CHARACTERISATION OF Na^+ CHANNEL BLOCKERS BY A SIMPLE, SPECTROPHOTOMETRIC ASSAY FOR THE DETERMINATION OF GLUTAMATE RELEASE FROM RAT CORTICAL SYNAPTOSOMES

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A correlation exists between the modulation of Na^+-channel activity and endogenous glutamate (GLU) release evoked by depolarization. Until recently, for the in vitro determination of GLU release a very low throughput enzyme-linked fluorometric assay based on the measurement of formation of NADPH in the presence of NADP and glutamate dehydrogenase (GDH) has been used. Here, we describe and validate a simple spectrophotometric method for GLU release from rat cortex synaptosomes suitable for medium throughput purposes.

Synaptosomal GLU release was measured from rat cortical synaptosomal preparation (P2 fraction, 1 mg protein/well) by continuous spectrophotometric monitoring of β-$NADP^+$ (1.2 mM) reduction by GDH (5 Units/well) in 96-well plates incubated at 37^{o}C (incubation volume 200 µl) using plate reader (Labsystems iEMS Reader MF) at 340nm. As depolarizing agents (Na^+ channel site 2 agonists) veratridine and aconitine were used. Tetrodotoxin (TTX), crobenetine, lifarizine, riluzole, eperisone, tolperisone, inaperisone, lamotrigine and phenytoin as Na^+ blockers were tested.

Increase in absorbance (i.e. GLU release) strongly depended on the synaptosomal protein content in the presence of depolarizing agents: > 0.8 mg protein/well was needed to detect the release of GLU. Basal GLU release was 0.75±1.4 nmol GLU/mg protein/10 min. Veratridine and aconitine caused a concentration-dependent release of endogenous GLU from synaptosomes. Maximal induction was evoked by 20 µM veratridine. Submaximal concentration of veratridine (3 µM) elicited GLU release of 13.5±1.2 nmol GLU/mg protein/10 min. All sodium channel blockers inhibited veratridine (3 µM) evoked GLU release in a concentration-dependent manner. TTX was the most potent (IC_{50}=0.022±0.001µM) followed by crobenetine (IC_{50}=0.29±0.15 µM). Lifarizine (IC_{50}=4.7±0.9 µM), riluzole (IC_{50}=8.4±0.9 µM) were active at low micromolar concentration while eperisone (IC_{50}=39.3±4.1 µM), tolperisone (IC_{50}=41.9±7.7 µM), inaperisone (IC_{50}=75.2 µM), lamotrigine (IC_{50}=95.5 µM) and phenytoin (IC_{50}=178.6 µM) were active in the high micromolar concentrations. A competitive interaction between veratridine and inhibitors such as tolperisone, crobenetine on GLU release was found. A good correlation between the potency of the drugs to inhibit the increase of GLU release induced by veratridine, their binding affinity for the [^{3}H]Batrachotoxin ([^{3}H]BTX) binding in rat cortical synaptosomes and inhibition of veratridine induced intracellular Ca^{2+} elevation measured by Ca^{2+} sensitive fluorescent dye, fluo-4 was observed.

This simple, spectrophotometric 96-well plate technique allowed medium throughput and still accurate determination of the effects of various drugs on depolarization evoked GLU release. There is a close correlation between the inhibitory activity of drugs on veratridine evoked glutamate release and displacement of [^{3}H]BTX binding and inhibition of veratridine evoked increase of intracellular [Ca^{2+}] level.