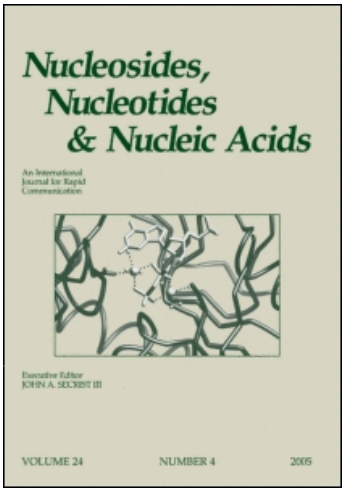

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Possible Agonistic Interaction of Defibrotide, A DNA Derivative, with Adenosine Receptors "In Vitro"

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POSSIBLE AGONISTIC INTERACTION OF DEFIBROTIDE, A DNA DERIVATIVE, WITH ADENOSINE RECEPTORS "IN VITRO".

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Abstract. Defibrotide (DFT), a single-stranded DNA, dose-dependently displaced only 3H-CHA and 3H-NECA (markers of adenosine receptors), among 37 radioligands tested. On isolated gastro-intestinal smooth muscle, DFT caused a dose-dependent relaxation, that was antagonized by 8-phenyltheophylline, a mixed A1- and A2-receptor antagonist.

Defibrotide (DFT) is a single-stranded polydeoxyribonucleotide derivative, obtained by controlled depolymerization of mammalian DNA. The actual substance is a family of chains of different lenght, with a Gaussian-like distribution of mean molecular weights centered between 15 and 30 kD. This substance enhances prostacyclin release from isolated vascular segments and whole organs (heart and kindey) and displays anti-ischaemic effects "in vivo", with salvage of cellular energetic pools.

Since a molecular mechanism of action for DFT has not yet been described, the present piece of work investigates binding affinity for various receptor sites (using radioligand displacing techniques) and possible correlated pharmacologic effects on isolated organs. Among a series of 37 radioligands, only 3H-CHA and 3H-NECA (markers of adenosine A1- and A2-receptors) were up to 100% and dose-dependently displaced by DFT, in the concentration range 1x10-6 to 1x10-4 M (comparable to that of theophylline). In the isolated rat stomach, continuously perfused with Krebs solution containing antagonists for alpha, beta, muscarine, histamine and serotonin receptors, addition of low concentrations of DFT (from 0.01 to 1 mcg/ml/min for 60 min) caused dose-dependent tissue relaxation from resting tone ($p < 0.01$ vs 19 control tissues), whereas higher concentrations (3 to 30 mcg/ml/min) gradually reversed such effect, resulting in a bell-shaped

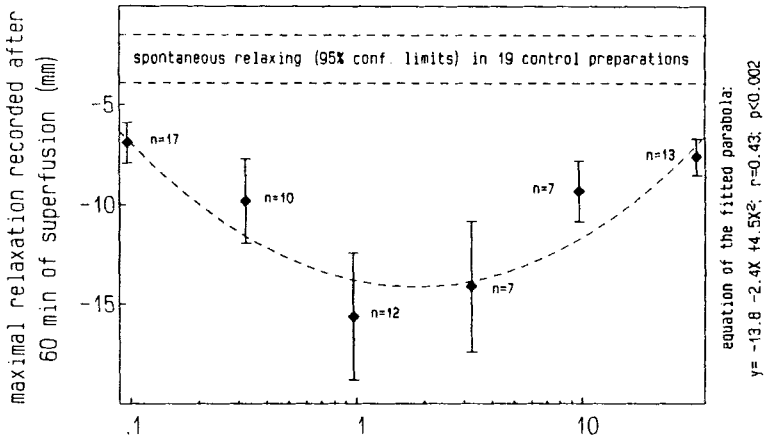

FIG. 1 Relaxing effects of defibrotide on the resting tone of the superfused rat stomach strip (DFT concentration = mcg/ml/min).

dose-response relationship (FIG. 1). The greatest relax observed (at 1 mcg/ml/min; n=12) was 15.6 ± 3.2 mm, corresponding to 40% of the maximum possible relaxation, determined by adding 1x10-5 M papaverine.

This effect was not blunted by adding indomethacin (2 mcg/ml/min) to the perfusion medium, but was totally abolished by 1 mcg/ml/min of 8-phenyltheophylline (8-PT), a mixed antagonist of A1- and A2-receptors. In 8 control preparations, spontaneous relax after 1 hour (quoted as % of the maximum possible) was 19%±3, a figure similar to that observed after perfusion with 8-PT alone (n=7) and 8-PT plus DFT (n=10) (17%±3 and 19%±3, respectively; p=n.s.), whereas DFT alone (n=8) relaxed 35%±4 ($p < 0.01$ vs all other groups).

These results suggest that DFT discriminates among different receptor systems and selectively interacts with the adenosine A1- and A2-sites, causing a pharmacologic effect (gastro-intestinal smooth muscle relaxation), which is typical of adenosine agonists. Since reversal of such effect was observed at higher doses, we also suggest an agonistic interaction with the so-called P-site, which is supposed to inactivate adenylate cyclase.

In conclusion, we provide the first evidence to our knowledge that single-stranded DNA chains (as for DFT) display binding affinity for adenosine receptors and cause agonistic-like pharmacologic effects.