

# 8

## EXPERIMENT EIGHT

# Guinea Pig Ileum

The classic guinea pig ileum preparation has many uses in undergraduate student laboratory classes, ranging from dose–response studies to demonstration of selective antagonism.

The ileum is innervated by the enteric, sympathetic, and parasympathetic divisions of the autonomic nervous system. Among other neurotransmitters, the parasympathetic and enteric fibres release acetylcholine, which acts on muscarinic receptors. It is thought that opioids inhibit release of acetylcholine, by binding pre-synaptically to G-protein coupled  $\mu$  receptors. This effect presumably underlies the unwanted constipatory effects of opioid analgesics. The action of morphine and other opioids in this preparation can be assessed by comparing electrically-induced contractions (presynaptic release of acetylcholine to produce contraction) with exogenous acetylcholine application (postsynaptic contractile response).

### Dissection

A convenient length of ileum is removed and transferred to a dish containing warmed Krebs' solution. The gut must be handled carefully, with fingers rather than with forceps, to avoid damage. When the tissue has relaxed, 2–3 cm long segments are cut and the lumen gently washed with physiological saline to remove the contents. If the animal is not fed for 24 hours prior to the experiment, this may leave the gut clean enough to use without washing. The segments are then attached to a gut tissue holder and mounted under 1 g wt tension in an organ bath containing physiological saline at 32 °C.

Plate 13 shows the ileum segment attached to a special holder: a perspex half-cylinder with a support pole for the tissue, through which one stimulating electrode passes, and another support for the second electrode in the arrangement. The ileum is carefully slid over the intraluminal electrode and the rimmed post it passes through, and tied in place. The other end of the ileum is attached by a thread to the force transducer. The external electrode is fixed at one end, but is flexible. Once the tissue is in place, it is bent to position it close to but not touching the ileum.

# Experiment

## Chart Settings

This is a single-channel arrangement with a PowerLab, Bridge Amp, and force transducer. In Chart, the range should be chosen to suit the maximum force to be exerted on the transducer. The final scale after units conversion and so on should be 0 to 5 g wt. The sampling rate should be at least 20/s. The view compression should be 10:1. A high-pass filter of 10 or 20 Hz should be chosen in the Bridge Amplifier dialog box. Data Pad miniwindows can be set up to show comment number and text.

## Stimulation

Field stimulation of autonomic nerves within the intestine may be accomplished merely with a pair of platinum wire electrodes, one on each side of the midpoint of the gut segment. More elaborate, and perhaps more efficient, arrangements are also used: either passing the tissue through a ring electrode at the top and bottom, or transmural stimulation with one electrode in the lumen and the other placed anywhere in the bath. The stimulus frequency (typically 0.2–0.25 Hz) is chosen to be just faster than the frequency of any spontaneous contractions. The stimulus width should be 0.2–0.5 ms.

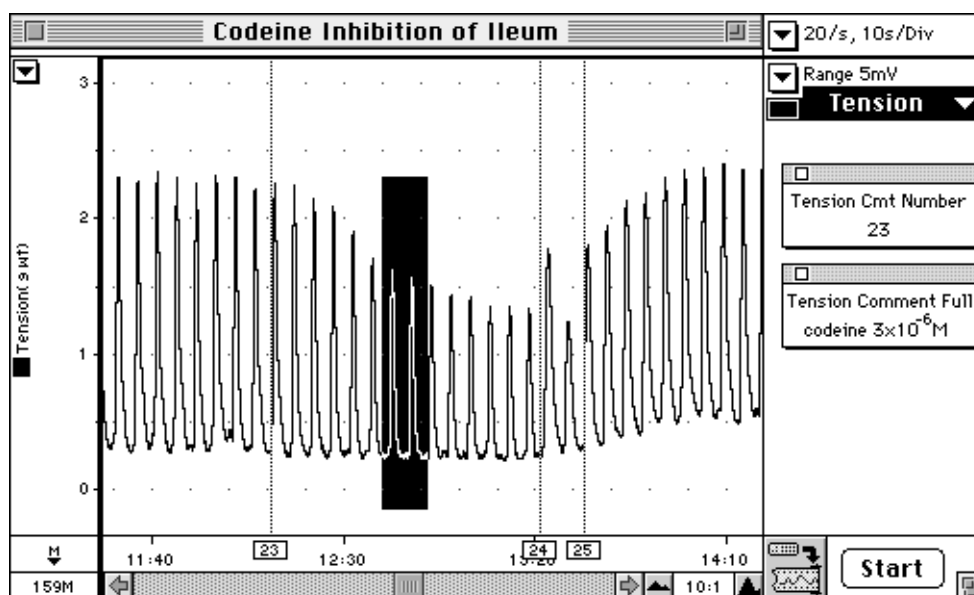
## Protocol

By trial and error, find a stimulus strength that gives strong, reproducible contractions. Equilibrate the tissue while stimulating, for 30 minutes.

Obtain dose–response curves with morphine and codeine to find the dose that produces a 50% inhibition of contraction (IC<sub>50</sub>). A suitable starting concentration for each drug is  $3 \times 10^{-8}$  M. Figure 8–1 shows contractions of guinea pig ileum in response to nerve stimulation, showing reversible inhibition by codeine.

**Figure 8–1**

Contractions of guinea pig ileum in response to nerve stimulation. Codeine ( $3 \times 10^{-6}$  M) added at comment #23 inhibits the response. The effect is reversible, as shown by the washout at comments #24 and 25.



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Test the ability of  $5 \times 10^{-8}$  M noradrenaline to produce an IC<sub>50</sub> response: if necessary adjust the dose to obtain correct level of inhibition. Check that the IC<sub>50</sub> doses of all drugs give rise to reproducible 50% inhibition.

Incubate with the opioid antagonist naltrexone ( $5 \times 10^{-8}$  M) for 20 minutes. Test the IC<sub>50</sub> doses of morphine, codeine, and noradrenaline. Wash out after each test, remembering to add naltrexone again. Naltrexone antagonises the opioid inhibitory effects with little or no change to the inhibition by noradrenaline.

Wash out naltrexone thoroughly. Without electrical stimulation, find a dose of acetylcholine that gives a good contraction (around  $10^{-7}$  M) and measure the response. Repeat in the presence of the opioid. The expected result is that the opioid has no effect, showing that its effect is not postsynaptic.

## Further Work

The guinea pig ileum also contains cannabinoid receptors. The effects of cannabinoid agonists such as CP55940 can be assessed by measuring evoked or spontaneous contractions or both. Suitable protocols are available in the literature<sup>1</sup>.

## Reference

1. R.G. Pertwee et al., Further evidence for the presence of cannabinoid CB1 receptors in guinea-pig small intestine, *British Journal of Pharmacology* 118(8): 2199–2205 (1996).

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**Plate 13.** Guinea pig ileum preparation, with transmural stimulating electrodes: one electrode passes through the support post so that it is in the lumen of the ileum, the other is positioned close to but not touching the ileum.

