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## **Nucleosides, Nucleotides and Nucleic Acids**

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### **Relative Contribution of Nerve Endings to the Release of Adenine Nucleotides in the Innervated from Sartorius Muscle**

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RELATIVE CONTRIBUTION OF NERVE ENDINGS TO THE RELEASE OF ADENINE  
NUCLEOTIDES IN THE INNERVATED FROG SARTORIUS MUSCLE

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**Abstract:** An evoked release of adenine nucleotides was observed at the frog innervated sartorius muscle, at least one third coming from nerve endings. Coformycin and  $\alpha,\beta$ -methylene ADP inhibited the hydrolysis of endogenously formed AMP as well as of exogenously added AMP.

Released adenine nucleotides might contribute to the pool of endogenous adenosine that inhibits neuromuscular transmission<sup>1</sup>. At the innervated frog sartorius muscle, exogenous ATP can be sequentially degraded into AMP and then either into IMP or into adenosine<sup>2</sup>. In the present work we investigated whether a complete blockade of AMP degradation could be achieved and if under those conditions adenine nucleotides are released upon nerve stimulation.

The preparations were mounted in a 2 ml bath at room temperature (22-25°C). The bathing solution (pH 6.8) contained (mM): NaCl 117, KCl 2.5, MgCl<sub>2</sub> 1.2, CaCl<sub>2</sub> 1.8, NaH<sub>2</sub>PO<sub>4</sub> 1, Na<sub>2</sub>HPO<sub>4</sub> 1, and the inhibitor of adenosine uptake, dipyridamole (0.5  $\mu$ M). Throughout the assays air was bubbled into the bath to facilitate diffusion. For the kinetic experiments, AMP (10  $\mu$ M) was incubated with the preparations at zero time. Samples of 75  $\mu$ l were collected from the bath at different times up to 90 min, and analysed by RP-HPLC<sup>3</sup>. The effect of a drug was assessed by comparison of AMP progress curves in the absence and in the presence of the drug in the same preparation. For the release experiments, the preparations were perfused (3.5 ml/min) during 60 min with bathing solution. The perfusion was stopped and a 30 min incubation period with no stimulation was followed by a 30 min nerve stimulation period (0.2 Hz, 14 V, 20  $\mu$ s). Samples (210  $\mu$ l) were

collected from the bath at 5, 10, 15, 20 and 30 min after starting stimulation and analysed by RP-HPLC<sup>3</sup>.

AOPCP (50-200  $\mu$ M), an inhibitor of 5'-nucleotidase, decreased in a concentration-dependent manner adenosine formation from AMP. A complete blockade of AMP degradation into adenosine was achieved with 200  $\mu$ M AOPCP (n=4). Coformycin (1-200  $\mu$ M), an inhibitor of 5'-AMP deaminase, decreased in a concentration-dependent manner IMP formation from AMP. A complete blockade of AMP degradation into IMP was achieved with 200  $\mu$ M coformycin (n=3). The simultaneous presence of AOPCP (200  $\mu$ M) and coformycin (200  $\mu$ M) completely blocked AMP degradation (n=3). In the presence of these enzyme inhibitors, AMP was detected in the bath from non-stimulated preparations, the concentration after 30 min being  $77 \pm 18$  nM (n=14). Upon nerve stimulation, the concentration of AMP after 30 min increased to  $106 \pm 22$  nM. Addition of tubocurarine (5  $\mu$ M), to block muscle contraction, decreased by  $60 \pm 8\%$  the nerve-evoked release of adenine nucleotides. Under these conditions, evoked release of adenine nucleotides was the greater the higher the frequency (0.2-1 Hz) of nerve stimulation. In the absence of AOPCP and coformycin, AMP was still detected in the bath but at a lower concentration ( $19 \pm 10$  nM, n=8).

The results suggest that one third of the amounts of adenine nucleotides released upon nerve stimulation come from nerve endings and that their degradation is prevented by AOPCP and coformycin.

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