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Effect of verapamil and diltiazem on isolated gastro-oesophageal sphincter of the rat

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The effect of verapamil and diltiazem on the contraction induced by agonists on the rat lower oesophageal sphincter in-vitro has been studied. Both calcium entry blockers inhibited the contractile response to acetylcholine, carbachol and KCl. The potency of the inhibitory action was diltiazem > verapamil. The results give substance to the use of calcium entry blockers in the treatment of oesophageal spasm.

Calcium antagonists such as verapamil and diltiazem were originally described as coronary vasodilators but it is now well known that these drugs have effects on other organs and tissues, such as smooth muscle of blood vessels and airways. Weiser et al (1978) have indicated that nifedipine (a calcium entry blocker) also exerts a relaxant effect on oesophageal smooth muscle and they have suggested the possibility of a role for this drug in the treatment of oesophageal spasm. Several investigators (Blackwell et al 1981; Bortolotti & Labo 1981; Nasrallah 1982) have demonstrated clinically, the usefulness of drugs therapy with calcium-antagonists in relieving the dysphagia of patients with achalasia. However little attention is paid to in-vitro studies on action of calcium antagonists on gastrointestinal smooth muscle.

In common with other smooth muscle, the contraction of the lower oesophageal sphincter (LES) have been shown to be dependent on calcium. Cohen & Green (1973) showed that the peak force and maximal velocity of shortening were diminished by reducing the external calcium concentration. De Carle et al (1977) demonstrated that LES tone was also dependent on Ca^{2+}.

In this communication we describe the effect of verapamil and diltiazem on contraction induced by acetylcholine, carbachol and KCl in the LES of the rat in-vitro.

Methods

Albino rats (200–250 g) of either sex were killed by a blow to the head and exsanguinated. The abdomen was opened and the stomach, together with 1·5 cm of oesophagus, was removed and immersed in a room-temperature aerated Krebs solution (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 10. According to Takayanagi & Kasuya (1977), transverse strips (approximately 7 mm × 3·5 mm) were taken from the gastro-oesophageal junc-

tion and mounted in a jacketed organ bath, containing 20 ml of Krebs solution gassed with 5% CO_2 in oxygen at 37 °C (pH 7·5). One end of each strip was fixed and the other end was attached to an isometric force-displacement transducer Hewlett Packard FTD-100-1 connected to a recorder. The initial tension was 1 g and all preparations were allowed to equilibrate for at least 30 min after which the strips were exposed to KCl (50 mM), carbachol (0·5 mM) or acetylcholine (0·5 mM). In preliminary experiments no significant differences were found between the contractile effects produced by these doses of agonists (80% of maximum contraction). After the bath had been washed out with oxygenated Krebs solution, either verapamil or diltiazem was added 10–15 min before a new dose of agonist.

Responses in the presence of antagonists were expressed as percentages of inhibition of the control response induced by the agonist. All results are the mean ± s.e.m. of at least 5 experiments. Linear least square regression analysis was used to obtain the line of best fit through the average of data points. The IC50 was calculated from the plot. The significance of differences was assessed by the *t*-test, $P < 0.05$ was accepted as the level of significance.

Drugs used were: acetylcholine chloride and carbachol chloride (Sigma), verapamil (Knoll) and diltiazem (kindly supplied by Dr. Esteve, S.A.).

Results

Acetylcholine (0·5 mM), carbachol (0·5 mM) and KCl (50 mM) produced contraction of the rat isolated LES. The effects of the drugs were: acetylcholine 106 ± 12·2 mg (n = 35) carbachol 135 ± 30·8 mg (n = 32), KCl 125 ± 26·7 mg (n = 30). The differences between these values were not statistically significant.

Either verapamil or diltiazem significantly reduced these responses in a dose-dependent manner (Fig. 1).

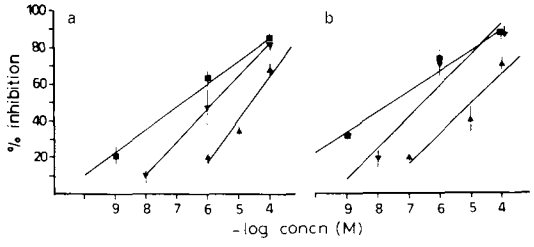

FIG. 1. Effects of various concentrations of (a) verapamil and (b) diltiazem on the oesophageal contraction induced by (▲) acetylcholine 0·5 mM (▼), carbachol 0·5 mM and (■) KCl 50 mM. At least 5 animals were used for each agonist and concentration of antagonist.

The IC50 values of verapamil were $3·8 \times 10^{-5}$, $1·52 \times 10^{-6}$ and 1×10^{-7}M vs acetylcholine, carbachol and KCl respectively. The IC50 values of diltiazem were $1·0 \times 10^{-5}$, $3·8 \times 10^{-7}$ and $3·02 \times 10^{-8}$M. The dose-ratio verapamil/diltiazem was 3·8 vs acetylcholine, 4·0 vs carbachol and 3·31 vs KCl.

Discussion

Our results showed that both Ca^{2+} entry blockers inhibited the contractile response induced by acetylcholine, carbachol and KCl on the LES of the rat. The inhibitory action of diltiazem was greater than that of verapamil the dose-ratio (verapamil/diltiazem) being similar for all agonists. Based on IC50 values, diltiazem is 3–4 times more effective in producing relaxation of the LES than verapamil.

Verapamil and diltiazem inhibited the contractile response to potassium more than the response to cholinomimetic agonists. These results were in agreement with the finding that in most vascular tissues, K^+-induced contractions are more sensitive to calcium entry blockers than contractions induced by other agonists (Shimizu et al 1980; Van Breemen et al 1981). In support of this finding, Cauvin et al (1983) have reported that electrically gated Ca^{2+} channels are more sensitive to calcium entry blockers than the Ca^{2+} pathways regulated by receptors.

The IC50 of both drugs tested for acetylcholine is greater than IC50 for carbachol. These results could be interpreted as meaning that extracellular Ca^{2+} does not appear to be an essential requirement for the direct post-synaptic contractile effect of acetylcholine, while the carbachol contraction has a partial dependence on influx of external Ca^{2+} concentration (Fox & Daniel 1979). On the other hand carbachol may release acetylcholine by depolarizing the presynaptic receptors in a calcium dependent manner (Ramaswamy et al 1978).

Thus, the result shown here support the clinical use of calcium entry blocker in the treatment of oesophageal spasm.

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