Table 1 Comparison of drug effects on cGMP response and [^{3}H]-QNB binding

Agonist	*EC_{50}–μM for cGMP increase*	*Ki for inhibition ^{3}H-QNB binding—μM*
Oxotremorine	5.6	6.4
Arecoline	22.0	61.0
Carbachol	120.0	115.0
Antagonist	*IC_{50}–nM against cGMP (100 μM oxotremorine)*	*Ki for ^{3}H-QNB binding–nM*
Scopolamine	5.6	5.8
QNB	23.0	5.0
Atropine	54.0	7.7
Clozapine	600.0	350.0
Thioridazine	1300.0	940.0
Chlorpromazine	9300.0	7700.0

(10 μM), but was completely prevented by atropine (1 μM) or QNB (0.1 μM). The muscarinic response was abolished if calcium ions were omitted from the incubation medium, and was additive to the increase in cGMP elicited by sodium azide.

The agonists oxotremorine, arecoline and carbachol yielded EC_{50} values for increasing GMP which agreed well with the corresponding values for these compounds in displacing [^{3}H]-QNB binding (Table 1). The cGMP response thus appears to correspond directly to muscarinic receptor occupancy.

The muscarinic antagonists QNB, scopolamine and atropine and the neuroleptic drugs thioridazine, clozapine and chlorpromazine inhibited the cyclic GMP response to 100 μM oxotremorine. The potencies of the various antagonists in the cGMP and [^{3}H]-QNB tests showed a less close agreement than seen for agonists, and calculated apparent Ka values suggested that the antagonists were more potent in blocking the cGMP response than in displacing [^{3}H]-QNB.

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Hyperpolarization of myenteric neurones by enkephalin

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In the guinea-pig myenteric plexus, morphine and the enkephalins inhibit the neuronal firing which is induced by a glass suction electrode (Dingledine & Goldstein, 1976; North & Williams, 1976). The inhibitory action of morphine in the myenteric plexus has been attributed to a membrane hyperpolarization (Dingledine & Goldstein, 1976; North & Tonini, 1976). The aim of the present experiments was to determine the effects of the enkephalins upon the membrane properties of myenteric neurones.

Intracellular recordings were made from myenteric ganglia which were removed from the ilea of adult guinea-pigs. The isolated ganglia were immobilized and perfused as previously described (Nishi & North, 1973). Cells were impaled under visual control (Nomarski optics, × 500) with glass micro-electrodes which contained 2M potassium chloride. Drugs were applied by changing the perfusing solution to one which differed only in its content of the drug(s). The majority of Type 1 cells were hyperpolarized by met-enkephalin (100–300 nM). The hyperpolarization began as soon as the met-enkephalin reached the tissue and continued throughout the period of exposure (up to 5 min) and rapidly reversed when the tissue was washed with drug-free Krebs solution. The hyperpolarization ranged in amplitude from 3 to 40 mV, the larger effects being more often seen in cells with low resting membrane potentials. In most cells the hyperpolarization was associated with a fall in membrane resistance—this was evident as a reduction in the amplitude of the membrane potential

displacement caused by passing a hyperpolarizing current pulse through the recording electrode by means of a bridge circuit. Cells which responded to met-enkephalin also responded to normorphine (300 nM–1 μM); cells which were unaffected by met-enkephalin were also unaffected by normorphine. Leu-enkephalin had qualitatively similar effects but appeared to be less potent. The hyperpolarization produced by met-enkephalin was reversed by changing to a solution which contained both met-enkephalin and naloxone (20–50 nM); exposure to naloxone alone did not affect the membrane potential but it prevented the action of enkephalin.

Type 2 cells seldom showed any effects of met-enkephalin. A few cells showed a small hyperpolarization (2–5 mV) which passed off during the continued presence of the met-enkephalin.

The functional significance of these effects in the myenteric plexus is not clear. Although the myenteric plexus contains enkephalins (Elde, Hökfelt, Johansson & Terenius, 1976; Smith, Hughes & Kosterlitz, 1976) inhibitory synaptic potentials have not been observed in this tissue. On the other hand, the hyperpolarization provides a basis for the inhibition of spike firing by enkephalins.

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Intracellular recording of the effects of 5-hydroxytryptamine on rabbit superior cervical ganglion cells

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The complex actions of 5-hydroxytryptamine (5-HT) at sympathetic ganglia, which include facilitation and depression of transmission and ganglion cell depolarization (see Wallis & Woodward, 1974), are difficult to elucidate by examining the response of a population of cells. In the work reported here, intracellular recordings were made from rabbit superior cervical ganglia. Rabbits were killed by air embolism and the excised ganglia superfused with Krebs solution at 37°C. Microelectrodes were filled with 2M KCl.

Superfusion of the whole ganglion with 5-HT (10^{-6}–10^{-4}M) produced only small or negligible changes in resting membrane potential and membrane resistance. In 24 tests on 20 cells, 5-HT had no apparent effect on nine occasions, caused a small depolarization (< 5 mV) on eleven occasions and a small hyperpolarization on four occasions. The first application of 5-HT more often produced effects than subsequent ones, suggesting the variability in responsiveness might be related to the rapid tachyphylaxis to 5-HT previously reported for these cells (Wallis & Woodward, 1975).

On the other hand, iontophoresis of 5-HT on to the ganglion cell membrane induced substantial depolarizations with a fall in membrane resistance in 29 of 41 cells tested. The iontophoresis electrode was filled with 20 mM 5-HT creatinine sulphate and positioned near an impaled cell, using a separate micromanipulator. Some cells responded to ejection currents of 20 nA for 100 ms, others only after passing greater currents for several seconds. Only outward current evoked depolarizations. Twelve cells were apparently unresponsive to 5-HT. Lack of response was not easy to establish unequivocally because of difficulty in visualizing the position of the iontophoresis electrode with respect to the impaled cell.

Iontophoresis of 5-HT induced depolarizations which were graded in amplitude (up to 19 mV) as the duration or the intensity of the ejection current was altered. The time to peak of the depolarization was typically 1–3 seconds. Membrane resistance fell by 15–55% at the peak of the depolarization. An after-hyperpolarization sometimes followed the depolarization by which time membrane resistance had returned to control values. The depolarization was sometimes clearly divided into an initial and a late depolarization, evident as a hump on the falling phase of the initial response. The 5-HT depolarization did not decline in