Stereoselective synthesis of oxazolidinonyl-fused piperidines of interest as selective muscarinic (M₁) receptor agonists


Supplementary Data

Studies of the allosteric effect of piperidine 64.
Report on M₁ receptor activity of piperidine 64 on rat duodenum

Aims
1. To evaluate piperidine 64 (MGN) as an allosteric agonist at M₁ muscarinic receptors of rat duodenum.
2. To evaluate MGN as an antagonist of muscarinic M₃ receptors in guinea-pig ileum.

Methods
M₁ receptor-mediated functional responses were measured as the relaxation responses of rat duodenum as characterized in this laboratory previously (Hamrouni, Gudka and Broadley, 2006). Isolated segments (1-2cm) of rat duodenum or guinea-pig ileum were set up in tissue baths containing Tyrodes solution (mM): NaCl, 137; KCl, 2.68; CaCl₂, 1.82; NaHCO₃, 5.9; MgCl₂, 1.0; NaH₂PO₄, 0.42; glucose, 5.6 gassed with O₂ 95% and CO₂ 5% and maintained at 37°C. Isometric tension was recorded by connecting one end of the tissue to a tissue holder and the other to a transducer, by means of a cotton thread. Duodenum was progressively stretched to a resting tension of 1.5g while ileum had a resting tension of 0.5g applied. Isometric tension was measured by force transducers (Ormed, Welwyn Garden City, Hertfordshire, UK) coupled to a PowerLab/4SP computer system (AD Instruments, Charlgrove, Oxfordshire, UK) for data collection. Data was analysed using Chart v.4.1.1 software (AD Instruments, Charlgrove, Oxfordshire, UK).

Concentration-response curves were constructed in the duodenum by adding either McN-A-343 or MGN to the bath non-cumulatively in increasing half logarithmic concentrations. Each dose was left in the bath for 1 min or until a maximum effect was produced. It was then washed from the bath and a 10 min interval allowed before the next dose was introduced. To examine the effect of MGN on responses to McN-A-343, a concentration-response curve for McN-A-343 was obtained first and in the same tissue repeated in the presence of MGN (10⁻⁷ M). MGN was added to the bath 15 min before each dose of McN-A-343.

Responses of the duodenum were measured as the maximum fall in tension (g) from the maximum baseline tension observed prior to addition of McN-A-343.

Concentration-response curves in the ileum to methacholine, a muscarinic agonist, were constructed by cumulative addition of increasing doses until the maximum contraction was achieved. MGM-M1-10A (0.1µM) or its vehicle (DMSO) were added to the tissue bath and allowed to equilibrate for 15 min before a second curve was constructed in their presence. Responses of the ileum were measured as the increase in contraction above the pre-concentration-response curve base line.

Results
MGN (0.1µM) did not affect the resting rhythmic activity of the rat duodenum, indicating that there was no direct agonist (orthosteric) activity at M₁ receptors. However, in its presence there was a shift of the dose-response curve for the relaxation by McN-A-343 to the left (Fig 1). This indicates POTENTIATION of the responses. By contrast, the vehicle for MGM-M1-10A, DMSO, had no effect on the dose-response curves for the M₁ receptor agonist (Fig 2).
The same concentration of MGM-M1-10A (0.1µM) had a small inhibitory effect on the concentration-response curve for methacholine contractions on the guinea-pig ileum (Fig 3). However, this shift to the right was not significant.

![Graph](MGN_vs_McN-A-343.png)

Figure 1 Effect of MGM-M1-10A (0.1µM) on relaxation responses of rat duodenum to the selective M₁ receptor agonist McN-A-343. * significantly different from values in the absence of MGM-M3-10A p<0.05 Student’s paired t-test.

![Graph](DMSO_vs_McN-A-343.png)

Figure 2 Effect of DMSO on relaxation responses of rat duodenum to the selective M₁ receptor agonist McN-A-343.
Figure 3 Contractions of isolated guinea-pig ileum to methacholine, added cumulatively. Effects of MGN-M1-10A (0.1µm) and DMSO on the concentration-response curve for methacholine. After addition of MGN/DMSO, methacholine was added to the bath cumulatively.

**Conclusion**

Piperidine 64 (MGN) appears to be a positive allosteric modulator of the muscarinic M₁ receptor as it potentiates the effects of an M₁ receptor agonist without causing agonist activity on its own or antagonistic activity. There was minimal activity at M₃ receptors of the guinea-pig ileum as the contractions to methacholine were slightly shifted to the right by MGN-M1-10A.

**Reference**