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Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Gonçalves, M. L. , Pinto, F. and Ribeiro, J. A.(1991) 'Adenosine Inhibition of 45 Ca Uptake by Synaptosomes as Function of Time of Electrical Stimulation', Nucleosides, Nucleotides and Nucleic Acids, 10: 5, 1161 — 1163

To link to this Article: DOI: 10.1080/07328319108047261 URL: http://dx.doi.org/10.1080/07328319108047261

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ADENOSINE INHIBITION OF ⁴⁵Ca UPTAKE BY SYNAPTOSOMES AS FUNCTION OF TIME OF ELECTRICAL STIMULATION

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Abstract. The effect of adenosine on 45 Ca uptake by rat brain synaptosomes electrically stimulated was studied as function of time of stimulation (10, 30, 120 s). Inhibition of 45 Ca uptake was more evident for 120 s.

Adenosine partially decreases calcium uptake into rat brain synaptosomes depolarized by potassium. I, 2 In guineapig ileal synaptosomes it was found that the uptake of $^{45}\mathrm{Ca}$ induced by electrical stimulation through pulses applied during 60 s was completely abolished by adenosine. 3 In this work it was investigated the effect of adenosine on $^{45}\mathrm{Ca}$ uptake by rat brain synaptosomes stimulated by electrical pulses applied during different periods of time.

Synaptosomes prepared from rat brain homogenates were incubated with adenosine (25 nM and 1 uM) in a medium (pH 7.4) containing (mM): NaCl 132; KCl 5; MgCl $_2$ 1.3; NaH $_2$ PO $_4$ 1.2; CaCl $_2$ 1.2; glucose 10. Ca uptake was induced by electrical stimulation applied through platinum electrodes. Rectangular pulses of 20 V amplitude and 400 μ s duration with a frequency of 10 pulses/s were applied during 10, 30 and 120 s. The uptake was terminated by adding a stop buffer solution (EGTA) followed by filtration through GF/C glass fiber filters. The radioactivity associated with the synaptosomes was determined.

TABLE 1 shows the mean values for ^{45}Ca uptake by rat brain synaptosomes induced by electrical stimulation during 10, 30 and 120 s in the absence (control) and in the presence of adenosine. Adenosine (25 nM and 1 μ M) inhibited

TABLE 1. Effect of adenosine (ADO) on electrically stimulated (E.S.) $^45\mathrm{Ca}$ uptake by rat brain synaptosomes during 10, 30 and 120 s.

Conditions	net ⁴⁵ Ca uptake nmol/mg protein	% Inhibition
E.S. 10 s (control)	0.79±0.14 (n=5)	-
ADO 25 nM	0.52±0.12 (n=5)	33
ADO 1 μM	0.38±0.17 (n=5)	52
E.S. 30 s (control)	2.28±0.22 (n=5)	-
ADO 25 nM	1.31±0.19 (n=5)	43
ADO 1 µM	1.06±0.14 (n=5)	54
E.S. 120 s (control)	2.52±0.56 (n=5)	-
ADO 25 nM	1.19±0.58 (n=4)	53
ADO 1 µM	0.23±0.19 (n=4)	91

The results are the mean \pm s.e. mean

in a concentration-dependent manner, 45 Ca uptake by rat brain synaptosomes stimulated during different periods of time. The inhibitory effect of adenosine was observed during the first period of stimulation (10 s). The effect was more evident when the time of stimulation was 30 s and a further increase in the inhibition of 45 Ca uptake was observed for 120 s of electrical stimulation. For adenosine 1 μ M the reduction increased from 52% after 10 s of stimulation to 91% after 120 s of electrical stimulation. Whether this increase in inhibition of 45 Ca uptake by synaptosomes in the presence of adenosine after 120 s stimulation might include also increase in 45 Ca efflux, it cannot be answered from the present work.

The present results support the view that adenosine inhibits entry of calcium during depolarization.

The authors acknowledge the competent technical assistance of Mr. Artur Costa.

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