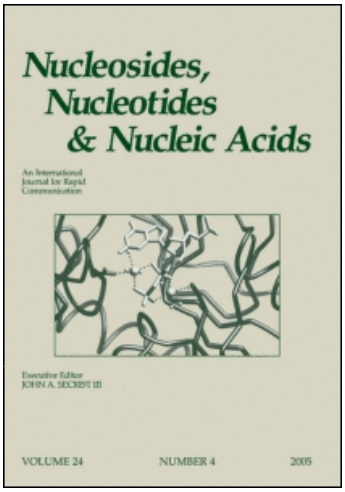

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Potentiation of ATP Responses by α,β-Methyleneadp in the Rabbit Ear Artery

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POTENTIATION OF ATP RESPONSES BY α,ß-METHYLENEADP IN THE RABBIT EAR ARTERY.

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Abstract: α,ß-methyleneADP, the ectonucleotidase inhibitor, produced a potentiation and kinetic stabilization of ATP induced contractions of the rabbit ear artery. However, studies with stable and unstable agonists indicated that this effect was not due to inhibition of purine metabolism.

A common problem in the classification of purine-receptors is the metabolic instability of many of the agonists used. The availability of agents which inhibit ectonucleotidases and therefore allow meaningful analysis of such agonists in organ bath experiments would be clearly desirable. α,ß-methyleneADP is an inhibitor of 5'-nucleotidase[1] and attempts have been made to use this compound to inhibit ATP metabolism in pharmacological experiments[2,3], without success. We have studied the effect of α,ß-methyleneATP on the contractile responses of both stable and unstable purines in the rabbit ear artery.

The central ear artery from male NZW rabbits was dissected out and the endothelium physically denuded. 5mm rings were suspended in Krebs' containing 2.8×10^{-6}M indomethacin and 8-sulphophenyltheophylline (300 μM) gassed with 95% O_2 : 5% CO_2 and maintained at 37°C. Changes in tension were measured isometrically. Concentration effect, E/[A], curves to ATP, α,ß-methyleneATP, ß,γ-methyleneATP, and ADP were constructed alone and in the presence of α,ß-methyleneADP (30μM, 60 min). The contractile effect of α,ß-methyleneADP itself was also studied. Changes in curve location ($p[A_{50}]$) were assessed by one-way analysis of variance.

α,ß-methyleneADP produced contractile responses in the range 3-1000μM. 30μM was chosen for the potentiation studies since this concentration produced negligible contractions. In the presence of this concentration of α,ß-methyleneADP, the E/[A] curve to ATP was markedly displaced to the left

($\Delta p[A_{50}]$ 1.88 ± 0.17, n=5*), and the nature of the response was changed from a phasic to a tonic contraction. In contrast, only small leftward displacements occurred in the E/[A] curves to α,ß-methyleneATP (0.15 ± 0.13, n=5 (n.s.)), ß,γ-methyleneATP (0.41 ± 0.14, n=5*) and ADP (0.30 ± 0.15, n=6 (n.s.)), nor were there changes in the kinetics of the contractions. (* significant $p<0.05$).

These results contrast with previous findings[3]. However, although ATP responses were potentiated by α,ß-methyleneADP pretreatment, the lack of potentiation observed with ADP implies that the effect seen with ATP is not due to inhibition of 5′-nucleotidase. The reduction in the fade of the contraction to ATP, induced by α,ß-methyleneADP, may explain the leftward displacement of the E/[A] curves[4]. However, this seems unlikely as the contractions to ß,γ-methyleneATP and ADP, both of which produce rapidly fading responses, were unaffected. Newby & Nakazawa[5] have found that ATP can act as a source of phosphate for the conversion of α,ß-methyleneADP into α,ß-methyleneATP. Under organ bath conditions ATP could produce its effects by in situ conversion to α,ß-methyleneATP. Since, α,ß-methyleneATP produces a more sustained as well as potent response than ATP, this conversion would explain the change in kinetics as well the left shift of the ATP E/[A] curve. As ADP, ß,γ-methyleneATP and α,ß-methyleneATP, could not act as sources of phosphate for the phosphorylation of α,ß-methyleneADP, formation of α,ß-methyleneATP would not occur and so no apparent potentiation would be observed. Whether this hypothesis explains our data requires further experiments.

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