have suggested that oestrogens enhance clotting factor synthesis through a fast-acting mechanism (Rama Rao et al 1964; Owens & Cimino 1984), or over a longer timecourse (Matschiner & Willingham 1974; Siegfried et al 1979). Our present findings suggest that in addition to these possibilities, there may be a more marked rate of catabolism of vitamin K-dependent clotting factor precursors in male rats than in female rats. Whether this effect is the result of a greater turnover of plasma proteins in male rats, compared with female rats, or to the specific breakdown of vitamin K-dependent clotting factor precursors, remains to be determined.

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The effect of meptazinol on the guinea-pig sphincter of Oddi in-vitro

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Meptazinol causes a dose-dependent contraction of the guinea-pig sphincter of Oddi in-vitro. This was antagonized by atropine in concentrations which blocked the contractile response to acetylcholine but not that to KCl. Naloxone was unable to block the response of the tissue to meptazinol, and other opioid drugs had inconsistent effects. Although meptazinol has significant anticholinesterase activity on this preparation, comparison with neostigmine suggests that this is irrelevant to its contractile action.

Meptazinol is a mixed agonist antagonist opioid analgesic which has additional cholinergic analgesic activity (Bill et al 1983). Cholinergic activity is potentiated by meptazinol in smooth muscle preparations (Stephens et al 1978) and in skeletal muscle preparations (Strahan et al 1985). These actions can be explained, at least in part, by meptazinol's anticholinesterase activity (Galli 1985).

Mixed agonist antagonist opioid analgesics are reputed to be less likely to cause spasm of the sphincter of Oddi than pure agonists such as fentanyl and morphine (McCammon et al 1984). However, since the sphincter of Oddi contracts powerfully to acetylcholine (Persson 1972) it is possible that meptazinol might have greater effects on this tissue than other opioids. This

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possibility has been examined in-vitro using the guinea-pig sphincter of Oddi preparation.

Methods

Male and female tricolour guinea-pigs were killed by a blow to the head. The common bile duct, cystic duct, gall bladder and a small piece of duodenum surrounding the sphincter of Oddi were dissected out. A cannula was introduced into the common bile duct through a hole made in the gall bladder and through the cystic duct and secured so that the tip of the cannula was 5 mm above the sphincter of Oddi. The preparation was perfused through the cannula with Krebs-Henseleit solution oxygenated with 5% CO_2 in oxygen at 37 °C at 0·5 mL min^{-1} and immersed in oxygenated Krebs-Henseleit solution at 37 °C. Pressure changes in the perfusate were measured just above the tissues using a pressure transducer connected to a Grass 79C recorder. The apparatus was calibrated daily with a mercury manometer. The resting pressure of the preparations was between 5 and 25 mm Hg (mean 14 mm Hg). Drugs were applied to the tissue as a bolus in 0·1 mL of 0·9% NaCl (saline) to the inner surface of the preparation or in the perfusing Krebs solution. The outside of the

preparation was washed after bolus doses had produced an effect.

Measurement of cholinesterase inhibition. The cholinesterase activity of homogenates of the guinea-pig sphincter of Oddi preparations was assayed using the method described by Ellman et al (1961). Various concentrations of meptazinol hydrochloride or neostigmine bromide were added to the cuvette and the inhibition of cholinesterase measured.

Meptazinol hydrochloride (Wyeth Laboratories), morphine hydrochloride (MacFarlan Smith Limited), neostigmine bromide (Sigma Chemical Company Limited), naloxone hydrochloride (Endo Laboratories Inc.), atropine sulphate (MacFarlan Smith Limited), and acetylcholine chloride (Sigma Chemical Company Limited) were freshly prepared by dissolving in saline or Krebs-Henseleit solution. The doses and concentrations *in the text refer to the salts.* Fentanyl citrate was obtained from the commercially available ampoule containing 0.05 mg mL^{-1} fentanyl base (Sublimaze, Janssen Pharmaceutical Limited).

Results

Some preparations were quiescent whilst others exhibited spontaneous activity such as rhythmic spikes of pressure changes or undulations. The response of the preparation to bolus doses of drug was a rise in basal pressure with superimposed spikes (Fig. 1). Results have been expressed as the maximum increase in pressure produced by the drug, as the duration and detailed characteristics of the response were too variable to quantify. All preparations tested responded to bolus doses of acetylcholine, meptazinol and neostigmine. These three agents caused a dose-dependent increase in pressure (Fig. 2).
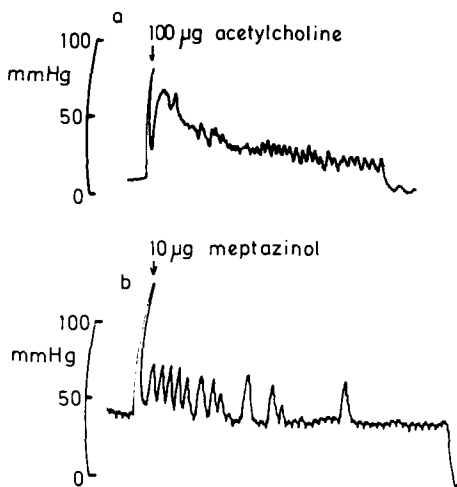
FIG. 1. Example of the response of the guinea-pig isolated sphincter of Oddi to (a) acetylcholine 100 µg and (b) meptazinol 10 µg.
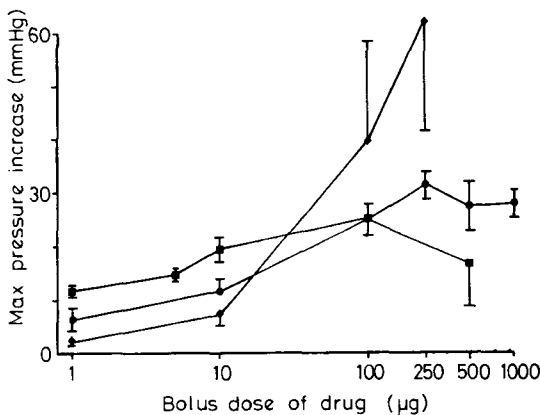
FIG. 2. Dose-response relationship for the maximum increase in pressure produced by acetylcholine (●), meptazinol (■) and neostigmine (◆) on the guinea-pig isolated sphincter of Oddi. Results are expressed as means ± s.e.m. of not less than 8 preparations.

Perfusion of the preparation with atropine 10 and 100 ng mL^{-1} reduced the responses to both acetylcholine and meptazinol but did not affect the response of the preparation to a bolus dose of potassium chloride (Table 1).

The response to acetylcholine was constant or slightly increased over several hours whilst that to meptazinol tended to decrease with time (Table 2). The time interval between doses was about 1 h.

Naloxone hydrochloride 10 µg mL^{-1} did not affect the response of the tissue to acetylcholine but it did appear to reduce the response to meptazinol more than was seen in the time matched control (Table 2). However, increasing the concentration of naloxone to 40 µg mL^{-1} caused no further reduction in the response to meptazinol.

The ability of the concentrations of naloxone used to block opioid receptors on this preparation could not be tested as only 22 out of 44 preparations responded to

Table 1. Effect of atropine sulphate on the response of the guinea-pig sphincter of Oddi to meptazinol, acetylcholine and potassium chloride in-vitro. Results are mean responses ± s.e.m. of not less than 8 preparations. *$P <$ 0.05 paired t-test.

	Maximum increase in pressure (mm Hg)		
	Control	Atropine 10 ng mL^{-1}	100 ng mL^{-1}
Meptazinol			
10 µg	18.4 ± 2.6	4 ± 2.1*	3 ± 1.4*
100 µg	16.1 ± 2.1	4.7 ± 2.1*	0
Acetylcholine			
100 µg	25.0 ± 3.1	12.2 ± 3.4*	6.1 ± 1.7*
250 µg	29.1 ± 3.5	23.8 ± 3.8*	17.2 ± 7.6*
KCl 350 µg	10.1 ± 1.2	8.8 ± 2.1	9.7 ± 1.6

Table 2. Effect of naloxone 10 μg mL^{-1} on the response of the guinea-pig sphincter of Oddi to acetylcholine and meptazinol in-vitro. Results are mean response ± s.e.m. of not less than 8 preparations. The second administration either with or without naloxone was 1 h after the first administration. * Significant (paired t-test) change from control response $P < 0.05$.

	Maximum increase in pressure (mm Hg)	
	Control response	Naloxone
Acetylcholine 250 μg	30·6 ± 4·7	34·6 ± 4·8
Meptazinol 10 μg	18·1 ± 2·4	7·6 ± 2·5*
Meptazinol 100 μg	19·2 ± 2·6	10·7 ± 2·3*
	Time-matched control	
	1st Admin.	2nd Admin.
Acetylcholine 250 μg	27·9 ± 4·6	33·4 ± 5·5
Meptazinol 10 μg	21·2 ± 5·9	17·9 ± 4·8
Meptazinol 100 μg	24·1 ± 3·6	15·0 ± 2·1*

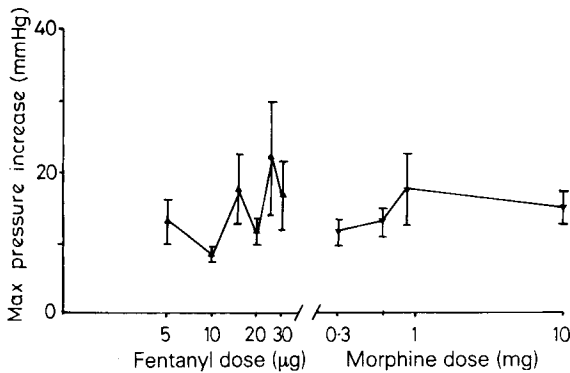

FIG. 3. Dose-response relationship for the maximum increase in pressure produced by fentanyl (▲), and morphine (▼) on the guinea-pig isolated sphincter of Oddi. Results are expressed as means ± s.e.m. of not less than 8 preparations.
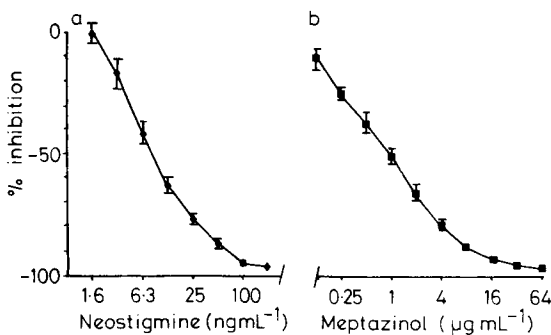

FIG. 4. Inhibition of cholinesterase from guinea-pig homogenized sphincter of Oddi preparations by (a) neostigmine and (b) meptazinol. Results are expressed as means ± s.e.m. of not less than 6 preparations.

morphine and 52 out of 112 responded to fentanyl. Tissues which did respond to these opioids did not exhibit any clear dose-response relationship (Fig. 3) and tolerance or tachyphylaxis occurred rapidly. Furthermore, a tissue which responded to fentanyl did not necessarily respond to morphine and vice versa.

The mean acetylcholinesterase activity of the homogenized guinea-pig sphincter of Oddi preparation was 6·22 ± 0·43 (n = 13) mol L^{-1} min^{-1} × 10^{-6} g^{-1} of tissue.

Both neostigmine and meptazinol inhibited cholinesterase in the sphincter of Oddi homogenate. However neostigmine was effective in ng mL^{-1} quantities whilst meptazinol required μg mL^{-1} quantities (Fig. 4).

Discussion

Meptazinol consistently induced an increase in pressure of the fluid perfusing the sphincter of Oddi. It is unlikely that this was mediated solely via an opioid receptor as naloxone antagonism was incomplete and other opioid drugs failed to increase the pressure in 50% of preparations. The poor response of in-vitro preparations of the sphincter of Oddi to opioids in general has been reported by others (Persson 1972).

The response to meptazinol was abolished by atropine in concentrations that showed selectivity for muscarinic receptors. Whilst meptazinol is an effective anticholinesterase agent on the sphincter of Oddi preparation it is doubtful whether this could explain its stimulant action. Neostigmine is almost a thousand times more potent than meptazinol as an anticholinesterase agent on the preparation but was approximately equipotent with meptazinol with respect to increasing the perfusion pressure of the tissue. The bolus dose of neostigmine necessary to produce a response from the sphincter of Oddi is compatible with doses of neostigmine which have direct muscarinic activity (Pleuvry & Hunter 1968).

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