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27. Januar 1993

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Subject: TINUVIN 770

Pharmacological Experiments with TINUVIN 770 (Pharmacology Unit, Pharma Division, Basel).

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1) In a first experiment, heart cell membranes from guinea pigs were used in radioligand binding assays with ^{3}H-desmethoxyverapramil, a calcium-antagonist, and TINUVIN 770 as well as tetramethylpiperidine hydroxide (HTMP), one of the major metabolites of TINUVIN 770. TINUVIN 770, but not HTMP, is a potent inhibitor of the high-affinitiy binding of ^{3}H-desmethoxyverapramil to L-type calcium channels. TINUVIN 770 was calculated to be 11 times more potent than diltiazem, a Ca^{2+}-antagonist of low potency. In this experiment, the receptors for calcium-antagonist binding, which are located on the inner, cytoplasmic side of the membrane, are easily accessible to ligands. The inhibitory concentration (IC_{50}) is 17 nmol/l (Appendix 1). With this experiment, the results of Glossmann have been confirmed.

2) In a second experiment, guinea pig atria (heart) were isolated in order to evaluate the influence of TINUVIN 770 on the force and rate of atrial contraction, a calcium dependent process in the isolated organ. TINUVIN 770 decreased the force and rate of contraction at a high concentration of 10 µmol/l, but not at lower concentrations. The effects are most likely due to calcium-antagonist properties of TINUVIN 770. In the intact organ, TINUVIN 770 proved to be 10 times less active than diltiazem, and showed thus weak calcium-antagonistic properties. (Appendix 2).

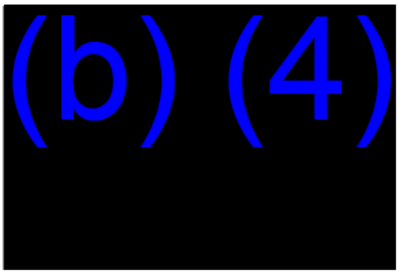
3) In a third experiment, perfused mesenteric arteries were injected with noradrenalin (NA) or KCl for vasoconstriction. When added to the perfused arteries, TINUVIN 770 inhibited KCL-induced vasoconstriction only at the highest testable concentration of 10 µmol/l, but not at lower concentrations. The NA induced vasoconstriction was not inhibited, indicating a true calcium-antagonistic effect. In this experiment, TINUVIN 770 was about 20 times less active than diltiazem (Appendix 3).

Conclusion:

When tested on isolated heart cell membrane fragments TINUVIN 770, but not its major metabolite HTMP, showed strong binding to L-type calcium-channels in the nanomolar range, about 11 times more potent than the reference substance diltiazem.

However, when tested on isolated organs with intact cells, TINUVIN 770 exhibited properties of a weak calcium-antagonist, about 10-20 times less active than the reference substance diltiazem. It is worth noting that TINUVIN 770 was active onyl at a concentration of 10 µmol/l, the highest testable concentration.

The high affinity binding of TINUVIN 770 to L-type calcium-channels of isolated heart cell membranes turned out to be of minor biological significance, as shown by functional experiments on isolated intact organs. It is assumed that the functional effects in isolated organs are limited by poor cellular uptake of the compound.



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