POTENTIATION BY CHOLINESTERASE INHIBITORS OF CHOLINERGIC ACTIVITY IN RAT ISOLATED STOMACH

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Cholinesterase (ChE) inhibitors increase gastrointestinal motility, but related side effects are common. These may reach a 53 % (nausea) or 12 % (diarrhoea) incidence with the acetyl-ChE (AChE) and butyryl-ChE (BuChE) inhibitor neostigmine and possibly lower with the selective AChE inhibitor donepezil (Imbimbo, 2001). However, the AChE inhibitor and dopamine D2 receptor antagonist itopride seems well tolerated (e.g. Sawant et al., 2004). We compared their AChE inhibitory activity and their gastric prokinetic-like activity in vitro, by measuring changes in cholinergically-mediated contraction, evoked by electrical field stimulation (EFS; Bassil et al., 2006).

Longitudinal forestomach preparations from adult, fed male CD rats (200 - 250 g) were suspended between two ring electrodes in Krebs’ buffer (5% CO2/ 95% O2; pH 7.4; 37°C) under tension for isometric recording. TTX (1 µM)- and scopolamine (10 µM)-sensitive contractions were evoked by EFS (25 V, 2.5 Hz, 0.5 ms pulse duration, for 10 s every 1 min). Compounds were added cumulatively (at least 10 min contact) to the bathing solution. AChE activity was measured over 5 min, using AChE from human erythrocytes incubated with 5,5'-dithiobis-(2 -nitro-benzoic acid) before addition of acetylthiocholine (Ellman et al., 1961). Data are means ± standard error of the mean; n = animals used (stomach) or number of experiments (erythrocytes) per compound.

Itopride, donepezil and tegaserod increased the amplitude of the EFS-evoked contractions at concentrations as low as 0.01 µM without effecting baseline muscle tension. Neostigmine acted similarly, at concentrations as low as 0.01 µM, but increased baseline muscle tension at 1 µM. The shapes of the concentration-response curves (CRCs) and their maximal activities varied widely. The CRC for tegaserod was bell-shaped (max 35 ± 5.2% increase at 1 µM; 10 µM caused inhibition, n=4); the presence of the 5-HT4 receptor antagonist SB-204070 (0.3 µM; Wardle et al., 1994) greatly reduced this excitatory activity (max 12 ± 2.7% increase at 1 µM, n = 4). The CRCs for donepezil and neostigmine were approximately linear without a defined maximum (respectively, 195 ± 87 % increase at 10 µM, n = 5 and 754 ± 337 % at 1 µM, n = 4). The CRC for itopride appeared biphasic: 23 ± 10 % - 71 ± 32 % increase at 0.01-1 µM, rising to 188 ± 84 % at 10 µM, n = 8); SB-204070 0.3 µM had no effect on these responses. The selective BuChE inhibitor iso–OMPA (Liston et al., 2004) gave a maximum increase of 36 ± 5 % at 10 µM, (n = 3). As inhibitors of AChE activity, the pIC50 values were 4.7 ± 0.1, 5.6±0, 7.6 ± 0.05, 7.6 ± 0.05 and <4 (n = 2 each) for tegaserod, itopride, donepezil, neostigmine and iso-OMPA, respectively.

Each ChE inhibitor exerted gastric prokinetic-like activity, but with marked differences in the levels of activity. The reasons for the differences are not clear, but may be related to variations in the activity of these compounds at AChE within the stomach and / or an interaction between AChE and BuChE inhibition (in the case of neostigmine).