Br. J. Pharmac. (1985), **85**, 277–283

Similarity between μ-opioid receptors in mouse vas deferens and guinea-pig ileum

Jacqueline Goodall, I.E. Hughes[1] & D. Mackay

Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds 2, LS2 9JT, Yorkshire.

1 The effects of the opioid receptor agonist RX783006 and of the opioid receptor partial agonist (+)-meptazinol have been examined on electrically-induced twitch responses of the guinea-pig isolated ileum and of the mouse isolated vas deferens.

2 Log_{10} concentration-tissue state curves were determined for (+)-meptazinol and for RX783006, alone, in combination and, when appropriate, in the presence of naloxone (30 nM).

3 Analysis of these log_{10} concentration-tissue state curves using the null equations derived and verified in the previous paper allows quantitation of the characteristics of the interaction of (+)-meptazinol with the opioid receptors in these tissues.

4 The results indicate that the apparent differences in the actions of (+)-meptazinol on isolated electrically-stimulated guinea-pig ileum and mouse vas deferens can be accounted for without the need to postulate differences between μ-opioid receptors in these two tissues.

Introduction

Twitch responses induced by field stimulation of the mouse isolated vas deferens and of the guinea-pig isolated ileum are both inhibited by compounds which stimulate μ-opioid receptors and it is generally thought that these receptors are identical in the two tissues. However, there is evidence which suggests that this may not be so. For example, certain irreversible μ-opioid receptor blockers have been reported to antagonize the effect of morphine in guinea-pig ileum but not in mouse vas deferens (Sayre *et al.*, 1983). In addition, the affinity constant of naltrexone (an opioid receptor blocker) is said to be different for the μ-receptors in the two tissues (Takemori & Portoghese, 1984).

The μ-opioid partial agonist meptazinol (Stephens *et al.*, 1978) is thought to show some selectivity (Blurton *et al.*, 1984) between the two types of μ-receptor (μ_1 and μ_2) which have been postulated to exist (Schultz & Wuster, 1981; Pasternak, 1982; Wood *et al.*, 1982) and we have previously shown that the (+)-isomer of meptazinol appears to show opioid agonist activity on the guinea-pig ileum but not on the mouse vas deferens (Duchesne *et al.*, 1984). This could also be interpreted as indicating that the μ-receptors are not identical in the two tissues. However, experiments with meptazinol on guinea-pig ileum and on mouse vas deferens are complicated by a cholinergic action which meptazinol is known to possess (Bill *et al.*, 1983) and by the ability of meptazinol to increase the evoked release of noradrenaline (Bill *et al.*, 1981).

These functional interactions interfere with the estimation of affinity constants for the interaction of meptazinol with its receptors if classical methods are used. However, in the preceding paper (Hughes & Mackay, 1985) we described a method of estimating affinity constants and of quantitating the functional effects of compounds which have mixed competitive antagonist and functional interactant actions in a tissue. This method has now been used to study the interaction of (+)-meptazinol with its receptors in mouse vas deferens and guinea-pig ileum.

Methods

Guinea-pig isolated field-stimulated ileum

Male guinea-pigs (200–400 g) were stunned and killed by cervical dislocation. A 2 cm portion of ileum taken from 10 cm above the ileo-caecal junction was removed, cleared of adherent tissue, and mounted between two 5 mm coils of platinum wire (one above

[1] Author for correspondence

and the other below the tissue) in physiological saline (composition (mM): NaCl 134, KCl 2.68, $CaCl_2$ 1.80, $MgSO_4$ 1.05, NaH_2PO_4 0.032, $NaHCO_3$ 11.9 and glucose 5.5; gassed with 5% CO_2 in O_2 and also containing mepyramine (0.1 μM) and hexamethonium (69 μM)). The temperature was maintained at 36°C and changes in length of the tissue in response to constant current electrical stimulation (300 mA, 2 ms duration 0.1 Hz) were recorded isotonically (load 0.5 g). Reproducible responses to electrical stimulation were established after about 1 h.

Mouse isolated field-stimulated vas deferens

Male mice (T.O. strain; 30–35 g) were stunned and killed by cervical dislocation. The whole vas deferens was removed, cleared of adherent tissue and mounted in physiological saline (composition (mM): NaCl 128, KCl 5.63, $CaCl_2$ 2.16, NaH_2PO_4 1.19, $NaHCO_3$ 25, glucose 11.1 and sucrose 13.1; gassed with 5% CO_2 in O_2). Changes in length of the tissue in response to constant current electrical stimulation (300 mA, 2 ms duration 0.1 Hz) were recorded isotonically (load 0.15 g). Reproducible responses were established after about 45 min.

Experimental procedure

Log_{10} concentration-tissue state curves were established, the magnitude of the response to electrical stimulation being taken as a measure of tissue state. In guinea-pig ileum a concentration of agonist was applied every 15 min, allowed to equilibrate with the tissue for 3 min and then removed by washing. In mouse vas deferens the cumulative technique was used, increasing concentrations of agonist being added at 3 min intervals. When appropriate (+)-meptazinol and/or the opioid antagonist naloxone were added to the physiological saline and allowed to remain in contact with the tissue for 2 min before agonists were added.

Analysis of results

For each experiment smooth curves were drawn by eye through the experimentally determined points and equi-effective concentrations of agonist were read from the smoothed curves. These equi-effective agonist concentrations were substituted into the appropriate null equations to obtain values of drug-receptor affinity constants and other parameters.

The following null equations have been used, where K and f represent the affinity constants and intrinsic efficacies of the receptor-drug complexes indicated by the appropriate subscript. Square brackets are used to denote molar concentrations of the molecular species indicated by the symbols within the brackets.

(a) Comparison of $\log_{10}$ concentration-tissue state curves of a full agonist A with a partial agonist B (Mackay, 1966a; 1966b).

$$\frac{1}{[A]} = \frac{\psi_{AB}}{[B]} + I_{AB} \quad (1)$$

where $I_{AB} = K_A(f_A/f_B + 1)$

$\psi_{AB} = f_A K_A / f_B K_B$

and $K_B = I_{AB}/\psi AB$ if $f_A >> f_B$ (2)

(b) Comparison of $\log_{10}$ concentration-tissue state curves of agonist A with that of the same agonist in the presence of a constant concentration [B] of a partial agonist B (Mackay, 1966b: Kenakin & Black, 1978).

$$[A]' = L\,[A] + N \quad (3)$$

where $L = (1 + K_B[B])\,(1 - f_B/f_A)$

and $N = -K_B f_B [B]/K_A f_A$

A plot of [A]′ versus [A] should therefore give a straight line with a positive slope L and a negative intercept N.

If $f_A >> f_B$ then the slope of a plot of [A]′ versus [A] is equal to $1 + K_B[B]$, from which K_B can be calculated.

(c) Comparison of the $\log_{10}$ concentration-tissue state curve for agonist A in the presence of a fixed concentration of antagonist I_1 with the curve for agonist A in the presence of the same concentration of I_1 together with a fixed concentration of a pure competitive antagonist I_2. The equieffective agonist concentrations are designated $[A]_2$ and $[A]_3$ respectively. I_1 may be either a pure competitive antagonist or a mixed antagonist of A.

$$\frac{[A]_3}{[A]_2} = 1 + \frac{K_{I2}[I_2]}{1 + K_{I1}[I_1]} \quad (4)$$

The dose-ratio $[A]_3/[A]_2$ produced by I_2 therefore depends on $K_{I1}[I_1]$. If K_{I2} has been determined from separate (competitive antagonist) studies then K_{I1} can be estimated from $[A]_3/[A]_2$ (Hughes & Mackay, 1985).

(d) If there is evidence that I_1 is a competitive antagonist with the extra properties of a functional antagonist or synergist then the appropriate null equation is

$$\frac{[A]_2}{[A]_1} = \alpha_{21} + \beta_{21}[A]_2 + \gamma_{21}/[A]_1 \quad (5)$$

where $[A]_2$ and $[A]_1$ are the equieffective concentrations of agonist in the presence and absence of I_1 respectively;

$$\begin{aligned} \alpha_{21} &= \alpha_x(1 + K_{I1}[I_1]) \\ \beta_{21} &= \beta_x \\ \text{and } \gamma_{21} &= \gamma_x(1 + K_{I1}[I_1]) \end{aligned} \quad (6)$$

If K_{I1} has been estimated as described in section (c) then values for the functional parameters α_x, β_x and γ_x can be estimated from the values of α_{21}, β_{21} and γ_{21} (Hughes & Mackay, 1985).

Where appropriate, all values are expressed as mean ± s.e.mean with the number of observations (n) contributing to the mean value indicated in parentheses. Tests of statistical significance (t and t' tests) were carried out as described by Snedecor & Cochran (1967).

Drugs

The following were used: hexamethonium bromide (Sigma), mepyramine maleate (Sigma), and naloxone hydrochloride (Sigma). (+)-Meptazinol hydrochloride was supplied by Wyeth Pharmaceuticals. Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (RX783006) was obtained from Cambridge Research Chemicals, dissolved in oxygen-free water and stored at −70°C.

Results

Guinea-pig ileum

Twelve experiments were carried out on guinea-pig ileum to test the effect of (+)-meptazinol on the response to electrical stimulation. In each case (+)-meptazinol (0.5 to 10 μM) produced a small inhibition of the electrically induced twitch response, an effect which is compatible with a partial agonist action on opioid receptors. However, in only 5 experiments was the effect sufficiently great to allow construction of acceptable $\log_{10}$ concentration-tissue state curves. A typical example is shown in Figure 1. Each such curve was analysed as described in 'Analysis of results', section (a) and the derived values are shown in Table 1.

Table 1 Values of I_{AB} and ψ_{AB} estimated from plots of 1/[A] vs 1/[B] where [A] and [B] are molar concentrations of RX783006 and (+)-meptazinol which produce equivalent tissue states in the stimulated guinea-pig ileum preparation.

Experiment number	$I_{AB} \times 10^{-8}$ M	ψ_{AB}	$K_B \times 10^{-6}$ M
1	1.57	43	3.65
2	0.72	136	0.53
3	1.05	197	0.53
4	1.28	210	0.61
5	0.86	135	0.63
Mean values ± s.e.mean	1.10 ± 0.15	144 ± 30	1.19 ± 0.62

Experiments were also carried out on guinea-pig ileum to investigate the effect of a constant concentration of (+)-meptazinol, acting as a partial agonist, on the $\log_{10}$ concentration-tissue state curve to the opioid-receptor agonist Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$OH (RX783006). The data from one of these experiments are shown in Figure 2. Using the appropriate null equation ('Analysis of results', section (b)), gave a value of $(4.71 \pm 1.9) \times 10^6 M^{-1}$ ($n = 5$) for the affinity constant of the (+)-meptazinol-opioid receptor complex. In all 5 experiments plots of [A]′ versus [A] gave negative intercepts in accord with the model.

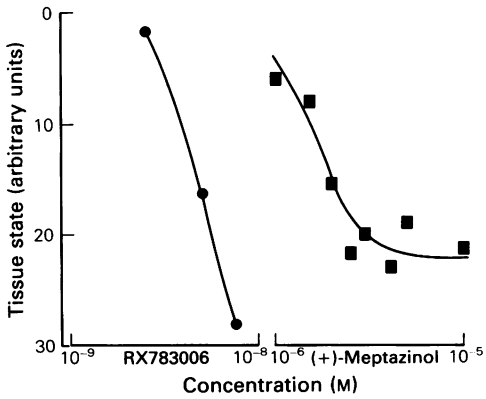

Figure 1 Log_{10} concentration-tissue state curves for RX783006 (●) and for (+)-meptazinol (■) determined on the electrically-stimulated guinea-pig ileum preparation.

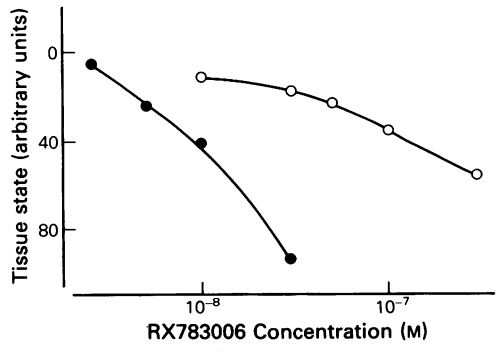

Figure 2 Log_{10} concentration-tissue state curves determined on the electrically-stimulated guinea-pig ileum preparation for RX783006 alone (●) and in the presence of (+)-meptazinol (5 μM; ○).

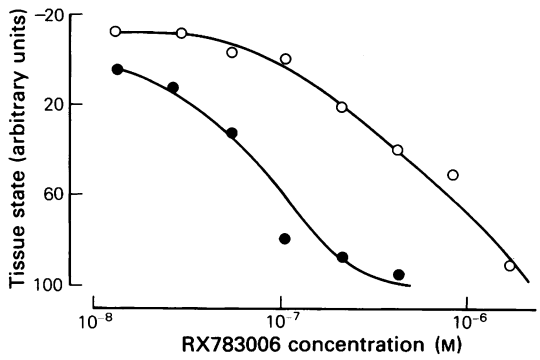

Figure 3 Log_{10} concentration-tissue state curves determined on the electrically-stimulated mouse vas deferens preparation for RX783006 alone (●) and in the presence of (+)-meptazinol (5 μM; ○).

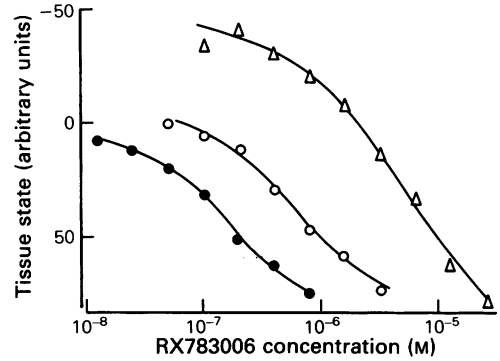

Figure 4 Log_{10} concentration-tissue state curves determined on the electrically-stimulated mouse vas deferens preparation to RX783006 alone (●), in the presence of (+)-meptazinol (5 μM; ○) and in the presence of both (+)-meptazinol and naloxone (30 nM) together (△).

Mouse vas deferens

Since (+)-meptazinol has no apparent opioid agonist activity on mouse vas deferens (Duchesne *et al.*, 1984) it was tested on this tissue in 6 experiments as an antagonist of RX783006. In 5 experiments the curve obtained for RX783006 in the presence of a fixed concentration of (+)-meptazinol was raised initially but became roughly parallel to the control curve at higher concentrations of RX783006 (see Figure 3). In the remaining experiment the log_{10} concentration-tissue state curve to RX783006 was not raised and actually became less steep at higher concentrations of RX783006 producing a pattern quite similar to that shown for guinea-pig ileum in Figure 2. Analysis of these results showed that they all fitted equation 3 (section b). However in 5 cases out of 6 the values of N were strongly positive indicating that on this tissue (+)-meptazinol was acting neither as a pure partial agonist nor as a pure competitive antagonist.

A series of experiments was therefore carried out to estimate the affinity constant of (+)-meptazinol for opioid receptors by the method described in 'Analysis of results', section (c) and to estimate functional interaction parameters as described in section (d). Log_{10} concentration-tissue state curves obtained in a representative experiment of this type are shown in Figure 4. The 'pure' competitive antagonist used (I_2) was naloxone.

The estimated affinity constants of (+)-meptazinol

Table 2 Estimated values of K_B the affinity constant of (+)-meptazinol for opioid receptors, and of other quantities calculated for isolated electrically stimulated vas deferens of the mouse.

Experiment number	*Curve 3 cf. Curve 2* α_{32}	$\gamma_{32} \times 10^7 M^{-1}$	$K_B \times 10^{-6} M$	*Curve 2 cf. Curve 1* α_X	$\gamma_X \times 10^8 M^{-1}$	*Curve 1* pD_2
1	2.06	1.67	2.20	0.31	0.11	7.28
2	2.82	6.19	1.14	0.80	8.90	7.07
3	6.79	32.1	0.22	2.08	2.78	6.80
4	4.10	16.4	0.59	1.83	5.10	6.64
5	3.08	1.87	0.97	1.25	0.57	7.78
Mean	3.77	11.6	1.02	1.25	3.94	7.11
± s.e.mean	± 0.82	± 5.8	± 0.34	± 0.33	± 1.33	± 0.20

The log_{10} [RX783006] vs tissue state curves were:
Curve 1: RX783006 alone
Curve 2: RX783006 + (+)-meptazinol (5 μM)
Curve 3: RX783006 + (+)-meptazinol (5 μM) + naloxone (30 nM)
In all cases β_X has been taken to be zero and K_I for naloxone was $0.41 \times 10^9 nM^{-1}$.
K_B in this table corresponds with K_{11} in equation 4.

Table 3 Results of calculations of expected effects of (+)-meptazinol on isolated field-stimulated vas deferens of the mouse

Experiment number	*Observed pD_2 for RX783006*	*Expected maximal effects of (+)-meptazinol expressed as a % of the maximal effect produced by RX783006*		
		Assuming observed pD_2 but no functional interaction	Assuming $pD_2 = 7.78$ and functional interaction parameters as in Table 2	Allowing for observed pD_2 values and observed functional interaction parameters
1	7.28	0	18	0
2	7.07	0	0	0
3	6.80	0	0	0
4	6.64	0	0	0
5	7.78	23	0	0

The values for I_{AB} and ψ_{AB} have been taken to be $1.10 \times 10^8 M^{-1}$ and 144 respectively (see Table 1). The experiment numbers refer to the sets of results shown in Table 2.

for the opioid receptors and the other parameters calculated are presented in Table 2.

If the receptors with which (+)-meptazinol interacts on mouse vas deferens are assumed to be identical with those in guinea-pig ileum then the values of I_{AB} and ψ_{AB} obtained in the latter tissue can be applied to mouse vas deferens. The expected $\log_{10}$ concentration-tissue state curve for (+)-meptazinol on the mouse vas deferens can then be calculated from that obtained for the agonist RX783006 by re-arranging equation 1 to the form

$$[B] = \frac{\psi_{AB}}{1/[A] - I_{AB}} \quad (7)$$

where [B] represents the equieffective molar concentration of (+)-meptazinol and [A] the molar concentration of RX783006. From such curves the maximal effects expected for (+)-meptazinol, acting only on these opioid receptors, can be calculated. The results are shown in Table 3. The effects of a superimposed functional antagonism of the magnitudes indicated in Table 3 can also be deduced. To accomplish this, values of α_x and γ_x were estimated for these concentrations of (+)-meptazinol, calculated as described above using equation 7, assuming the values of $(\alpha_x - 1)/(\alpha_x + 1)$ and of γ_x to be proportional to the concentration of (+)-meptazinol. Concentrations of RX783006 required to produce the same reduction in response to electrical stimulation in the presence of such functional antagonism, $[RX]_2$, were then obtained from

$$[RX]_2 = \alpha_x[RX]_1 + \gamma_x \quad (8)$$

where $[RX]_1$ is the concentration of RX783006 which produces the same effect in the absence of functional interaction. These values of $[RX]_2$ were then used to estimate revised values of the corresponding equieffective concentrations of (+)-meptazinol and so on, iteratively, until the calculated (+)-meptazinol concentrations converged to a constant value or became negative, a negative concentration meaning that no real concentration of (+)-meptazinol is capable of producing such a response. Using this technique the maximal effects to be expected from (+)-meptazinol, acting on a highly sensitive mouse vas deferens ($pD_2 = 7.78$ for RX783006), have been calculated assuming the various degrees of functional antagonism indicated by the respective pairs of values of α_x and γ_x shown in Table 2. These maximal expected effects are presented in Table 3.

Discussion

Null equations are generally derived on the assumption that the drugs being studied are acting at sites in or on the cells (of the tissue) whose state is being recorded. In the studies described here the actions of the opioid drugs are on opioid receptors located on the nerve endings and the state of the latter is being deduced indirectly from the contractile response of the smooth muscle to nerve stimulation. Functional interaction is also assumed to occur at the nerve endings, although functional interaction due to a non-opioid action of meptazinol on the smooth muscle would probably also be described adequately by the null equation used here for mixed antagonism. The results presented in Table 1 summarize the partial agonist properties of meptazinol in field stimulated guinea-pig ileum. Since I_{AB} and ψ_{AB} contain the parameters f_A, f_B, K_A and K_B, which should all be characteristic of the receptor-agonist complexes, the former should also be

characteristic of the receptor type. In fact ψ_{AB} becomes the relative potency of two agonists if they produce parallel $\log_{10}$ concentration-tissue state curves.

The ratio I_{AB}/ψ_{AB} gives an approximate estimate of the affinity constant (K_B) of meptazinol for its receptors in the field stimulated guinea-pig ileum, the mean value being $(1.19 \pm 0.62) \times 10^6 M^{-1}$ ($n = 5$). Experiments in which the agonist action of RX786003 on field-stimulated guinea-pig ileum was studied in the presence of meptazinol acting as a partial agonist gave a value of $(4.71 \pm 1.9) \times 10^6 M^{-1}$ ($n = 5$) for K_B. The discrepancy between the two results is appreciable though not statistically significant ($0.2 > P > 0.1$; t' test). It must also be remembered that the latter experiments involved a prolonged contact of the tissue with a high concentration of meptazinol and the phenomenon of acute tolerance to opioid agonists is well established. Another possible explanation of the discrepancy is that the latter experiments may have allowed a non-opioid action of meptazinol (Duchesne *et al.*, 1984) to become effective.

Since meptazinol had no visible opioid agonist effect on field stimulated mouse vas deferens it was initially tested as an antagonist against RX783006. However, the relative positions of the curves obtained to RX783006 in the absence and presence of a fixed concentration of (+)-meptazinol was neither that expected for a pure antagonist nor that expected for a partial agonist (compare Figures 3 and 2). Plots of [A]′ vs [A] gave good straight lines but in 5 out of 6 cases the intercepts N were strongly positive. This is in contrast with the results of similar experiments on guinea-pig ileum in which all 5 values of N were negative, in accord with a partial agonist action. Because of these complications an alternative method has been used to estimate K_B, the affinity constant of (+)-meptazinol for opioid receptors. The method is based on competition between a pure competitive antagonist and a mixed antagonist, in this instance naloxone and (+)-meptazinol respectively. The mean value of K_B obtained for mouse vas deferens using this technique is $(1.02 \pm 0.34) \times 10^6 M^{-1}$ (Table 2), which is not significantly different ($P > 0.5$; t test) from the value of $(1.19 \pm 0.62) \times 10^6 M^{-1}$ (Table 1) obtained by direct comparison of the agonist actions of (+)-meptazinol and RX783006 on field-stimulated guinea-pig ileum.

The method mentioned above for estimating K_B on mouse vas deferens also permits estimation of the functional interaction parameters α_x, β_x and γ_x. In these experiments the functional antagonistic effect of (+)-meptazinol against RX783006 was important only at low concentrations of RX783006, no change being observable in the maximum effect produced by the latter compound. This corresponds to a situation in which the main functional interaction parameters are α_x and γ_x. The value of β_x in these experiments was therefore taken to be zero and only values of α_x and γ_x have been calculated (Table 2).

The results discussed so far provide no convincing evidence that (+)-meptazinol has a different affinity for its receptors in guinea-pig ileum and for those in mouse vas deferens. Nevertheless, (+)-meptazinol does appear to be a partial agonist in the former tissue but not in the latter. If the receptors in the two tissues are the same then the lack of agonist action of (+)-meptazinol on mouse vas deferens could be due to an inadequate density of opioid receptors (Duchesne *et al.*, 1984) or to the functional antagonistic effects of the drug, or both. These possibilities have been tested by calculation, the results being summarized in Table 3. It will be seen that if no allowance is made for functional interaction, then only the tissue most sensitive to RX783006 (experiment no. 5) would be expected to produce an observable response to (+)-meptazinol. On the other hand if the various degrees of functional interaction indicated by the values of α_x and γ_x in Table 2 are assumed to apply to this most sensitive tissue then only the lowest degree of functional antagonism (experiment no. 1) would allow any detectable response to meptazinol. It follows that the lower sensitivity of mouse vas deferens to RX783006, as compared with guinea-pig ileum, together with the general degree of functional interaction indicated by Table 2 can explain why (+)-meptazinol has no apparent opioid agonist action on mouse vas deferens.

One final piece of evidence which tends to support the idea that (+)-meptazinol acts as a sub-threshold partial agonist on mouse vas deferens can be deduced from the positive values of γ_{32} presented in Table 2 and from graphs such as that shown in Figure 4. In this type of experiment naloxone is being used as a competitive antagonist in the presence of a fixed concentration of (+)-meptazinol and so curve 3 would be expected to be parallel to curve 2. Although the data have been analysed on the basis of this assumption this condition is in fact not entirely fulfilled. It will be seen that naloxone seems to increase the functional antagonistic effect of (+)-meptazinol. This could be explained by (+)-meptazinol having a barely detectable agonist action in this tissue.

No convincing evidence has therefore been obtained that the affinity constant of (+)-meptazinol for its opioid receptors in mouse vas deferens differs from that in guinea-pig ileum. The lack of partial agonist action of (+)-meptazinol on the field-stimulated mouse vas deferens can be explained by the functional antagonistic properties of the drug and the lower sensitivity of the tissue to opioid receptor stimulation compared with the guinea-pig ileum preparation. The fact that in these experiments naloxone seems to be capable of potentiating the functional antagonistic properties of meptazinol on mouse vas deferens supports these conclusions since such effects would be consistent with a sub-threshold partial agonist effect of meptazinol in this tissue.

References

BILL, D., COWLRICK, I.S., FOX, J., TODD, M.H., WARD, P.J., WOOD, M.D. & WYLLIE, M.G. (1981). Does meptazinol bind to opiate receptors? *Br. J. Pharmac.*, **74**, 866P.

BILL, D., HARTLEY, J.E., STEPHENS, R.J. & THOMPSON, A.M. (1983). The antinociceptive activity of meptazinol depends on both opiate and cholinergic mechanisms. *Br. J. Pharmac.*, **79**, 191–199.

BLURTON, P., WOOD, M.D. & WYLLIE, M.G. (1984). The analgesic meptazinol displays a selective interaction at the opioid mu_1-site. *Br. J. Pharmac.*, **81**, 59P.

DUCHESNE, R.J., GOODALL, JACQUELINE & HUGHES, I.E. (1984). Pharmacological effects of meptazinol and its enantiomers on guinea-pig ileum and mouse vas deferens. *J. Pharm. Pharmac.*, **36**, 560–562.

HUGHES, I.E. & MACKAY, D. (1985). Quantification of the characteristics of antagonists exhibiting both competitive antagonism and functional interaction. *Br. J. Pharmac.*, **85**, 271–275.

KENAKIN, T.P. & BLACK, J.W. (1978). The pharmacological classification of practolol and chloropractolol. *Mol. Pharmac.*, **14**, 607–623.

MACKAY, D. (1966a). A general analysis of receptor-drug interaction. *Br. J. Pharmac.*, **26**, 9–16.

MACKAY, D. (1966b). A new method for the analysis of drug receptor interactions. In *Advances in Drug Reasearch.*, Vol. 3, pp. 1–20. ed. Harper, N.J. & Simmonds, A.B. New York: Academic Press.

PASTERNAK, G.W. (1982). High and low affinity opioid binding sites: relationship to mu and delta sites. *Life Sci.*, **31**, 1301–1306.

SAYRE, L.M., PORTOGHESE, P.S. & TAKEMORI, A.E. (1983). Difference between mu-receptors in the guinea-pig ileum and the mouse vas deferens. *Eur. J. Pharmac.*, **90**, 159–160.

SCHULTZ, R. & WUSTER, M. (1981). Are there sub-types (isoreceptors) of multiple opiate receptors in the mouse vas deferens. *Eur. J. Pharmac.*, **61–66.**

SNEDECOR, G.W. & COCHRAN, W.G. (1967). In *Statistical Methods*, 6th. Edition. Ames, Iowa: Iowa State University Press.

STEPHENS, R.J., WATERFALL, J.F. & FRANKLIN, R.A. (1978). A review of the biological properties and metabolic disposition of the new analgesic agent, meptazinol. *Gen. Pharmac.*, **9**, 73–78.

TAKEMORI, A.E. & PORTOGHESE, P.S. (1984). Comparative antagonism by naltrexone and naloxone of mu, kappa and delta agonists. *Eur. J. Pharmac.*, **104**, 101–104.

WOOD, P.L., RICHARD, J.W. & THAKUR, M. (1982). Mu-opioid isoreceptors: differentiation with kappa agonists. *Life Sci.*, **31**, 2313–2317.

(*Received November 26, 1984. Accepted December 23, 1984.*)