorenzo, P., Moro, M.A., 2002. Inhibition of glutamate release via recovery of ATP levels accounts for a neuroprotective effect of aspirin in rat cortical neurons exposed to oxygen–glucose deprivation. Stroke 33, 261–267.

Dienel, G.A., Hertz, L., 2001. Glucose and lactate metabolism during brain activation. J. Neurosci. Res. 66, 824–838.

Dixon, M., 1953. The determination of enzyme inhibitor constant. Biochem. J. 55, 170–171.

Drejer, J., Larsson, O.M., Schousboe, A., 1982. Characterization of L-glutamate uptake into and release from astrocytes and neurons cultured from different brain regions. Exp. Brain Res. 47, 259–269.

Drejer, J., Honore, T., Meier, E., Schousboe, A., 1986. Pharmacologically distinct glutamate receptors on cerebellar granule cells. Life Sci. 38, 2077–2085.

Fischer, E., von Mering, J., 1903. Ueber eine neue Klasse von Schlafmitteln. Ther. Ggw. 44, 97–101.

Ghosh, J., Quastel, J.H., 1954. Narcotics and brain respiration. Nature 174, 28–31.

Gutman, M., Singer, T.P., Reinert, H., Casida, J.E., 1970. Reaction sites of rotenone, piericidin A and amytal in relation to the nonheme iron components of NADH dehydrogenase. Proc. Natl. Acad. Sci. U.S.A. 65, 763–770.

Hertz, L., Dienel, G.A., 2002. Energy metabolism in the brain. In: Dwyer, D. (Ed.), Glucose Metabolism in the Brain, International Review of Neurobiology. Academic Press, New York, NY, in press.

Hertz, L., Swanson, R.A., Newman, G.C., Mariff, H., Juurlink, B.H.J., Peng, L., 1998. Can experimental conditions explain the discrepancy whether or not glutamate stimulates aerobic glycolysis? Dev. Neurosci. 20, 339–347.

Hodes, J.E., Soncrant, T.T., Larson, D.M., Carlson, S.G., Rapoport, S.I., 1985. Selective changes in local cerebral glucose utilization induced by phenobarbital in the rat. Anesthesiology 63, 633–639.

Huang, R., Hertz, L., 1995. Neuroprotective effect of phenylsuccinate, an inhibitor of cytosolic glutamate formation from glutamine, under anoxic conditions but not during exposure to exogenous glutamate. Neurosci. Lett. 183, 22–26.

Huang, R., Hertz, L., 2000. Receptor subtype and dose dependence of demedetomidine-induced accumulation of [^{14}C]glutamine in astrocytes suggests glial involvement in its hypnotic-sedative and anesthetic-sparing effects. Brain Res. 873, 297–301.

Huang, R., Chen, Y., Yu, A.C.H., Hertz, L., 2000. Dexmedetomidine-induced stimulation of glutamine oxidation in astrocytes—a possible mechanism for its neuroprotective activity. J. Cereb. Blood Flow Metab. 20, 895–898.

Juurlink, B.H.J., Hertz, L., 1992. Astrocytes. In: Boulton, A.A., Baker, G.B., Walz, W. (Eds.), Neuromethods: vol. 23, Cell Cultures. Humana Clifton, New York, NY, pp. 269–321.

Kawakami, M., Sekiguchi, M., Sato, K., Kozaki, S., Takahashi, M., 2001. Erythropoietin receptor-mediated inhibition of exocytotic glutamate release confers neuroprotection during chemical ischemia. J. Biol. Chem. 276, 39469–39475.

Khan, N., Hajek, M., Antonini, A., Maguire, P., Muller, S., Valavanis, A., Leenders, K.L., Regard, M., Schiess, R., Wieser, H.G., 1997. Cerebral metabolic changes (^{18}F-DG PET) during selective anterior temporal lobe amobarbital test. Eur. Neurol. 38, 268–275.

Koinig, H., Vornik, V., Rueda, C., Zornow, M.H., 2001. Lubeluzole inhibits accumulation of extracellular glutamate in the hippocampus during transient global cerebral ischemia. Brain Res. 898, 297–302.

Larrabee, M.G., 1965. Discussion in Bunker, J.P., Vandam, L.D. Effects of anesthesia on metabolism and cellular functions. Pharm. Rev., 207–211.

Larsen, M., Langmoen, I.A., 1998. The effect of volatile anaesthetics on synaptic release and uptake of glutamate. Toxicol. Lett. 100–101, 59–64.

Larsen, M., Grondahl, T.O., Haugstad, T.S., Langmoen, I.A., 1994. The effect of the volatile anesthetic isoflurane on Ca(2+)-dependent glutamate release from rat cerebral cortex. Brain Res. 663, 335–337.

Levi, G., Aloisi, F., Ciotti, M.T., Gallo, V., 1984. Autoradiographic localization and depolarization-induced release of acidic amino acids in differentiating cerebellar granule cell cultures. Brain Res. 290, 77–86.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

MacIver, M.B., Mikulec, A.A., Amagasu, S.M., Monroe, F.A., 1996. Volatile anesthetics depress glutamate transmission via presynaptic actions. Anesthesiology 85, 823–834.

Mathers, D.A., Barker, J.L., 1980. (−)Pentobarbital opens ion channels of long duration in cultured mouse spinal neurons. Science 209, 507–509.

McIlwain, H., 1953. The effect of depressants on metabolism of stimulated cerebral tissues. Biochem. J. 53, 403–412.

McQueen, J.K., Martin, M.J., Fink, G., 1984. Comparison of the effects of Althesin and sodium pentobarbitone on the regional uptake of 2-deoxyglucose by the brain and the pituitary gland of the rat: selective effects on pars intermedia. Neuroendocrinology 38, 237–242.

Meier, E., Schousboe, A., 1982. Differences between GABA receptor binding to membranes from cerebellum during postnatal development and from cultured cerebellar granule cells. Dev. Neurosci. 5, 546–553.

Miao, N., Frazer, M.J., Lynch III, C., 1995. Volatile anesthetics depress Ca^{2+} transients and glutamate release in isolated cerebral synaptosomes. Anesthesiology 83, 593–603.

Miao, N., Nagao, K., Lynch III, C., 1998. Thiopental and methohexital depress Ca^{2+} entry into and glutamate release from cultured neurons. Anesthesiology 88, 1643–1653.

Michenfelder, J.D., 1974. The interdependency of cerebral functional and metabolic effects following massive doses of thiopental in the dog. Anesthesiology 41, 231–236.

Michenfelder, J.D., Theye, R.A., 1970. The effects of anesthesia and hypothermia on canine cerebral ATP and lactate during anoxia produced by decapitation. Anesthesiology 33, 430–439.

Miyazaki, H., Nakamura, Y., Arai, T., Kataoka, K., 1997. Increase of glutamate uptake in astrocytes: a possible mechanism of action of volatile anesthetics. Anesthesiology 86, 1359–1366.

Mortier, E., Struys, M., Herregods, L., 2000. Therapeutic coma or neuroprotection by anaesthetics. Acta Neurol. Belg. 100, 225–228.

Nava-Ocampo, A.A., Reyes-Periez, H., Bello-Ramirez, A.M., Mansilla-Olivares, A., Ponce-Monter, H., 2000. For ischemic brain damage, is preclinical evidence of neuroprotection by presynaptic blockade of glutamate release enough? Med. Hypotheses 54, 77–79.

Nicoll, R.A., Wojtowicz, J.M., 1980. The effects of pentobarbital and related compounds on frog motoneurons. Brain Res. 191, 225–237.

Nilsson, L., Siesjo, B.K., 1975. The effect of phenobarbitone anaesthesia on

blood flow and oxygen consumption in the rat brain. Acta Anaesthesiol. Scand., Suppl. 57, 18–24.

Nishiki, K., Erecinska, M., Wilson, D.F., 1979. Effect of amytal on metabolism of perfused rat heart: relationship between glycolysis and oxidative phosphorylation. Am. J. Physiol. 237, C221–C230.

Otsuka, T., Wei, L., Bereczki, D., Acuff, V., Patlak, C., Fenstermacher, J., 1991. Pentobarbital produces dissimilar changes in glucose influx and utilization in brain. Am. J. Physiol. 262, R265–R275.

Pearce, B.R., Currie, D.N., Beale, R., Dutton, G.R., 1981. Potassium-stimulated, calcium-dependent release of [^{3}H]GABA from neuron- and glia-enriched cultures of cells dissociated from rat cerebellum. Brain Res. 206, 485–489.

Peng, L., Hertz, L., 1993. Potassium induced stimulation of oxidative metabolism of glucose in cultures of intact cerebellar granule cells but not in corresponding cells with dendritic degeneration. Brain Res. 629, 331–334.

Peng, L., Juurlink, B.H.J., Hertz, L., 1991. Differences in transmitter release, morphology and ischemia-induced cell injury between cerebellar granule cell cultures developing in the presence and in the absence of a depolarizing potassium concentration. Dev. Brain Res. 63, 1–12.

Peng, L., Zhang, X., Hertz, L., 1994. High extracellular potassium concentrations stimulate oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes, but have no stimulatory effect in a GABAergic neuronal culture. Brain Res. 663, 168–172.

Pocock, J.M., Nicholls, D.G., 1998. Exocytotic and non-exocytotic modes of glutamate release from cultured cerebellar granule cells during chemical ischaemia. J. Neurochem. 70, 806–813.

Qu, H., Waagepetersen, H.S., van Hengel, M., Wolt, S., Dale, O., Unsgard, G., Sletvold, O., Schousboe, A., Sonnewald, U., 2000. Effects of thiopental on transport and metabolism of glutamate in cultured cerebellar granule neurons. Neurochem. Int. 37, 207–215.

Quastel, J.H., Wheatley, A.N.H., 1932. Narcosis and oxidations of the brain. Proc. R. Soc. Lond., Biol. 112, 60–71.

Rho, J.M., Donevan, S.D., Rogawski, M.A., 1996. Direct activation of $GABA_A$ receptors by barbiturates in cultured rat hippocampal neurons. J. Physiol. 497, 509–522.

Rose, C.R., Ransom, B.R., 1996. Regulation of intracellular sodium in cultured rat hippocampal neurones. J. Physiol. 499, 573–587.

Sanna, E., Garau, F., Harris, R.A., 1995. Novel properties of homomeric beta 1 gamma-aminobutyric acid type A receptors: actions of the anesthetics propofol and pentobarbital. Mol. Pharmacol. 47, 213–217.

Schmid-Elsaesser, R., Schroder, M., Zausinger, S., Hungerhuber, E., Baethmann, A., Reulen, H.J., 1999. EEG burst suppression is not necessary for maximum barbiturate protection in transient focal cerebral ischemia in the rat. J. Neurol. Sci. 162, 14–19.

Schousboe, A., 1999. Pharmacologic and therapeutic aspects of the developmentally regulated expression of GABA(A) and GABA(B) receptors: cerebellar granule cells as a model system. Neurochem. Int. 34, 373–377.

Schousboe, A., Hertz, L., 1987. Primary cultures of GABAergic and glutamatergic neurons as model systems to study neurotransmitter functions: II. Developmental aspects. In: Vernadakis, A., Privat, A., Lauder, J.M., Timiras, P.S., Giacobini, E. (Eds.), Model Systems of Development and Aging of the Nervous System. Martinus Nijhoff Publishers, Boston, MA, pp. 33–42.

Schousboe, A., Drejer, J., Hansen, G.H., Meier, E., 1985. Cultured neurons as model systems for biochemical and pharmacological studies on receptors for neurotransmitter amino acids. Dev. Neurosci. 7, 252–262.

Schousboe, A., Meier, E., Drejer, J., Hertz, L., 1989. Preparation of primary cultures of mouse (rat) cerebellar granule cells. In: Shahar, A., de Vellis, J., Vernadakis, A., Haber, B. (Eds.), A Dissection and Tissue Culture Manual for the Nervous System. Alan R. Liss, New York, NY, pp. 203–206.

Spetzler, R.F., Hadley, M.N., 1989. Protection against cerebral ischemia: the role of barbiturates. Cerebrovasc. Brain Metab. Rev. 1, 212–229.

Takahashi, S., Shibata, M., Fukuuchi, Y., 1997. Effects of increased extracellular potassium on influx of sodium ions in cultured rat astroglia and neurons. Dev. Brain Res. 104, 111–117.

Thompson, S.A., Whiting, P.J., Wafford, K.A., 1996. Barbiturate interactions at the human $GABA_A$ receptor: dependence on receptor subunit combination. Br. J. Pharmacol. 117, 521–527.

Varming, T., Christopherson, P., Schousboe, A., Drejer, J., 1997. Pharmacological characterization of voltage-sensitive calcium channels and neurotransmitter release from mouse cerebellar granule cells in culture. J. Neurosci. Res. 48, 43–52.

Warner, D.S., Takaoka, S., Wu, B., Ludwig, P.S., Pearlstein, R.D., Brinkhouse, A.D., Dexter, F., 1996. Electroencephalographic burst suppression is not required to elicit maximal neuroprotection from pentobarbital in a rat model of focal cerebral ischemia. Anesthesiology 84, 1475–1484.

Wong, E.H.F., Leeb-Lundberg, L.M., Teichberg, V.I., Olsen, R.W., 1984. γ-Aminobutyric acid activation of $^{36}Cl^-$ flux in rat hippocampal slices and its potentiation by barbiturates. Brain Res. 303, 267–275.

Yu, A.C.H., Schousboe, A., Hertz, L., 1982. Metabolic fate of (^{14}C)-labelled glutamate in astrocytes. J. Neurochem. 39, 954–966.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ.

Zhan, R.Z., Fujiwara, N., Taga, K., Fukuda, S., Endoh, H., Shimoji, K., 1997. NMDA induces a biphasic change in intracellular pH in rat hippocampal slices. Brain Res. 760, 179–186.

Zhan, R.Z., Fujiwara, N., Tanaka, E., Shimoji, K., 1998. Intracellular acidification induced by membrane depolarization in rat hippocampal slices: roles of intracellular Ca^{2+} and glycolysis. Brain Res. 780, 86–94.

Zuo, Z., 2001. Isoflurane enhances glutamate uptake via glutamate transporters in rat glial cells. NeuroReport 12, 1077–1080.