5 mg/kg; i.p.) and measuring hot plate reaction times and respiratory rates at peak effect. Dependence was measured by challenging pretreated mice with naloxone (1 mg/kg). Hot plate reaction times and respiratory rates were measured for one hour following injection. Frequency of jumping, 'wet dog' shakes, shivering, weight loss, and other signs of withdrawal were also noted.

The increase in hot plate reaction times and depression of respiratory rates in animals pretreated with either morphine or ethylketocyclazocine for between 12 and 72 h prior to acute administration of either morphine or ethylketocyclazocine, were significantly lower than in control animals pretreated with vehicle. The degree of dependence was difficult to assess, but similar signs of withdrawal following administration of naloxone were seen in animals pretreated with either morphine of ethylketocyclazocine.

Pieces of guinea-pig ileum were set up for transmural stimulation (0.1 Hz, 0.5ms duration, supramaximal voltage). Tolerance was assessed by a comparison of the ID_{30} agonist dose to inhibit twitch height in pretreated animals with the ID_{30} agonist dose derived from equivalent controls. Degree of dependence was assessed using a method similar to that described by Schultz & Herz (1976) in which segments of ileum from pretreated guinea-pigs contract when challenged with naloxone.

Tolerance, cross-tolerance, and dependence was seen in animals treated with morphine or ethylketocyclazocine for 6–96 hours.

S.J.W. is an S.R.C. student.

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Characterization of opiate receptors in the isolated rat rectum

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The isolated rectum of the rat has been reported to contract in response to low concentrations of several opioid peptides (Nijkamp & van Ree, 1978). In the present study a field stimulated rat rectum preparation was developed in which the electrically-evoked contractions were shown to be inhibited by both the classical opiate normorphine, and the opioid peptides leucine-and methionine-enkephalin and β-endorphin.

Segments of rat rectum were induced to contract by applying trains of pulses (1 ms pulses at 2 pulses/s for 2 s every 40 s) by means of ring electrodes above and below the tissue. The contractions so induced were biphasic, and the first component was inhibited by opiates (IC_{50} for leu-enkephalin = 10.8 ± 2.67 nM)

Table 1 Comparison of agonist potency and reversibility by naloxone of representative opiates in three *in vitro* preparations

	Guinea Pig Ileum[a]		*Mouse Vas Deferens*[a]		*Rat Rectum*	
	Potency[b]	*Ke naloxone* (nM)	*Potency*[b]	*Ke naloxone* (nM)	*Potency*[b]	*Ke naloxone* (nM)
Leu-enkephalin	1	1.74	1	21.4	1	21.7 ± 2.34
Met-enkephalin	2.1	1.94	0.28	28.3	1.98 ± 0.33	19.7 ± 1.76
β-endorphin	8.9	2.53	0.138	30.5	0.198 ± 0.037	28.8[c]
Normorphine	4.52	1.83	0.07	4.75	0.141 ± 0.038	3.68 ± 1.01

[a] Data adapted from Shaw & Turnbull (1978).
[b] Potencies are expressed as ratios to the IC_{50} of leu-enkephalin.
[c] Mean of 2 observations. All other figures represent the mean of at least 5 determinations.

and by atropine. The response to opiates, but not that to atropine, was antagonized by naloxone.

The receptors involved were then characterized using the μ and δ-receptor classification of Lord *et al.*, (1977). The opiate μ-receptor, as found in the guinea-pig ileum, is equally sensitive to the enkephalins and the classical opiate agonist, normorphine, whilst the δ-receptor, as found in the mouse vas deferens, is considerably less sensitive to normorphine than to the enkephalins. In addition, the concentration of naloxone required to antagonize the enkephalins is ten times higher in the mouse vas deferens than in the guinea pig ileum whilst normorphine is reversed by low concentrations of naloxone in both tissues.

From Table 1 it is clear that the rat rectum is considerably more sensitive to the enkephalins than to normorphine and that the effect of the enkephalins, but not that of normorphine, is relatively resistant to antagonism by naloxone. Thus, in both these respects, the rectum is similar to the mouse vas deferens. The opiate receptors in the rat rectum would thus appear to be of the δ-type.

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Effects of peptides on neurones in the substantia nigra and nucleus accumbens

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It is now clear that peptides play an important role in the brain. Indirect evidence suggests that TRH (pyroglutamyl-histidyl-prolineamide) and MIF (prolyl-leucyl-glycineamide) might influence the activity of the nigro-striatal and mesolimbic dopaminergic pathways (Barbeau, 1975; Miyamoto & Nagawa, 1977). Both Substance P (Mroz & Leeman, 1977) and angiotensin-converting enzyme (Arregui, Emson & Spokes, 1978) occur in the substantia nigra and Substance P has been shown to have direct effects on neurones in the substantia nigra (Davies & Dray, 1976). In the present investigations we have studied the effects of substance P, TRH, MIF and angiotensin II on neurones in the rat substantia nigra and nucleus accumbens.

Female Wistar rats (150–200 g) were anaesthetised with urethane (1.5 g/kg). Action potentials were recorded extracellularly and drugs were applied microiontophoretically using 6-barrelled micropipettes as previously described (Crossman, Walker & Woodruff, 1974). The recording barrel contained pontamine sky blue (2%) in NaCl soln. (0.2 M). The other barrels each contained one of the following: Substance P (0.7 mM); angiotensin II (10 mM) (each in 20 mM acetic acid, pM 4.5); TRH (48 mM, pH 4.5); MIF (55 mM, pH 4.5); dopamine HCl (200 mM, pH 4.0). All peptides were ejected as positive ions. In the case of MIF, experiments were carried out to ensure that the drug was expelled from the micropipette under the conditions used. Each peptide was tested on between 14 and 96 neurones.

Both MIF and TRH (up to 100 nA for 120 s) failed to influence the activity of neurones in either the nucleus accumbens or in the zona compacta (SNC) or the zona reticulata (SNR) of the substantia nigra. The inhibitory of dopamine in the nucleus accumbens was unaffected by MIF or TRH. Angiotensin II was similarly inactive on substantia nigra neurones when applied with currents of up to 100 nA for 160 seconds. Substance P, on the other hand, caused a powerful, dose-dependent, stimulation of neurones in the substantia nigra. The excitations produced by Substance P were of slow onset and long duration and were apparent both in the SNC and in the SNR. A total of 65 of the 96 cells tested were excited by Substance P.

Our results are consistent with a functional role for Substance P in the substantia nigra but provide no evidence to support the hypothesis that TRH, MIF or angiotensin II might be transmitters in the areas investigated.

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