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KINETIC PROPERTIES OF GLUTAMINASE FROM CEREBRAL CORTEX*

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The rates of phosphate activated glutaminase activity in finely homogenised cerebral cortex and synaptosomes were measured. Activity was 25–50% higher at pH 7.0 than at pH 8.0. Glutamate inhibited activity with a K_i of 2–3 mM while aspartate had little effect. Calcium (1 mM) activated the enzyme but magnesium was without action. The pH profiles of the effects of these modulators of glutaminase activity in these finely ground preparations showed that all agents were more effective at pH 7.0 than at pH 8.0.

INTRODUCTION

Phosphate-activated glutaminase is responsible for the production of a large proportion of glutamate from glutamine in brain, and part of this glutamate appears to be employed in the glutamatergic transmitter system and part for the formation of transmitter GABA (1–4). Several studies have appeared on the endogenous factors which control the activity of this enzyme in brain which could therefore be key controlling factors in the formation of transmitter glutamate (5–8). In the present study we report on the pH dependance of the modulating effects of glutamate, aspartate, and calcium on brain glutaminase. The results obtained in this study differ in certain key respects from data previously reported (5, 6, 9).

* Dedicated to Henry McIlwain.

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EXPERIMENTAL PROCEDURE

Animals. Female Sprague Dawley rats (200–250 g body wt) were used throughout.

Tissue Preparations. Homogenates were prepared from cerebral cortex by homogenizing with 100 strokes at 0–4°C in a tight-fitting motar and pestle in 50mM borate buffer at the appropriate pH. This procedure produced a very fine and uniform suspension which allowed accurate sampling. Synaptosomes were prepared by the method described previously (10) and were then homogenized in 50 mM borate buffers as described above. They were then treated as for cerebral homogenates.

Glutaminase Assay. Glutaminase activity measured either by following ammonia production (1), or by measuring the glutamate formed by employing the glutamate dehydrogenase reaction. In the latter case, the reaction mixture contained: glutamine, 2 mM; potassium phosphate buffer, 20 mM; borate buffer, 50 mM; EDTA, 1 mM, and tissue at 1 to 4 mg wet weight $ml.^{-1}$ The rates were linear over at least 8 min. Glutaminase activity was calculated from the rates of appearance of ammonia or glutamate measured by taking aliquots of suspension at 0, 4, and 8 min and plotting a time course of reaction. Protein was measured by the method of Lowry et al. (11) employing serum albumin as standard.

RESULTS

The pH Profile of Glutaminase Activity. The activity of glutaminase in thoroughly-homogenised cerebral cortex and homogenates of cortical synaptosomes incubated in hypotonic media was found to decrease between pH 7.0 and pH 8.0. The activity in the presence of 10 mM phosphate was measured either as total glutamate formation (Figure 1A) or as ammonia formation (Figure 1B). The total variation in activity between pH 7.0 and 8.0 was 20–50% (see also Figure 3).

Glutamate Inhibition of Glutaminase. As previously reported (1, 5, 6), glutamate will inhibit glutaminase activity. In agreement with our previous findings (1), 3-5 mM glutamate was necessary to effect 50% inhibition at pH 7.0–7.4 (Figure 2). The pH dependence of this inhibition in cerebral homogenates is shown in Figure 1B. Glutamate (10 mM) was a much more effective inhibitor (80%) at pH 6.8 and 7.0 than at pH 7.6 or 8.0 (10%).

Aspartate was found to be ineffective as an inhibitor at all pH values examined (1) and, in fact, caused a small but significant enhancement of activity in cerebral homogenates under the conditions employed (data not shown).

Calcium Activation of Glutaminase. Others have described the ability of calcium to enhance glutaminase activity (6, 9). The data of Figure 3 confirms this stimulatory action of calcium (1 mM) in cortex homogenates (15–50%) which was most effective at pH 7.0, and least effective at pH 8.0. The glutaminase rates were higher at 2 mM substrate concentration than at 0.5 mM as expected (7), and the stimulatory action of calcium

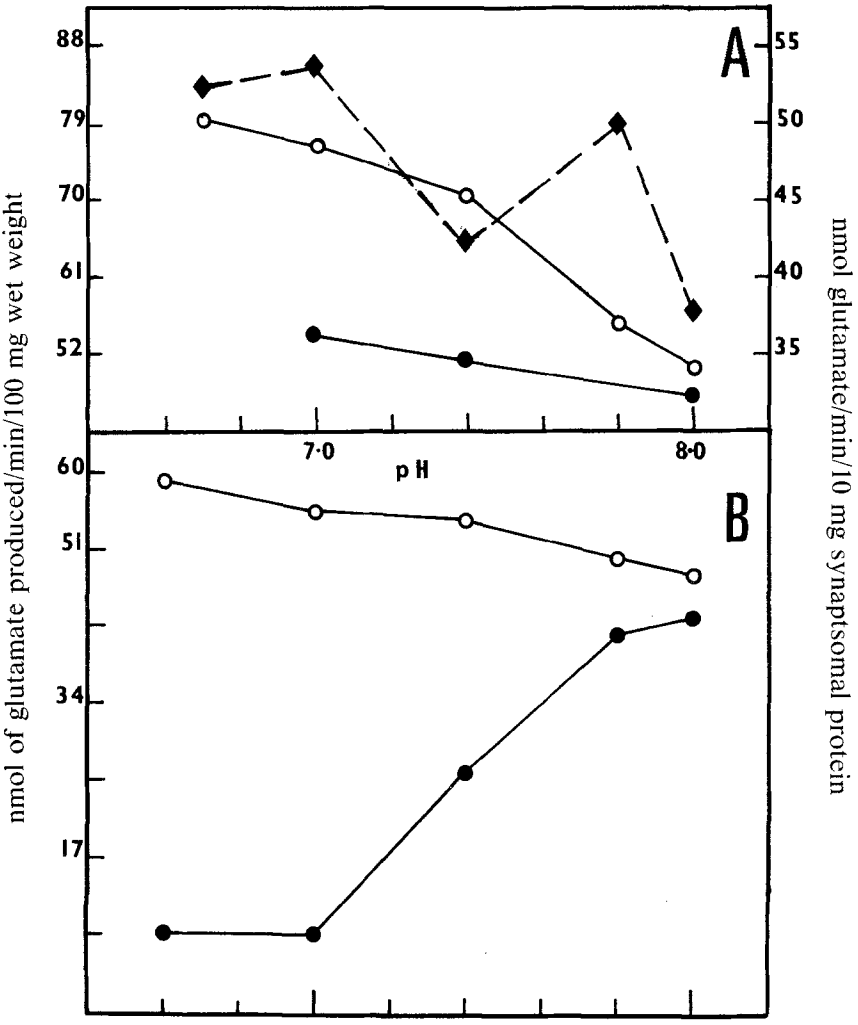

FIG. 1. Change in the activity of phosphate-stimulated glutaminase with variation in pH. Cortex homogenates were incubated at 37°C as described in the methods section in borate-phosphate buffers. (A) glutaminase was measured as the glutamate formed over 8 min. The response of two preparations is shown. Data are the mean of 6 (●) or two (○) measurements. The lower plot (●) is the more typical response. The broken line (◆) represents the activity in synaptosomal homogenates (means of two measurements). (B) glutaminase activity of cortex homogenates was measured as ammonia produced over 8 min in presence (●) or absence (○) of glutamate (10 mM); values are mean of 4 measurements and are typical of data from two preparations.

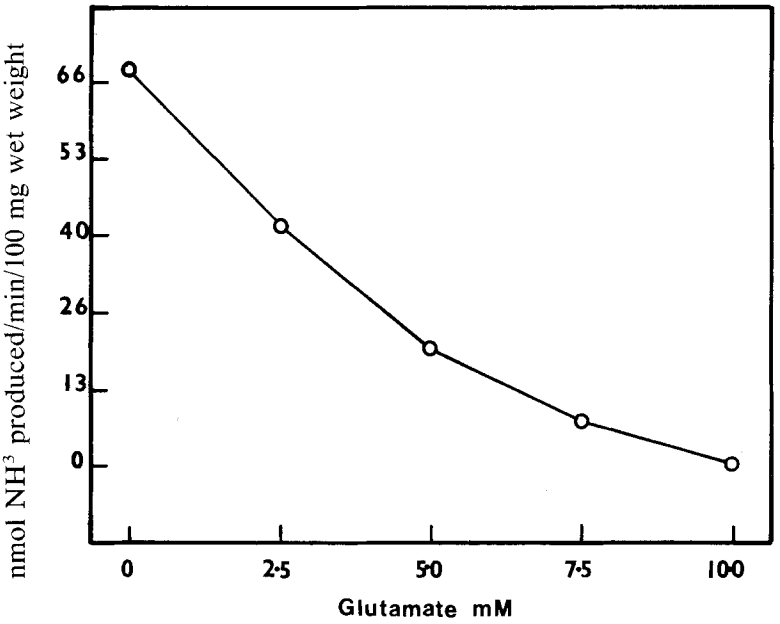

FIG. 2. Effect of sodium glutamate on glutaminase activity of cortical homogenates at pH 7.1. Incubation conditions are given in the methods section. Values are mean of 4 measurements and typical of data from 2 experiments.

was also higher at the 2 mM glutamine concentration. Magnesium at identical concentrations was without effect.

DISCUSSION

pH Dependence of Glutaminase. It has been reported that phosphate stimulated glutaminase in cortical homogenates and in crude or purified synaptosomes increases substantially (2 fold) over the pH range 7 to 8 (5, 6). This is in contrast to our current findings where activity was found not to change to such a large extent over this pH range, and was significantly higher (35–50%) at pH 7 than at pH 8. Essentially the same results were obtained whether glutamate or ammonia formation was monitored, suggesting that measuring the total glutamate formation under the present conditions (ie no glucose present) was an acceptable measure of glutaminase activity. Previous authors have measured glutaminase by following the formation of [^{14}C]glutamate from [U-^{14}C]glutamine (5, 6).

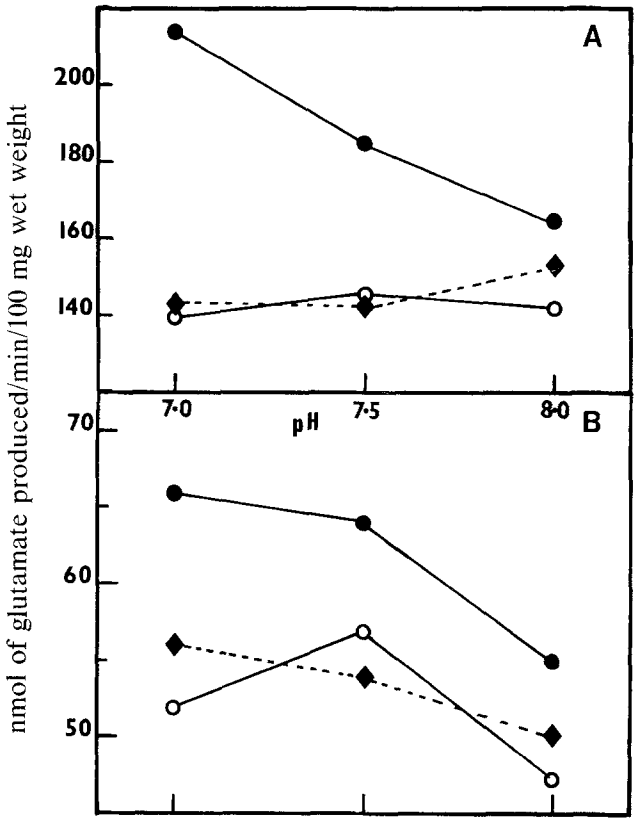

FIG. 3. Effect of calcium and magnesium on glutaminase activity of cortical homogenates at varying pH levels. (A) glutamine concentration 2.0 mM (B) glutamine concentration 0.5 mM. Glutaminase was measured as glutamate formation over 8 min in the presence of 1 mM $CaCl_2$ (●), 1 mM $MgCl_2$ (◆) or 2 mM KCl (○). Other incubation conditions are given in the methods section. Values are mean of two measurements.

Also, the results reported here were obtained employing cerebral cortex or synaptosomes thoroughly ground in hypotonic borate buffers in a pestle and mortar, to rupture all cytoplasm-containing bodies. This was followed by incubation at 37°C in hypotonic medium (221 milliosmols). Ruptured synaptosomes were chosen since the cytoplasmic barrier between incubation medium and mitochondria is removed, and mitochondria are directly exposed to pH and modulating substances (example glutamate, Ca). Cortical tissue homogenised and incubated in isotonic Krebs-Ringer showed a quite different pH profile of activation (6).

Under present conditions inhibition of glutaminase by both glutamate and calcium were more effective at pH 7.0 than pH 8.0, which is again in contrast to results of previous authors (5, 6). The K_i for glutamate inhibition at pH 7.1 was between 3 and 5 mM which is similar to the values previously determined (1, 12). This value is much higher than those reported by others, eg 0.45 mM (6), and could indicate a high degree of cell intactness in the preparations used by these authors. Reinvestigation has shown that the final pH of the buffer employed for the glutaminase assay in our previous study (1) was closer to pH 7.0 than pH 8.0 due to the buffering action of the activating phosphate ions.

In summary, whilst the inhibitory action of glutamate and the stimulatory actions of calcium, on phosphate-activated glutaminase reported by others (1, 5, 6) has been confirmed, the pH profile of this inhibition was found to be radically different in the present study. This is possibly due to the difference in cellular intactness in the preparations employed here and previously (5, 6, 9).

Where intact synaptosomes are present, the role of glutamine transport systems and the intervention of organised cytoplasm could critically effect rates of glutaminase activity.

Since the pK_2 of phosphoric acid is 7.2 (13) a change in the stimulatory action of phosphate on enzymes or on transport systems could be expected to occur over the pH range 7.0 to 8.0, with the increase in concentration of the phosphate ion species carrying two negative changes.

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