Organic Calcium Channel Blockers Enhance [3H]Purine Release from Rat Brain Cortical Synaptosomes

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The release of [3H]purines was investigated in a crude mitochondrial fraction (P₂ fraction) from rat brain cortex pre-loaded with [3H]adenosine for 30 sec at 37°C in vitro. Potassium, veratridine and glutamate were used as depolarizing agents to evoke the release of [3H]purines. Ca²⁺ removal, the addition of EGTA, and treatment with organic or inorganic Ca²⁺ antagonists did not inhibit [3H]purine release in this preparation. On the other hand, Ca²⁺ removal and the addition of EGTA greatly enhanced [3H]-purine release induced by glutamate. D-600 and dil-tiazem enhanced K⁺-evoked [3H]purine release, and nifedipine increased veratridine evoked [3H]purine release indicating that either these Ca²⁺ antagonists have different sites of action, or that K⁺ and veratridine may release [3H]purine from different metabolic pools. Organic Ca²⁺ antagonists failed to enhance the [3H]purine release evoked by glutamate, further supporting the notion that various depolarizing agents may release [3H]purines from different cellular compartments.

INTRODUCTION

Adenosine is known to depress neuronal activity at many levels of the neural axis. Adenosine and its derivatives have been shown to inhibit firing rates of rat brain cortical neurons in the intact brain (1) and evoked

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potentials in hippocampal slice preparations (2, 3). The behavioral depressant effect of adenosine was shown by intraventricular injection of a number of its analogs (4). It has been proposed that adenosine may play a neuromodulatory role in the central nervous system (5, 6) via inhibition of stimulus induced release of dopamine, 5-hydroxytryptamine and nor-epinephrine (7).

To exert its action, endogenous adenosine must presumably be released from structure(s) associated with nerve terminals. Indeed, adenosine-like material was released from various tissue preparations by nerve stimulation (8-10). The release of adenosine and related substances from in vivo (11-14) and in vitro (15-18) rat brain preparations has also been shown. The amounts of adenosine (and related purines) released into the extracellular space during nerve stimulation may be appreciable, as purine substances were released in estimated concentrations of up to 0.1 mM in the synaptic cleft at the neuromuscular junction (19) and in a concentration range of 0.15 ~ 0.4 mM in the central nervous system (15). McIlwain and his colleagues showed that electrical stimulation and depolarization induced by high potassium of cerebral tissue in vitro released a small fraction of [14C]adenine derived labeled material as adenine nucleotides, with adenosine accounting for about 50% of the released radiolabeled purines (16, 20–22). Fredholm and Vernet (18) obtained similar results with hypothalamic synaptosomes using electrical stimulation and depolarization by high potassium or veratridine. The release of adenosine induced by high potassium from cortical synaptosomes was calcium dependent (16), whereas the release from hypothalamic synaptosomes was largely calcium-independent (18). Potassium evoked release from cortical synaptosomes was not altered by the sodium channel blocker tetrodotoxin (23) whereas veratridine evoked release from cortical and hypothalamic synaptosomes was shown to be calcium dependent and was blocked by tetrodotoxin (18, 23). Bender et al. (24) showed that in brain cortical synaptosomes preloaded with rapid uptake of 3H-adenosine, the release of adenosine was enhanced by EGTA. This indicated that the removal of calcium by EGTA increased the release of [3H]adenosine, suggesting the release of [3H]adenosine was not dependent upon extracellular calcium. In the present report, we examined the effect of extracellular Ca²⁺ on adenosine release from rat brain cortical synaptosomes. The effect of various organic channels blockers and Ca²⁺ antagonists on adenosine release was also investigated.

EXPERIMENTAL PROCEDURE

Male Sprague Dawley rats (weighing 200–300 g) were sacrificed and their brains removed. The brain was placed on an ice-cold stage and the cerebral cortices were dissected out. The