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Effects of δ-aminolaevulinic acid, porphobilinogen, amino acids and barbiturates on calcium accumulation by cultured neurons

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Episodes of acute neurological dysfunction which occur in the hereditary hepatic porphyrias are accompanied by the overproduction of the porphyrin precursors, δ-aminolaevulinic acid (ALA)* and porphobilinogen (PBG). The aetiology of the neurological disturbance is unknown but several mechanisms have been postulated at a neurochemical level [1, 2]. There is ample evidence to suggest that ALA and PBG may exert direct neurotoxic effects thus contributing to the neuropathology of porphyria [3–6].

ALA has been shown to be capable of interfering with neurotransmitter release. ALA increases γ-aminobutyric acid (GABA) and glutamic acid release from rabbit and rat brain cortical synaptosomes [6, 7] and reduces the K^+-stimulated release of GABA from synaptosomes [8]. ALA also inhibits the electrically evoked release of acetylcholine in rat phrenic nerve-hemidiaphragm preparations [9]. It has been suggested that ALA may affect neurotransmission by increasing membrane permeability to a variety of substances in a non-specific fashion [10]. Since neurotransmitter release is believed to be triggered by an increase in the intracellular calcium concentration [11], it was decided to examine whether ALA or PBG are capable of influencing calcium accumulation by neurons in culture. Furthermore, the possibility that ALA may modify the effects on calcium uptake of the amino acids, GABA and glutamic acid and the barbiturates, phenobarbitone and pentobarbitone was also investigated.

Materials and methods. Neuronal and glial cell cultures were prepared from chick embryo cerebral hemispheres as previously described [12]. $^{45}Ca^{2+}$ accumulation was determined according to the methods of Blaustein and Ector [15]. Cells were washed with buffer containing 137 mM NaCl, 5.4 mM KCl, 1.2 mM $CaCl_2$ 0.4 mM $MgSO_4$, 5.6 mM glucose and 10 mM Tris–HCl (pH 7.4) and then preincubated in 1 ml buffer for 15 min at 37° prior to addition of $^{45}Ca^{2+}$ (1–1.5 μCi/μmole calcium in final solution) in 1 ml buffer. Incubation was continued for a further 5 min. At the end of the incubation period, cells were rapidly washed with 150 mM choline chloride, 10 mM Tris–HCl (pH 7.4) and dissolved in 1M NaOH for 24 hr, at room temperature. Radioactivity was determined on aliquots of this solution to which 10 ml demilume-30 was added, with the aid of a Beckman model LS9000 liquid scintillation spectrometer. The protein content was determined according to Lowry *et al.* [16]. $^{45}Ca^{2+}$ uptake was also measured in buffer containing 70 mM KCl (final concentration, isomolar replacement of NaCl by KCl). When compounds were tested for interference with $^{45}Ca^{2+}$ accumulation by the cells, these substances were added to the cultures, in buffer at pH 7.4, after the 15 min preincubation period. The $^{45}Ca^{2+}$ aliquot was added either directly or after a further 15 min preincubation of the cells with the test compound. Statistical significances were calculated using Student's *t*-test.

Results. When 70 mM K^+ was included in the incubation medium, it produced a significant ($P < 0.001$) stimulation of $^{45}Ca^{2+}$ uptake into neurons (168 ± 6.9% of control values, mean ± S.E.M., $n = 11$). However, $^{45}Ca^{2+}$ accumulation by glial cultures was not affected by the addition of K^+. Control values for $^{45}Ca^{2+}$ accumulation by neurons and glia were similar and corresponded to 3.5–5 nmoles calcium/mg protein.

Preincubation of the neurons with 1 mM glutamic acid significantly ($P < 0.001$) inhibited the K^+-stimulated $^{45}Ca^{2+}$ uptake into neurons (18 ± 2.8% inhibition, $n = 10$), while preincubation with 1 mM ALA, PBG, GABA, phenobarbitone or ALA in combination with GABA or phenobarbitone was without effect. Pentobarbitone, however, produced a marked, dose-dependent inhibition of the K^+-stimulated $^{45}Ca^{2+}$ uptake into neurons (Fig. 1). At a concentration of 0.5 mM, pentobarbitone reduced the stimulated uptake by 45 ± 5.7 per cent ($n = 6$, $P < 0.01$). ALA did not modify the pentobarbitone effect in any way.

Glutamic acid significantly increased $^{45}Ca^{2+}$ uptake into non-K^+-stimulated neurons (Fig. 2). At a concentration as low as 10 μM, glutamic acid increased $^{45}Ca^{2+}$ uptake to 146 ± 3.7 per cent of control values ($n = 12$, $P < 0.001$). ALA, PBG and GABA did not alter $^{45}Ca^{2+}$ accumulation by non-K^+-stimulated neurons.

Discussion. Elevated extracellular K^+ was clearly shown to stimulate calcium influx into neurons in culture. Calcium accumulation by glia, however, remained insensitive to K^+ treatment. Glial release of GABA and glial membrane conductance have previously been shown to be unaffected by elevated extracellular K^+ concentrations [17, 18].

Pentobarbitone produced a marked inhibition of the K^+-stimulated $^{45}Ca^{2+}$ uptake into neurons, while phenobarbitone was without effect. These results agree with data reported for synaptosomal preparations [15] and the observation that pentobarbitone and not phenobarbitone inhibits the K^+-stimulated release of GABA from rat cerebral cortex slices [19]. Pentobarbitone is thought to interfere with neurotransmitter release by selectively inhibiting depolarization induced calcium influx into synaptosomes [15]. Barbiturates are known to precipitate acute attacks in porphyria and it was thought that ALA may enhance or interfere with their action on $^{45}Ca^{2+}$ uptake. However this was not the case.

ALA, PBG and GABA did not affect K^+-stimulated calcium uptake into neurons but glutamic acid, an excitatory neurotransmitter, reduced the stimulated uptake. Glutamic acid is known to stimulate neurotransmitter release and was shown to stimulate $^{45}Ca^{2+}$ influx into cultured neurons.

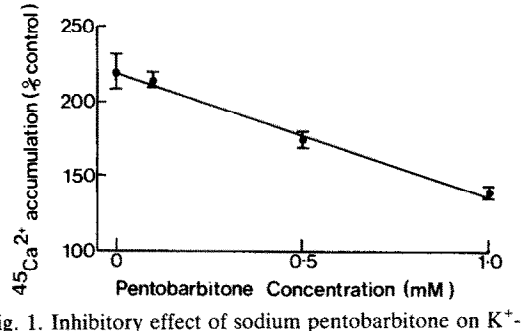

Fig. 1. Inhibitory effect of sodium pentobarbitone on K^+-stimulated $^{45}Ca^{2+}$ uptake into neurons. Results are the mean ± S.E.M. of 6 observations. Cells were preincubated for 15 min with various concentrations of sodium pentobarbitone prior to addition of $^{45}Ca^{2+}$ in 1 ml 135 mM K^+ solution.

* Abbreviations: δ-Aminolaevulinic acid: ALA; Porphobilinogen: PBG; γ-Aminobutyric acid: GABA.

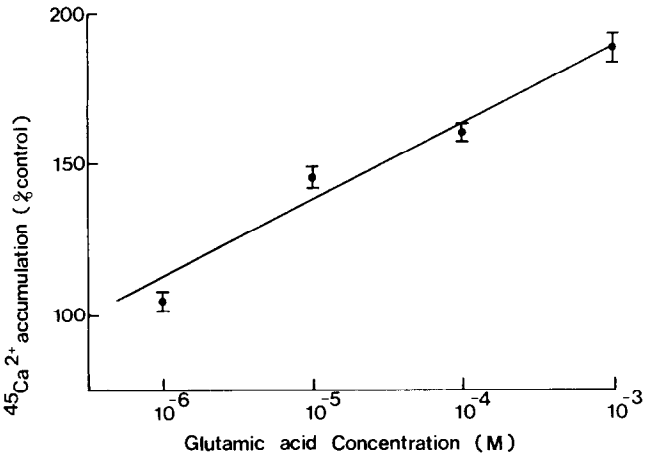

Fig. 2. Stimulatory effect of glutamic acid on $^{45}Ca^{2+}$ accumulation by cultured neurons. Results are the mean ± S.E.M. of 12 to 34 observations. Cells were preincubated in 1 ml buffer prior to addition of $^{45}Ca^{2+}$ in 1 ml buffer containing various concentrations of glutamic acid.

The excitatory effects of glutamic acid on synaptic transmission may be mediated by the increase in calcium uptake into the cells. Glutamic acid has been shown to inhibit calcium uptake into neuroblasts and reisolated neuronal cells, after being preincubated with the cells for 5 min [20], and to stimulate calcium uptake into synaptosomal preparations [21, 22]. ALA, PBG and GABA did not affect $^{45}Ca^{2+}$ accumulation by cultured neurons. Calcium accumulation has been shown to be insensitive to GABA in rat cortical synaptosomes [23] and in neuroblasts and reisolated neuronal cells [20].

In summary, PBG does not appear to affect calcium accumulation and, although ALA has been clearly shown to inhibit the K^+-stimulated release of GABA from synaptosomal preparations [8], it did not affect the K^+-stimulated influx of calcium into cultured neurons. ALA has further been shown to stimulate GABA and glutamic acid release from unstimulated synaptosomes [6, 7] yet ALA did not affect calcium accumulation by cultured neurons. The present findings therefore suggest that ALA exerts its effects on neurotransmitter release without altering membrane permeability to calcium ions.

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