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APPENDIX 1

Effect of Tinuvin 770 on ^{3}H-desmethoxyverapamil binding to guinea-pig heart membranes

Method

Heart membranes. Male Pirbright White guinea-pigs (Tif:DHP, (b) (4) [redacted] were killed by cervical dislocation. Hearts were rapidly removed and cleaned of connective tissue, and membranes were prepared as described (1), with the exception that $MgCl_2$, EDTA, and phenylmethylsulphonylfluoride were omitted from the buffers. The membranes were frozen in liquid nitrogen and stored at -80°C until use.

Radioligand binding assay. Competition-binding experiments were performed using [^{3}H]-desmethoxyverapamil (New England Nuclear, about 68 Ci/mmol) as ligand. Membranes (protein about 75 µg) were incubated for 60 min. at 25°C with the radioligand and competitors in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.4. After incubation, samples were rapidly diluted with 10 ml of ice-cold 50 mM Tris-HCl, pH 7.4, and filtered under vacuum through Whatman GF/C filters pretreated with 0.3% polyethylenimine. The filters were washed three times with 10 ml of the same buffer and radioactivity was measured by liquid scintillation counting. Binding in the presence of 10^{-5} M verapamil was defined as non-specific. Protein was determined by the method of Bradford (2) using bovine serum albumin as standard.

Data analysis. Dose-response curves were analysed by non-linear regression (3).

Results

Tinuvin 770 inhibits [^{3}H]-desmethoxyverapamil binding to guinea-pig heart membranes (IC_{50} = 17 nM; Table 1) whereas the 'Tinuvin 770-fragment' (b)(4) [redacted] (b) (4) [redacted] showed no notable effect in this test (Table 1).

Discussion

Receptor sites for the non-dihydropyridine Ca^{++}-antagonists at the L-type calcium channel of cardiac membranes can be labelled specifically with [^{3}H]-desmethoxyverapamil. This high-affinity binding is susceptible to displacement by non-dihydropyridine Ca^{++}-antagonists, such as verapamil, diltiazem, prenylamine, or lidoflazine. Tinuvin 770 potently inhibits [^{3}H]-desmethoxyverapamil binding to guinea-pig heart membranes, indicating that this compound has an affinity for L-type calcium channels equal to about 3 that of verapamil and about 11 times that of diltiazem. Since this binding site appears to be located at the cytoplasmic site of the Ca^{++}-channel and to bind compounds exclusively when the pore is open (4), only studies on intact cardiac tissue can show whether the effect is translated into a biological activity.

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(b) (4) [redacted]

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Table 1:

Effect of Tinuvin 770 and reference Ca++-channel blockers on ^{3}H-desmethoxyverapamil binding to guinea-pig heart membranes.

	IC_{50} (nM)	N
Tinuvin 770	17	2
(b) (4) [redacted]	>100 000	2
Verapamil	59	6
Diltiazem	180	8

References

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