J. Pharm. Pharmacol. 1989, 41: 168–172
Received June 15, 1988

Naloxone-insensitive Inhibitory and Excitatory Effects of Opioid Agonists in the Rat Isolated Uterus

S.E. OHIA* AND A. A. LANIYONU

Department of Pharmacology, University of Glasgow, Glasgow G128 QQ, UK

Abstract—Morphine ($3 \times 10^{-6} - 10^{-3}$ M) produced a concentration-dependent inhibition of both spontaneous rhythmic contractions and tonic contraction induced by potassium chloride (KCl) (50 mM) in rat isolated uterus. Uteri at the metoestrus phase were the most sensitive to the inhibitory effect of morphine. Morphine-induced relaxation was characterized by a post-wash tonic contraction. The μ-specific opioid agonist, D-alaglymepheglycol (DAGO) ($4 \times 10^{-8} - 1{\cdot}2 \times 10^{-5}$ M) did not affect spontaneous rhythmic contraction but produced a partial inhibition of the KCl depolarized uterus. There was no post-wash contraction following DAGO. In contrast to the inhibition produced by both morphine and DAGO, methionine enkephalin ($8 \times 10^{-7} - 2{\cdot}4 \times 10^{-5}$ M) produced a concentration-dependent contraction of the KCl depolarized uterus. Naloxone up to 100 μM, propranolol (10 μM), flurbiprofen (50 μM) and metiamide (10 μM) did not affect either the relaxation produced by morphine and DAGO or the contraction produced by methionine enkephalin. The results showed that the opioid agonists may have non-receptor mediated direct effects on the rat uterus.

Enkephalin-containing neurons which may subserve a physiological role have been demonstrated in the female genital tract (Lundberg et al 1980). In-vitro, morphine and some of the other opioid agonists have been shown to exert an inhibitory action on uterine smooth muscle (Acevedo & Contreras 1984; Silvalingham & Pleuvry 1985). Campbell et al (1961) reported that the use of therapeutic doses of morphine during labour resulted in prolongation of parturition. Furthermore, the central effects of morphine in-vivo may affect the degree to which the parturient is able to co-operate in delivery (Jaffe & Martins 1985). However, the mechanism(s) which underlie the effects of morphine and other opioids on the female reproductive system is unclear. Some opioid agonists like methadone (Huidobro et al 1971) and pethidine (Silvalingham & Pleuvry 1985; Fazackerley & Pleuvry 1987) have been shown to produce excitatory or inhibitory effects depending on the concentration used.

The aim of the present study, therefore, was to examine the action of some specific opioid agonists on the rat uterus under different hormonal conditions. The effects of morphine, methione enkephalin and DAGO have been investigated on the spontaneously-contracting and potassium-depolarized uteri during the proestrus, oestrus, metoestrus and dioestrus phases of the oestrus cycle.

Materials and Methods

Virgin female Wistar rats (180 g to 300 g) were used. Vaginal smears were taken immediately after animals were killed by stunning and exsanguination. The procedure involved injecting 0·5 mL of distilled water into the vagina from a Pasteur pipette and the fluid containing shed vaginal cells was then withdrawn, placed on a glass slide and air dried. The smears were stained with 0·1% (w/v) methylene blue and the four phases of the oestrus cycle—proestrus, oestrus, metoestrus and dioestrus were identified according to Ham (1969). 2–3 cm lengths from the mid-portion of each uterine horn was suspended in paired 10 mL organ baths containing modified Ringer-Locke solution at 37°C and gassed with pure oxygen. The Ringer-Locke solution had the following composition (mM); NaCl 154·7; KCl 5·63; $NaHCO_3$ 1·78; $CaCl_2$ 3·14 and glucose 5·55. Isometric tension was recorded via Grass FTO3 force-displacement transducers and displayed on a Grass Model 7D polygraph. A resting tension of 0·5 g was applied to each horn and the tissues were allowed to equilibrate for at least 1 h.

Uteri from the different phases of the oestrus cycle exhibited spontaneous rhythmic contractions on set up. There was a slight variation in the spontaneous rhythmic contractions during the oestrus cycle but time-matched controls were employed when each drug effect was examined. The effects of the opioid agonists, morphine, methione enkephalin (met-enkephalin) and DAGO were examined on both spontaneous rhythmic activity and on KCl-induced contraction. KCl (50 mM) caused a biphasic contractile response with an initial transient phase followed by a secondary sustained phase which lasted for more than 1 h. Cumulative concentration-response curves to the opioid agonists were constructed 15 min into the sustained (tonic) phase of the KCl response. In studies involving the use of antagonists, naloxone (in concentrations up to 100 μM), a β-adrenoceptor blocking drug, propranolol (10 μM), a cyclo-oxygenase inhibitor, flurbiprofen (50 μM) or a histamine H_2-receptor blocking drug, metiamide (10 μM) were incubated with the tissues for at least 30 min before the effect of each agonist was re-examined. Drugs used included: morphine hydrochloride (Macarthys), naloxone hydrochloride (Winthrop), propranolol hydrochloride (ICI), sodium flurbiprofen dihydrate (Boots), metiamide (SKF), methione enkephalin (Sigma), D-alaglymepheglycol (Sigma) and methylene blue (BDH). All values given are means ± standard error of the mean (s.e.m.). Student's t-test (two-tailed) and

* Present address: Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3V6.

Correspondence to: A. A. Laniyonu, University of Manitoba, Department of Oral Biology, Faculty of Dentistry, 780 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3.

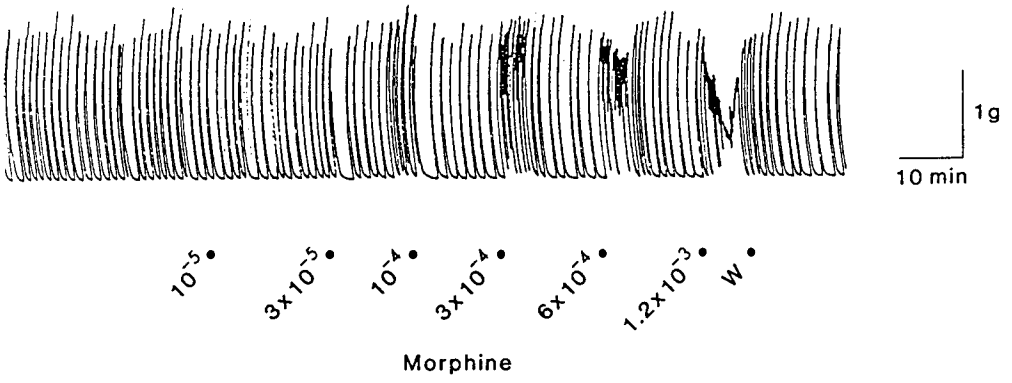

FIG. 1. Inhibitory effect of morphine on spontaneous rhythmic contractions of the rat isolated uterus. High concentrations of morphine abolished the spontaneous rhythmic contractions. Molar concentrations of morphine were added cummulatively. W = wash.

Kruskal-Wallis ANOVA by ranks tests were performed to compare results.

Results

When compared with time-matched controls, low concentrations of morphine (up to 10 μM) had no effect on spontaneous rhythmic contractions of the rat isolated uterus. However, higher morphine concentrations caused a decrease in tonus and frequency of the spontaneous contractions which was completely abolished at 3 mM. The inhibitory effect of morphine occurred throughout the oestrus cycle and Fig. 1 illustrates the typical effect observed in the oestrus phase. The effect induced by morphine in the four phases of the oestrus cycle was reversible by washing. Both met-enkephalin and DAGO (up to 10 μM) had no effect on the spontaneous rhythmic contractions.

In preliminary experiments, the effects of KCl concentrations between 3 and 100 mM were examined in uteri from the four phases of the oestrus cycle. The standard concentration of KCl chosen was 50 mM and the maximum tension (g) produced in the proestrus, oestrus, metoestrus and dioestrus were $2{\cdot}10\pm0{\cdot}35$ (n = 6), $2{\cdot}52\pm0{\cdot}37$ (n = 6), $1{\cdot}84\pm0{\cdot}49$ (n = 5) and $1{\cdot}90\pm0{\cdot}24$ (n = 6), respectively. There were no statistically ($P>0{\cdot}05$) significant differences in the maximum tension developed to KCl in the secondary sustained phase in uteri throughout the oestrus cycle. On uteri precontracted with KCl, morphine (30 μM–6 mM) produced a concentration-dependent relaxation in proestrus, oestrus, metoestrus and dioestrus (Fig. 2). The sensitivity of the uterine muscle to the inhibitory effect of morphine measured as the pD_2 value was highest at the metoestrus stage of the cycle and was significantly different ($P<0{\cdot}05$, Kruskal-Wallis test) from the value obtained during the proestrus stage (Table 1). Uteri from animals in dioestrus and oestrus exhibited intermediate sensitivity to morphine. Following wash-off of morphine and its replacement with fresh Ringer-Locke solution, there was an immediate tonic contraction of the uterus which was maintained for about 5 min before the spontaneous activity of the uterus resumed (Fig. 3A illustrates the relaxation of KCl-induced tone by morphine and the typical post-wash contracture). The post-wash tonic contraction was observed in all phases of the oestrus cycle.

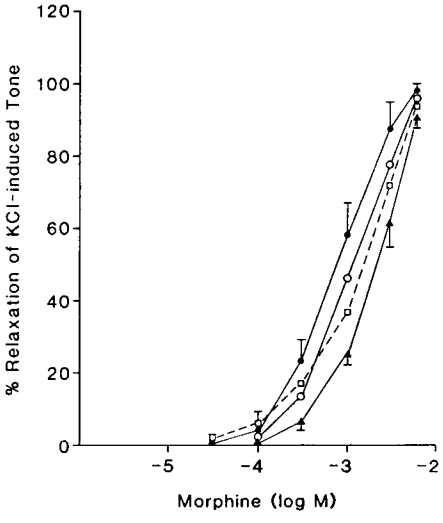

FIG. 2. The relaxation of rat uterus at different stages of oestrus cycle precontracted with KCl (50 mM) by the cumulative addition of morphine. ●——● metoestrus, ○——○ dioestrus, □ - - - - □ oestrus and ▲——▲ proestrus. The data points are mean ± s.e.m. (n = 4–6).

DAGO (40 nM–12 μM) caused a concentration-related relaxation of the KCl-induced tone during the oestrus cycle (Fig. 3B). The pD_2 values obtained for DAGO during the oestrus cycle are shown in Table 1. There was no statistically significant differences in the pD_2 values for DAGO in the four phases. In comparison with morphine, DAGO was more potent in eliciting relaxation of the rat uterus. However, DAGO was less efficacious than morphine and did not display a post-wash contractile response.

Unlike both morphine and DAGO, met-enkephalin (0·8–24 μM) produced a concentration-dependent contraction of KCl-depolarized uteri in the four phases of the oestrus cycle (Fig. 4). Following washout of met-enkephalin, there was a latency of about 5 min before spontaneous activity resumed in uteri from all four phases. Met-enkephalin potency ($pD_2 = -\log_{10}$ EC50) in eliciting contractions of KCl-depolarized uteri in proestrus, oestrus, metoestrus and dioestrus were $5{\cdot}98\pm0{\cdot}40$ (n = 5), $6{\cdot}02\pm0{\cdot}38$ (n = 6), $6{\cdot}36\pm0{\cdot}25$ (n = 5) and $6{\cdot}32\pm0{\cdot}36$ (n = 5), respectively. There were no statisti-

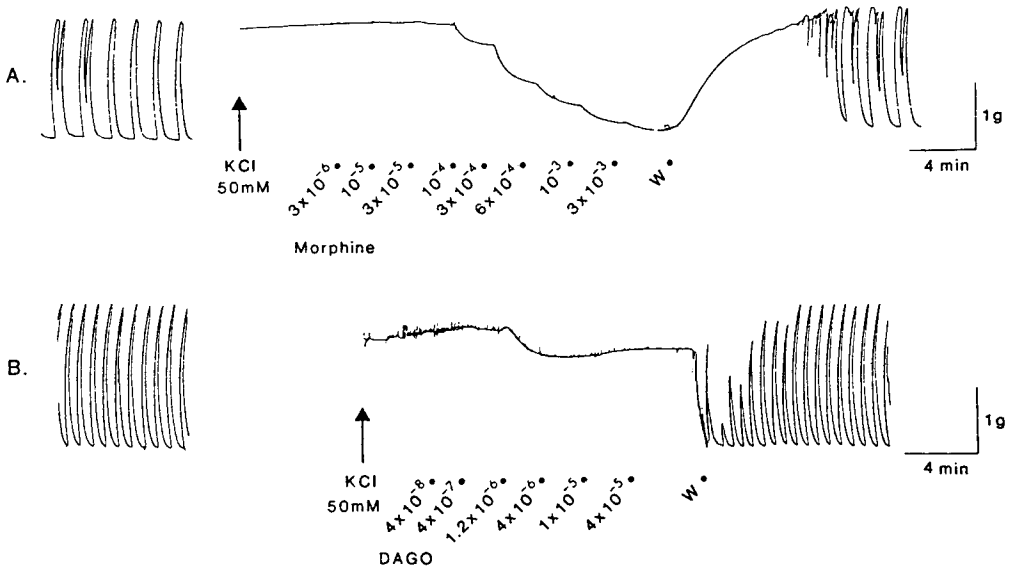

FIG. 3. Inhibition of KCl-induced contraction by morphine and DAGO. The uterus was precontracted with KCl (50 mM). Molar concentrations of morphine (upper panel A) or DAGO (lower panel B) were then added cummulatively. Following the wash out (W) of morphine, there was an immediate tonic contraction before spontaneous rhythmic activity resumed. There was no wash out tonic contraction with DAGO. Top and bottom tracings were from separate experiments.

Table 1. Sensitivity of the rat uterus measured as pD_2 values to the inhibitory effect of morphine during the various stages of the oestrus cycle.

	pD_2	
Phase	Morphine	DAGO
Proestrus	3·36±0·06*(6)	6·08±0·30 (5)
Oestrus	3·51±0·22 (5)	6·03±0·42 (5)
Metoestrus	4·24±0·31 (6)	6·51±0·33 (6)
Dioestrus	4·07±0·37 (4)	6·39±0·28 (4)

(a) $pD_2 = -\log_{10}IC50$, where IC50 is the effective concentration eliciting 50% relaxation of KCl-induced tone.
* Significantly different from metoestrus phase, $P < 0{\cdot}05$. Number of observations in parentheses.

cally ($P > 0{\cdot}05$) significant differences in the pD_2 values for met-enkephalin during the oestrus cycle.

Naloxone (in concentrations up to 100 μM), propranolol (10 μM) flurbiprofen (50 μM) and metiamide (10 μM) had no effect on either morphine- or DAGO-induced relaxations.

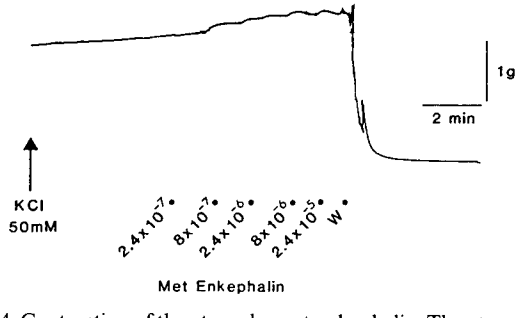

FIG. 4. Contraction of the uterus by met-enkaphalin. The uterus was contracted with KCl (50 mM). Cumulative addition of molar concentration of met-enkephalin resulted in further contractions. There was no post-wash contraction.

The post-wash contractile response observed with morphine was also not affected by these antagonists. Similarly, the met-enkephlain-evoked contractions of the uterus were unaffected by naloxone, propranolol, flurbiprofen or metiamide.

Discussion

The results of the present study demonstrate that opioid agonists produce both inhibitory and excitatory effects on the rat isolated uterus during the natural oestrus cycle. Morphine and DAGO elicited an inhibitory action, while met-enkephalin induced a stimulant effect on the uterus. Similar qualitative differences have also been produced by the opioid peptides on the cardiovascular system (Holaday 1983). The spasmolytic effect caused by morphine in the rat uterus is analogous to that reported by other workers in the cat (Huidobro et al 1971) and mouse (Acevedo & Contreras 1984) uterus. Acevedo & Contreras (1984) found that acute administration of morphine depressed the contractile response to acetylcholine in the mouse uterus. In our study, uteri from rats in the proestrus phase of the oestrus cycle were the least sensitive to the inhibitory effect of morphine. It may well be that the high concentrations of circulating oestrogens found in animals under this phase (Yoshinaga et al 1969) could be partially responsible for the decreased sensitivity in proestrus. Indeed, the antagonistic effect of oestrogens on morphine-induced responses has been demonstrated in other systems. Kasson & George (1984) showed that treatment of animals with oestradiol caused a reduction, whilst ovariectomy produced an increase in both morphine-induced antinociception and hypothermia.

Replacing the bathing medium with fresh Ringer-Locke solution after the maximal relaxant effect elicited by morphine on the KCl contraction resulted in a post-wash

stimulant action. The post-wash contractile response was not observed with DAGO or met-enkephalin in this study. Similarly, histamine and salbutamol produced maximal inhibition of KCl-induced tone without a post-wash stimulant action (Goyal & Verma 1982; Boyle & Ohia 1985). This post-wash contraction is similar to naloxone- or washout-induced contraction of the morphine-dependent guinea-pig ileum (Collier et al 1981; Schulz et al 1985; Johnson et al 1987) and may be indicative of development of morphine dependence by the uterus following brief in-vitro exposure.

Contractile responses in the potassium-depolarized rat uterus produced by met-enkephlain would tend to suggest that the opioid agonists may be utilizing different means in eliciting their effects in this preparation. In contrast to the above finding, Ottensen et al (1983) were unable to demonstrate any stimulatory or inhibitory effect of either met-enkephalin or leu-enkephalin on human uterine smooth muscle. Thus, species differences may exist in uterine response to met-enkephalin.

The opioid receptor antagonist, naloxone had no effect on the responses induced by morphine, DAGO and met-enkephalin. In addition, the potency of the drugs tested does not correlate with known potencies of these agents on other systems/receptors. It seems, therefore, that an opioid receptor mediated mechanisms may not be involved in the effects elicited by these agonists in the rat uterus. Silvalingham & Pleuvry (1985) also showed that the stimulant action of pethidine and the inhibitory effect of morphine on the rat uterus was insensitive to naloxone. In order to evaluate the involvement of other receptors, the effect of morphine was investigated in the presence of a β-adrenoceptor antagonist (propranolol), of histamine H_2-receptor antagonist (metiamide) and a cyclo-oxygenase inhibitor (flurbiprofen) at the concentrations previously shown to block the respective receptors or enzyme system in the rat uterus (Diamond & Brody 1966; Vane & Williams 1973; Bertaccini et al 1979; Ohia & Okpako 1981; Goyal & Verma 1982). None of the antagonists altered the action of morphine on the rat uterus. Furthermore, inhibition of Na^+/K^+-ATPase activity is unlikely to be involved in the morphine-induced effect since ouabain has been shown to have no effect on the inhibitory response in the mouse uterus (Acevedo & Contreras 1984). Thus it would appear that the opioid agonists may be producing their differential action on the uterus at a locus beyond the level of occupation of opioid receptors. One possible mechanism may be an interaction between the opioid agonists and uterine calcium function. Indeed, there is evidence that pethidine-induced contractions of the pregnant rat uterus is sensitive to verapamil indicating that mobilization of extracellular calcium is necessary for pethidine's action on this muscle (Fazackerley & Pleuvry 1987). Opioid agonists-induced effects on calcium function have also been demonstrated in other systems. Both Ross (1977) and Yamamoto et al (1978) showed that acute morphine treatment produced a selective decrease in the calcium binding capacity and calcium content of brain synaptosomes. Morphine and methadone have also been shown to cause a naloxone-insensitive inhibition of calcium-induced protein phosphorylation of synaptic membrane proteins in intact synaptosomes from the rat striatum (Clouet et al 1978).

The concentrations of morphine used in the present study are similar to those which inhibit depolarization-evoked calcium uptake in cerebrocortical slices (Bradford et al 1986). Therefore it is feasible that the inhibitory responses produced by morphine and DAGO in the rat uterus may involve a direct/indirect effect on calcium uptake in this muscle. At the moment, we are not in a position to speculate on the reasons for the qualitative differences in the observed responses between morphine and DAGO on one hand, and met-enkephalin on the other. The fact that they have affinity for different subtypes of opioid receptors may not suffice since their effects in any case were resistant to the blocking action of naloxone. For met-enkephalin, the possibility that more potent opioid δ-receptor antagonists than naloxone may be able to block its excitatory effect cannot be ruled out.

In conclusion, our results show that a non-receptor mediated relaxant effect of morphine on the uterus may partially explain the prolongation of labour caused by this drug. This effect of morphine is shared by the specific μ-agonist, DAGO, but not the δ-agonist, met-enkephalin. In general, the degree of effects elicited by the opioid agonists appear to be independent of the hormonal status of the animal.

References

Acevedo, C. G., Contreras, E. (1984) Effects of morphine on the responses to acetylcholine and adrenaline in the mouse uterus. Cell. Mol. Biol. 30: 239–242

Bertaccini, G., Molina, E., Vitali, T., Zappia, L. (1979) Action of histamine receptor agonists and antagonists on the rat uterus. Br. J. Pharmacol. 66: 13–17

Boyle, F. C., Ohia, S. E. (1985) Specificity of enhancement of salbutamol responses by cyclooxygenase inhibition in the rat isolated uterus. Ibid. 86: 684P

Bradford, H. F., Crowder, J. M., White, E. J. (1986) Inhibitory actions of opioid compounds on calcium fluxes and neurotransmitter release from mammalian cerebral cortical slices. Ibid. 88: 87–93

Campbell, C., Phillips, O. C., Frazier, T. M. (1961) Analgesia during labour: comparison of pentobarbital, meperedine and morphine. Obstet. Gynecol. 17: 714–718

Clouet, D. H., O'Callaghan, J. P., Williams, N. (1978) The effect of opiates in calcium-stimulated phosphorylation of synaptic proteins in intact synaptosomes from rat striatum. In: Ree, V., Terenius, L. (eds) Characteristics and Functions of Opioids. Elsevier Press, Holland, pp 351–352

Collier, H. O. J., Cuthbert, N. J., Francis, D. L. (1981) Model of opiate dependence in the guinea-pig isolated ileum. Br. J. Pharmacol. 73: 921–932

Diamond, J., Brody, T. M. (1966) Hormonal alteration of the response of the rat uterus to catcholamines. Life Sci. 5: 2187–2193

Fazackerley, J., Pleuvry, B. J. (1987) Stimulant action of pethidine on the pregnant rat uterus in-vitro. J. Pharm. Pharmacol. 39: 491–492

Goyal, R. K., Verma, S. C. (1982) Mechanism of actions of histamine in the estrogen-primed rat uterus. Eur. J. Pharmacol. 77: 237–242

Ham, A. W. (1969) In: Ham, A. W. (ed) Histology. 6th edn, J. B. Lippincott Press, Philadelphia, pp 918–922

Holaday, J. W. (1983) Cardiovascular effects of endogenous opiate systems. Ann. Rev. Pharmacol. Toxicol. 23: 541–594

Huidobro, F., Tamayo, L., Contreras, E. (1971) Effects of methadone on the action of catecholamines in isolated preparations. Arch. Int. Pharmacodyn. 192: 168–178

Jaffe, J. H., Martins, W. R. (1985) Opioid analgesics and antagonists. In: Gilman, A. G., Goodman, L. S., Rall, T. W., Murad, F. (eds) The Pharmacological Basis of Therapeutics. 7th edn, Macmillan, New York, pp 491–531

Johnson, S. M., Humphreys, C. M. S., Costa, M. (1987) Sustained

nerve-mediated contractions of guinea-pig ileum during morphine withdrawal by washout. Eur. J. Pharmacol. 141: 41–50

Kasson, B. G., George, R. (1984) Endocrine influences on the actions of morphine IV: Effects of sex and strain. Life Sci. 34: 1627–1634

Lundberg, J. M., Hokfelt, T., Anggard, A., Uvnas-Wallensten, K., Brimijoin, S., Brodin, E., Fahrenkrug, J. (1980) Peripheral peptide neurons: distribution, axonal transport and some aspects on possible function. In: Costo, E., Trabucchi, M. (eds) Neural Peptides and Neuronal Communication. Raven Press, New York, pp. 25–28

Ohia, S. E., Okpako, D. T. (1981) Effect of animal maturity on histamine receptor activity in the rat uterus. Br. J. Pharmacol. 74: 306–307P

Ottensen, B., Sondergaad, F., Fahrenkrug, J. (1983) Neuropeptides in the regulation of female genital smooth muscle contractility. Acta Obstet. Gynecol. Scand. 62: 591–592

Ross, D. H. (1977) Calcium content and binding in synaptosomes subfraction during chronic morphine treatment. Neurochem. Res. 2: 581–593

Schulz, R., Seidl, E., Herz, A. (1985) Opioid dependence in the guinea-pig myenteric plexus is controlled by non-tolerant and tolerant opioid receptors. Eur. J. Pharmacol. 110: 335–341

Silvalingham, T., Pleuvry, B. J. (1985) Action of morphine, pethidine and pentazocine on the oestrus and pregnant rat uterus in vitro. Br. J. Anaesth. 57: 430–433

Vane, J. R., Williams, K. I. (1973) The contribution of prostaglandin production to the contractions of the isolated uterus of the rat. Br. J. Pharmacol. 48: 629–639

Yamamoto, H., Harris, R. A., Loh, H. H., Way, E. L. (1978) Effects of acute and chronic morphine treatments on calcium localization and binding. J. Pharmacol. Exp. Ther. 205: 255–264

Yoshinaga, K., Hawkins, R. A., Stocker, J. F. (1969) Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy. Endocrinology 85: 103–112