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Stereospecific inhibition of 5-HT-induced increase of intracellular free calcium by (+)- and (−)-desmethoxyverapamil in human platelets

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The concentration of intracellular free calcium $[Ca^{2+}]_i$ in human platelets was measured by the quin-2 method. 5-Hydroxytryptamine (5-HT) at 10^{-5} M induced a rapid transient increase of $[Ca^{2+}]_i$ which was antagonized by 10^{-7} M ketanserin or cyproheptadine. The verapamil derivative, desmethoxyverapamil (D 888), showed stereospecific inhibition of the 5-HT-induced $[Ca^{2+}]_i$ increase. The IC_{50} for (−)-D 888 was approx. 2×10^{-8} M; (+)-D 888 was almost 50 times less potent.

Introduction Various stimuli (e.g. adenosine diphosphate (ADP), collagen, adrenaline, 5-hydroxytryptamine (5-HT)) increase the intracellular free calcium concentration $[Ca^{2+}]_i$ in platelets (Owen *et al.*, 1980; Rink & Hallem, 1984; Erne & Pletscher, 1985) and thus stimulate secretion of biologically active substances and induce platelet aggregation (Rink & Hallem, 1984). It has been suggested that these calcium-dependent processes may be modulated by calcium antagonists which are known to inhibit cellular Ca^{2+} influx through the potential-dependent calcium channels. The calcium antagonists nifedipine, verapamil and diltiazem have been reported to inhibit the aggregation of platelets induced by ADP (Han *et al.*, 1983) or collagen (Ikeda *et al.*, 1984) but at concentrations at least 1000 fold higher than those that are therapeutically effective.

The 5-HT-induced but not the ADP-dependent shape change of human platelets was shown to be inhibited by verapamil (Affolter *et al.*, 1985). Nifedipine and diltiazem had no effects on either the 5-HT- or ADP-induced shape change reaction. These authors suggest that the inhibitory effect of verapamil is due to an antagonistic effect at the 5-HT_2-receptor. This is supported by results indicating that verapamil competes with the 5-HT_2-receptor antagonists [^{3}H]-ketanserin and [^{3}H]-spiperone for their specific binding sites on membranes from rat cerebral cortex (Affolter *et al.*, 1985; Taylor & Defeudis, 1985).

In this communication we describe the stereospecific inhibition of the 5-HT-induced $[Ca^{2+}]_i$ increase by the verapamil derivative, D 888, in human platelets.

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Methods Citrated blood was obtained from healthy volunteers by venous puncture. After centrifugation at 100 *g* for 15 min the platelet rich plasma was incubated with 2×10^{-5} M quin-2-AM for 30 min at 37°C. Subsequently the quin-2-loaded platelets were passed through a Sepharose 2B-CL column pre-equilibrated with HEPES-buffer containing (mM): NaCl 145, KCl 5, $MgSO_4$ 1, Na_2HPO_4 0.5 and glucose 6. The peak fractions of the eluted platelets (about 2×10^8 platelets ml^{-1}) were supplemented with 1 mM $CaCl_2$ for 15 min and used for quin-2 measurements. The dye was excited at 340 mm and the emission was measured at 492 mm. The intracellular free calcium concentration was calculated as described by Tsien *et al.* (1982).

Desmethoxyverapamil (D 888) was kindly donated by Prof. Dr Kretzschmar (Knoll AG, Ludwigshafen, F.R.G.).

Results The $[Ca^{2+}]_i$ in unstimulated human platelets has been determined to be 128 ± 3 nM (n = 38). Upon addition of 10^{-5} M 5-HT a rapid increase of $[Ca^{2+}]_i$ up to 350n M was observed within 30 s. This increase was transient, the $[Ca^{2+}]_i$ declined after 7 min to about 190 nM. The 5-HT antagonists, cyproheptadine and ketanserin, at 10^{-7} M completely abolished the 5-HT-induced $[Ca^{2+}]_i$ rise (Ch. Bruns, unpublished results). Verapamil was able to inhibit the 5-HT-induced increase of $[Ca^{2+}]_i$ (Figure 1). At 5×10^{-5} M verapamil, $[Ca^{2+}]$ did not increase upon addition of 10^{-5} M 5-HT. As can be seen in Figure 1 the rise of $[Ca^{2+}]_i$ was effectively inhibited by (−)-D 888 with an IC_{50} of 2×10^{-8} M, whereas (+)-D 888 was nearly 50 times less potent ($IC_{50} = 1 \times 10^{-6}$ M).

Discussion Data from the literature reveal that verapamil interferes with 5-HT-induced processes. Auguet *et al.* (1984) showed, that verapamil inhibits the 5-HT-induced contraction of rabbit isolated aorta. The 5-HT-dependent shape change of human platelets was inhibited by verapamil (Affolter *et al.*, 1985) and verapamil competes for the specific binding of [^{3}H]-

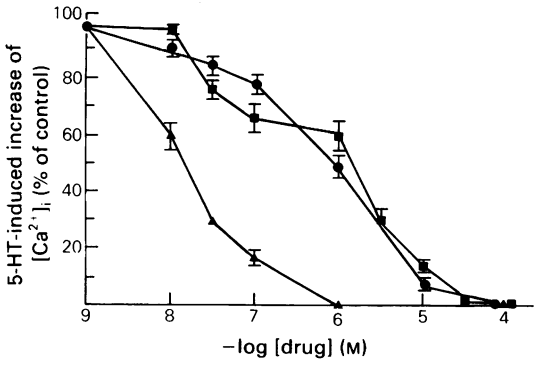

Figure 1 The concentration-dependent inhibitory effect of verapamil (■), (+)-D 888 (●) and (−)-D 888 (▲) on the 5-hydroxytryptamine-induced increase of the intracellular free calcium concentration. The values represent means of 3–5 individual experiments with s.e.mean shown by vertical lines.

spiperone to 5-HT_2-receptors from rat cerebral cortex (Taylor & Defeudis, 1985).

We were able to demonstrate that 5-HT causes a rapid transient increase of $[Ca^{2+}]_i$ within 30 s. This confirms a recent observation by Erne & Pletscher (1985). Verapamil inhibited this increase of $[Ca^{2+}]_i$. D 888, a verapamil derivative, showed a stereospecific inhibition of the 5-HT-induced rise of $[Ca^{2+}]_i$, (−)-D 888 was a very effective inhibitor ($IC_{50} = 2 \times 10^{-8}$ M) whereas (+)-D 888 and verapamil were almost 50 times less potent.

This stereospecificity of D 888 at 5-HT receptors coincides with the stereospecificity of D 888 at calcium channels (Goll *et al.*, 1984). The similar stereoselectivity of 5-HT receptors and calcium channels towards D 888 could indicate that both receptor systems have one element in common.

We have also tried to identify specific binding sites for [^{3}H]-verapamil at human platelet membranes. Our results indicate the existence of specific binding sites for [^{3}H]-verapamil independent of 5-HT_2- or α_2-adrenoceptors (Bruns & Marmé, 1985). Displacement experiments with (−)-D 888 and (+)-D 888 revealed however, that (−)-D 888 was less potent in displacing [^{3}H]-verapamil than (+)-D 888. These observations suggest that these specific [^{3}H]-verapamil binding sites are most probably not involved in the effective inhibition of $[Ca^{2+}]_i$-increase by (−)-D 888. It is more likely that (−)-D 888 interferes stereospecifically with 5-HT_2 receptors of human platelets.

These findings might have profound implications in the search for regulator mechanisms controlling intracellular free calcium concentrations independent of voltage-gated calcium channels. Both effects of (−)-D 888, antagonizing calcium-dependent vascular smooth muscle contraction (Nawrath & Raschack, 1984) and antagonizing 5-HT_2-dependent processes (this communication), could be related to the therapy of disturbances in cerebral microcirculation e.g. migraine.

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References

AFFOLTER, H., BURKARD, W.P. & PLETSCHER, A. (1985). Verapamil, an antagonist at 5-hydroxytryptamine receptors of human blood platelets. *Eur. J. Pharmac.*, **108**, 157–162.

AUGUET, M., DEFEUDIS, F.V. & CLOSTRE, F. (1984). Competitive-inhibition of 5-HT receptors, in rabbit isolated aorta by the Ca^{2+}-antagonist methoxyverapamil (D 600). *Neurochem. Int.*, **6**, 701–710.

BRUNS, C.H. & MARMÉ, D. (1985). Binding studies with calcium antagonists to a particulate fraction from human platelets. *Naunyn-Schmiedebergs Arch. Pharmac.*, **329**, 192.

ERNE, P. & PLETSCHER, A. (1985). Rapid intracellular release of calcium in human platelets by stimulation of 5HT_2-receptors. *Br. J. Pharmac.*, **84**, 545–549.

GOLL, A., FERRY, D.R., STRIESSNIG, J., SCHOBER, M. & GLOSSMANN, H. (1984). (−)-[^{3}H]Desmethoxyverapamil, a novel Ca^{2+} channel probe. *FEBS Letters*, **176**, 371–377.

HAN, P., BOATWRIGHT, C. & ARDLIE, N.G. (1983). Effect of calcium-entry blocking agent nifedipine on activation of human platelets and comparisons with verapamil. *Thromb. Haemostas.*, **50**, 513–517.

IKEDA, Y., KIKUCHI, M., TOVAMA, K., WATANABE, K. & ANDO, Y. (1981). Inhibition of human platelet functions by verapamil. *Thromb. Haemostas.*, **45**, 158–161.

NAWRATH, H. & RASCHACK, M. (1984). Calcium antagonistic effects of the radioligand (−)-desmethoxyverapamil on cardiac and vascular smooth muscle preparations. *Cell Calcium*, **5**, 316.

OWEN, N.E., FEINBERG, H. & LE BRETON, G.C. (1980). Epinephrine induces calcium uptake in human blood platelets. *Am. J. Physiol.*, **239**, 483–488.

RINK, T.J. & HALLEM, T.J. (1984). What turns platelets on? *TIBS*, May 1984, 215–119.

TAYLOR, J.E., DEFEUDIS, F.V. (1985). Inhibition of [^{3}H]-spiperone binding to 5-HT_2-receptors of rat cerebral cortex by the calcium antagonists verapamil and D 600. *Eur. J. Pharmac.*, **106**, 215–216.

TSIEN, R.Y., POZZAN, T. & RINK, T.J. (1982). Calcium homeostasis in intact lymphocytes: cytoplasmic free calcium monitored with a new, intracellularly trapped fluorescent indicator. *J. cell. Biol.*, **94**, 325–334.

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