Br. J. Pharmac. (1983), **80**, 371–376

Characterization of the opiate receptor population mediating inhibition of VIP-induced secretion from the small intestine of the rat

Ian M. Coupar

School of Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia

1 Net water transport was measured from the jejunum of anaesthetized rats by a re-circulation technique.

2 Several narcotic analgesics were administered intravenously and assessed for activity in reversing net fluid secretion induced by intra-arterial infusion of vasoactive intestinal peptide (VIP).

3 The μ-opiate agonists, morphine, RX 783006 and FK 33–824 produced full reversal of the secretory phase of the VIP response, but failed to restore totally fluid transport to the control level of net absorption.

4 The κ-agonist, ethylketocyclazocine, caused only partial reversal of VIP-induced secretion while the more selective κ-agonist, MR 2034, produced a small though non-significant antisecretory effect at high doses. The δ-agonist, D-Ala^2-D-Leu^5-enkephalin also had negligible antisecretory activity.

5 Naloxone caused a parallel displacement to the right of the antisecretory dose-response line to morphine. The '*in vivo* pA_2' value of naloxone was 7.14.

6 The results are compared with previously published antinociceptive activities of the opiate agonists and *in vivo* pA_2 values of naloxone. It is concluded that stimulation of μ-opiate receptors mediates inhibition of VIP-induced fluid secretion from the rat jejunal mucosa.

Introduction

Morphine inhibits small intestinal fluid secretion stimulated by cholera enterotoxin, prostaglandin E_1 (PGE_1), vasoactive intestinal peptide (VIP) and carbachol. The antisecretory effect of morphine is inhibited by naloxone in the instances where PGE_1, VIP and carbachol have been used as secretagogues. Furthermore, the antisecretory mechanism displays stereospecificity since it is activated by levorphanol but not dextrorphan (Valiulis & Long, 1973; Coupar, 1978; Beubler & Lembeck, 1979; Lee & Coupar, 1980a). As a consequence of these findings it has been proposed that the antidiarrhoeal effect of morphine is better explained by its ability to decrease secretion by acting on opiate receptors than by inhibiting the propulsive ability of the gut (Coupar, 1978; Beubler & Lembeck, 1979). Studies with newer antidiarrhoeal drugs such as loperamide, suggest that they also exert antisecretory activity on the intestinal mucosa by activating opiate receptors (Piercey & Ruwart, 1979; Sandhu, Tripp, Candy & Harries, 1981). However, it is now apparent that opiate receptors exist in multiple form peripherally as well as centrally. Although several subpopulations have been identified, the most significant are considered to be μ-, δ- and κ-opiate receptors at which morphine, D-Ala^2-D-Leu^5-enkephalin and ethylketocyclazocine are examples of prototype agonists respectively (see Wood, 1982 for review). The experiments described in this paper were undertaken in an attempt to define the opiate receptor population mediating inhibition of VIP-induced secretion from the rat jejunum. Such knowledge should help in predicting the effect of narcotic analgesics on the intestine and in developing selective antidiarrhoeal drugs.

Methods

Male and female hooded Wistar rats (220–300 g) were deprived of food overnight but were given free access to drinking water. The animals were anaesthetized with pentobarbitone (60 mg kg^{-1}, s.c.) and cannulae (PE 10) were introduced into the left com-

mon carotid artery for infusion of VIP and into the left external jugular vein for administration of the drugs under study.

A re-circulation technique was used to measure the net amount of water transported by the jejunum (20–30 cm) as has been described previously in detail (Coupar, 1978). Briefly, this involves using an isosmotic solution containing (gl^{-1}): NaCl 8.57, KCl 0.37, dextrose 1.0 and phenolsulphonphthalein 0.02 as a non-absorbable marker of water transport. This solution, at 37°C, is re-circulated through the lumen of the jejunum by gas lift, using moistened CO_2 (5%) in O_2, for a period of 20 min.

Drugs under investigation were injected intravenously (i.v.) 10 min before starting the perfusion of the jejunum. Secretion was initiated 5 min before perfusing the jejunum by an intra-arterial (i.a.) infusion of VIP at 2.4×10^{-10} mol min^{-1} and maintained for the duration of the 20 min perfusion. This infusion rate induces near maximal secretion (Lee & Coupar, 1980a).

In the experiments to determine the effect of naloxone on the antisecretory effect of morphine, both drugs were administered i.v. in the same solution 10 min before recirculating the luminal perfusing solution. Therefore, all drugs under study were injected 20 min before the mid-point of luminal perfusion. Some animals were respired artificially with an air pump at a rate of 53 strokes min^{-1} and a volume of 1 ml 100 g^{-1} body weight.

Results are expressed as the net amount of water absorbed (+) or secreted (−) per g wet weight of intestinal tissue in the 20 min perfusion.

Drugs

D-Ala^2-D-Leu^5-enkephalin (Bachem), ethylketocyclazocine methanesulphonate (Sterling-Winthrop), FK 33-824 (D-Ala^2-MePhe^4-Met^5(*o*)-ol-enkephalin, Sandoz), morphine hydrochloride (Macfarlane Smith), MR 2034 ((−)-(**1R**, **5R**, **9R**, 2″**S**)-5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6, 7-benzomorphan)-tartrate, Boehringer Ingelheim), naloxone hydrochloride (Endo), phenobarbitone (Nembutal, Abbott), RX 783006 (Tyr-D-Ala-Gly-MePheNH$(CH_2)_2$ OH, Cambridge Research Biochemicals) and VIP (vasoactive intestinal peptide, Karolinska Institute). All drugs for i.v. administration were dissolved in saline (0.9% w/v NaCl solution) and injected in volumes of 0.1 ml/100 g with the exception of 170 mg kg^{-1} of morphine plus naloxone which was administered in a volume of 0.2 ml 100 g^{-1} body weight. The doses of drugs available as salts are expressed in terms of the amount of free base. VIP was dissolved in saline to give the required infusion rate, using a volume rate of 0.04 ml min^{-1}.

Statistics

The differences between individual means and control were assessed for significance by Dunnett's *t* test. ED_{50} values and slopes of log dose-response lines were calculated by linear regression analysis. A Columbia Microcomputer, programmed for 'Pharmacologic calculations' (Tallarida & Murray, 1981) was used to compare the slopes of regression lines and to calculate the '*in vivo* pA_2'. Differences were considered significant when the value of *P* was smaller than 0.05.

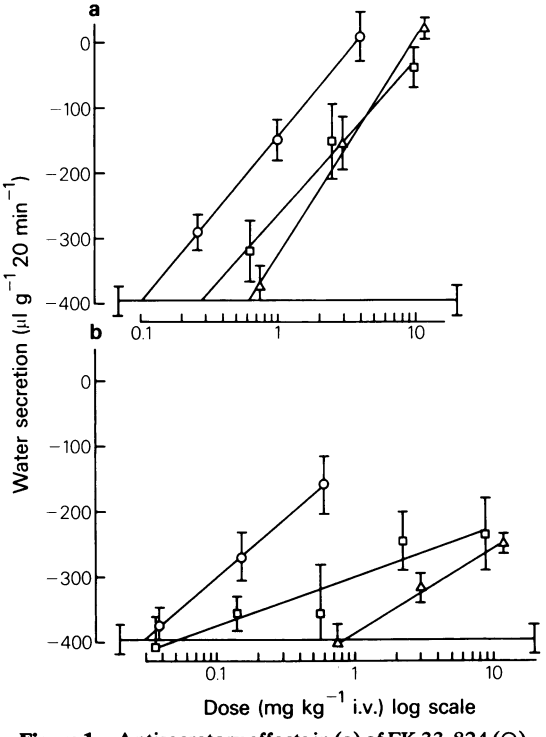

Figure 1 Antisecretory effects in (a) of FK 33-824 (○), morphine (□), RX 783006 (△) and in (b) of ethylketocyclazocine (○), MR 2034 (□) and D-Ala^2-D-Leu^5-enkephalin (△). The lower line is the control value of secretion induced by i.a. infusion of VIP at 2.4×10^{-10} mol min^{-1}. FK 33-824 at 16 mg kg^{-1} did not cause a greater restoration of water transport than at 4 mg kg^{-1} (see Results). In (b) the only mean of the 3 drugs tested that differs significantly from the control value of secretion is the highest dose of ethylketocyclazocine (0.6 mg kg^{-1}, $P < 0.05$, Dunnett's *t* test, 12 groups including VIP control). Points are means with s.e.means ($n = 5$).

Table 1 Antisecretory and previously published analgesic activities

	Antisecretory activity		*Analgesic ED_{50}*	
	ED_{50}	95% CL	*Heat*	*Pressure*
RX 783006	2.61	0.86 – 6.87	2.9*	
FK 33-824	0.61	0.13 – 2.64	0.4** (mice)	0.9**
Morphine	1.90	0.14 – 21.3	1.65*	
Ethylketocyclazocine	0.35	0.04 – 6.69	1.4***	0.14***
D-Ala^2-D-Leu^5-Enkephalin	—			
MR 2034	—		Inactive***	0.07***

Compounds are listed in decreasing order of slopes. Brackets indicate that the slopes are not significantly different. All analgesic values were estimated in rats unless specified in the table. ED_{50} values are in mg kg^{-1}.
* Tail flick test, measurement 15 min after i.v. administration (Handa, Lane, Lord, Morgan, Rance & Smith, 1981).
** Paw pressure test, measurement 30 min after s.c. injection. Mice were injected i.v. and tested using the tail flick 30 min later (Roemer, Buescher, Hill, Pless, Bauer, Cardinaux, Closse, Hauser & Huguenin, 1977).
*** Tail flick and paw pressure tests 30 min after s.c. injection (Tyers, 1980).

Results

Narcotic analgesics

The value of net water absorption in control animals injected i.v. and infused i.a. with saline, was $216 \pm 27\,\mu l\,g^{-1}$ ($n = 5$) in 20 min. This was reversed to a large net secretion of $396 \pm 24\mu l\,g^{-1}$ ($n = 5$) in 20 min by an i.a. infusion of VIP at 2.4×10^{-10} mol min^{-1}.

Figure 1a and b shows that all narcotic analgesics displayed some degree of reversal of VIP-induced secretion. The most effective antisecretory compounds were morphine and the two peptides RX 783006 and FK 33-824. The maximal effects of these drugs extended to complete block of secretion but they failed to restore net absorption to the control value. The possibility that a higher dose than shown in Figure 1a might cause full reversal of water transport was tested using the most potent member of this group, FK 33-824. Whereas 4 mg kg^{-1} of FK 33-824 induced a net absorption of $6.4 \pm 37\,\mu l\,g^{-1}$ ($n = 5$) in 20 min, 16 mg kg^{-1} actually showed less antisecretory activity resulting in a secretion value of $99 \pm 44\,\mu l\,g^{-1}$ ($n = 5$) in 20 min. Neither of these values was significantly different from zero ($P > 0.05$). For this reason, the maximal effect for the purpose of calculating antisecretory ED_{50} values shown in Table 1, was taken as a transport rate of $0\,\mu l\,g^{-1}$ in 20 min.

The least effective antagonists of VIP-induced secretion were MR 2034 and D-Ala^2-D-Leu^5-enkephalin which produced less than 50% reversal of secretion. The slope of the dose-response line to ethylketocyclazocine was not significantly different from either MR 2034 or D-Ala^2-D-Leu-enkephalin, but it produced greater than 50% reversal of secretion (Figure 1b). A comparison of the individual slopes is shown in Table 1, which also shows previously published analgesic ED_{50} values from heat (μ-sensitive) and pressure (μ- and κ-sensitive) methods.

Some compounds, notably the high doses of FK 33-824 and MR 2034, caused respiratory arrest. The number of animals requiring artificial respiration is shown in Table 2.

Table 2 Proportion of animals with respiratory arrest

	Dose (mg kg^{-1})	*Number with respiratory arrest*
RX-783006	12	0
D-Ala^2-D-Leu^5-enkephalin	12	0
Morphine	10	1
FK 33-824	4	1
	16	5
Ethylketocyclazocine	0.6	3
MR 2034	2.25	3
	9	5

$n = 5$ for all groups

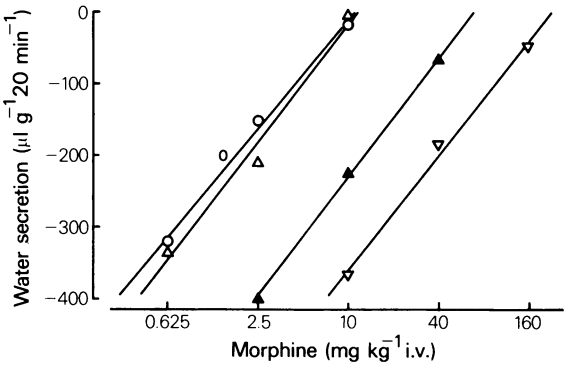

Figure 2 Effect of naloxone, 0.55 (△), 2.2 (▲) and 8.8 (▽) $\times 10^{-7}$ mol kg^{-1} on the antisecretory response to morphine (○) during i.a. infusion of VIP. $n = 5$ in each mean.

Naloxone

Naloxone caused a parallel displacement to the right of the antisecretory dose-response line to morphine (Figure 2). The ED_{50} values of morphine in the absence and presence of naloxone doses is shown in Table 3. The values of dose-ratios produced a Schild plot having a slope that was not significantly different from − 1. With the slope constrained to − 1 (Tallarida & Murry, 1981) the '*in vivo* pA_2' value was calculated to be 7.14 (95% CL, 5.94–8.35).

Discussion

The narcotic analgesics display varying activities in reversing VIP-induced fluid secretion in the small intestine. The most effective compounds are the predominantly μ-opiate receptor agonists, morphine, RX 783006 and FK 33-824 which fully block secretion. However, the response to VIP is not totally overcome, so that even 16 mg kg^{-1} FK 33-824 (at least 18 times larger than its antisecretory and analgesic ED_{50}) fails to restore completely the normal level of net absorption. It is possible that full reversal does not occur because the infusion of VIP used causes near-maximal secretion. However, it has been noted that morphine fully reverses near-maximal PGE_1-induced secretion (Coupar, 1978). It is more likely that the lack of full reversal of VIP by the μ-agonists is due to a difference between the mechanisms of VIP- and PGE_1-induced secretion. Some differences have already been noted, particularly that VIP causes greater secretion than PGE_1 (Lee & Coupar, 1980a) and that morphine inhibits the rise in 3',5'-cyclic AMP induced by PGE_1 (Beubler & Lembeck, 1980) but not to VIP (Lee & Coupar, 1980b). It is apparent that secretory and antisecretory mechanisms are complex and cannot be fully elucidated by the present method which measures net fluid transport only.

Table 3 Antagonism of morphine by co-administration of naloxone

Naloxone dose	*Morphine*		
($\times 10^{-7}$ mol kg^{-1})	*ED_{50}*	(95% CL)	*Dose-ratio*
0	1.8	0.2 – 15.2	
0.55	2.2	0.1 – 42.5	1.2
2.2	13.1	2.3 – 81.6	7.2
8.8	40.9	5.0 – 336.0	22.5

Binding studies have shown that morphine (Magnan, Paterson, Tavani & Kosterlitz, 1982) and RX 783006 (Handa, Lane, Lord, Morgan, Rance & Smith, 1981) are ligands with high affinity and selectivity at the μ-binding site. FK 33-824 has been classified mainly as a μ-ligand with some affinity also for the δ-site (Wood, Charleson, Lane & Hudgin, 1981). These agonists were the most effective antisecretory narcotic analgesics tested. There is also very good correlation between antisecretory ED_{50} values for morphine and RX 783006 and corresponding heat antinociceptive ED_{50}s determined in rats under comparable experimental conditions (see Table 1). The heat tests such as the tail flick using rodents are thought to show μ-agonist activity (Tyers, 1980; Upton, Sewell & Spencer, 1982). The antisecretory and analgesic ED_{50}s are also similar for FK 33-824 although the experimental conditions are not as comparable as with morphine and RX 783006.

Ethylketocyclazocine, MR 2034 and D-Ala^2-D-Leu^5-enkephalin represent a reasonably different group of compounds since they caused only partial reversal of VIP-induced secretion. The κ-agonist, ethylketocyclazocine was the most effective member of the group causing greater than 50% reversal of secretion with a slope significantly lower than RX 783006 but not different from FK 33-824 and morphine. The antisecretory ED_{50} of ethylketocyclazocine is closer to the ED_{50} in pressure than heat noxia tests using rats (Table 1). Since heat antinociception is believed to be mediated through μ-opiate receptors and pressure through μ- and κ-receptors (Tyers, 1980), this implies that inhibition of VIP-induced secretion involves κ-receptors as well as μ-receptors already identified by the high antisecretory activity of the μ-agonists. However, there are several results indicating that κ-receptors are not important in the antisecretory effect. For instance, binding studies show that ethylketocyclazocine has high affinity for μ- as well as κ-sites (Kosterlitz, Paterson & Robson, 1981; Magnan *et al.*, 1982). Although ethylketocyclazocine was originally clas-

sified as a competitive antagonist at μ-receptors in dogs (Martin, Eades, Thompson, Huppler & Gilbert, 1976), it appears to act as an agonist in rats as shown by its ability to induce strong cross-tolerance to morphine (Porreca, Cowan, Raffa & Tallarida, 1982). In both mice and rats, ethylketocyclazocine is an analgesic in heat tests. Tyers (1980) showed that ethylketocyclazocine was 10 times more active at inhibiting pressure than heat noxia but that other drugs such as ketocyclazocine, pentazocine and buprenorphine distinguished more clearly between κ- and μ-receptors. The most discriminating drug was MR 2034 and in the present experiments, was completely ineffective in reversing secretion over the dose-range known to cause stimulation of κ-receptors (Tyers, 1982). Only a small reversal which was not statistically significant occurred at high 'non-κ' doses. It is, therefore, reasonable to conclude that κ-receptors are not involved to any extent in the inhibition of VIP-induced secretion.

It has been proposed on the basis of electrophysiological findings, that the small intestinal mucosa of the rabbit (McKay, Linaker & Turnberg, 1981) and guinea-pig (Kachur & Miller, 1982) contain significant populations of δ-opiate receptors. Evidence linking δ-receptors to the antisecretory mechanism in the rat, however, is not conclusive. The δ-agonist, D-Ala^2-D-Leu^5-enkephalin appears to cause a small inhibition of VIP-induced secretion over the dose-range known to have anti-diarrhoeal activity in mice following s.c. injection (Miller & Cuatrecasas, 1979). However, D-Ala^2-D-Leu^5-enkephalin is relatively unstable in its biological activity. In mice the analgesic effect after i.v. administration of 15 and 20 mg kg^{-1} peaks at 10 min and wears off between 30–45 min (Wei, Tseng, Loh & Li, 1977). D-Ala^2-D-Leu^5-enkephalin also has affinity for μ-binding sites and μ-receptors (Miller & Cuatrecasas, 1979; Kosterlitz & Paterson, 1980; Kosterlitz *et al.*, 1981). Therefore, the possible involvement of δ-receptors in the control of fluid transport by the rat small intestine must await more stable and selective δ-agonists.

Previous suggestions that morphine mediates its antisecretory effect via opiate receptors has been based partly on the fact that its effect is inhibited by naloxone (Coupar, 1978; Beubler & Lembeck, 1979; Lee & Coupar, 1980a). With VIP as the secretagogue, it is now demonstrated that naloxone acts *in vivo* as a competitive antagonist as shown by parallel displacement of the dose-response lines to morphine. The '*in vivo* pA_2' was estimated to be 7.14 which is in very close agreement with '*in vivo* pA_2' values of naloxone against morphine in heat noxia tests in rats. For instance, values of 7.17 (Szekely, Dunai-Kovacs, Miglecz, Ronai & Bajusz, 1978) and 6.98 (Yaksh & Rudy, 1977) have been obtained by the tail flick method and a value of 7.04 using the hot-plate method (Yaksh & Rudy, 1977). The lapse-times employed between morphine plus naloxone administration and subsequent measurement of analgesia were similar to the time employed in the experiments described at present. This was 20 min to the mid-point of measuring water transport and is considered to approximate to peak effects of both drugs (Smits & Takemori, 1970; Tallarida, Harakal, Maslow, Geller & Adler, 1978).

These results and the demonstration that μ-opiate agonists have high potency and intrinsic activity, suggest that μ-receptors mediate inhibition of VIP-induced secretion from the rat small intestinal mucosa.

The author wishes to thank the drug companies responsible for their gifts of ethylketocyclazocine, FK 33-824, MR 2034 and naloxone. The work was supported by a Project Grant from the National Health & Medical Research Council of Australia.

References

BEUBLER, E. & LEMBECK, F. (1979). Inhibition of stimulated fluid secretion in the rat small and large intestine by opiate-agonists. *Naunyn-Schmiedebergs Arch. Pharmac.*, **306**, 113–118.

BEUBLER, E. & LEMBECK, F. (1980). Inhibition by morphine of prostaglandin E_1-stimulated secretion and cyclic adenosine 3',5'-monophosphate formation in the rat jejunum *in vivo*. *Br. J. Pharmac.*, **68**, 513–518.

COUPAR, I.M. (1978). Inhibition by morphine of prostaglandin-stimulated fluid secretion in rat jejunum. *Br. J. Pharmac.*, **63**, 57–63.

HANDA, B.K., LANE, A.C., LORD, J.A.H., MORGAN, B.A., RANCE, M.J. & SMITH, C.F.C. (1981). Analogues of β-LPH$_{61-64}$ possessing selective agonist activity at μ-opiate receptors. *Eur. J. Pharmac.*, **70**, 531–540.

KACHUR, J.F. & MILLER, R.J. (1982). Characterization of the opiate receptor in the guinea-pig ileal mucosa. *Eur. J. Pharmac.*, **81**, 177–183.

KOSTERLITZ, H.W. & PATERSON, S.J. (1980). Characterization of opioid receptors in neural tissue. *Proc. R. Soc. B*, **210**, 113–122.

KOSTERLITZ, H.W., PATERSON, S.J. & ROBSON, L.E. (1981). Characterization of the κ-subtype of the opiate receptor in the guinea-pig brain. *Br. J. Pharmac.*, **73**, 939–949.

LEE, M.K. & COUPAR, I.M. (1980a). Opiate receptor-

mediated inhibition of rat jejunal fluid secretion. *Life Sci.*, **27**, 2319–2325.

LEE, M.K. & COUPAR, I.M. (1980b). Inhibition of intestinal secretion without reduction of cyclic AMP levels. *Eur. J. Pharmac.*, **68**, 501–503.

MAGNAN, J., PATERSON, S.J., TAVANI, A. & KOSTERLITZ, H.W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn Schmiedebergs Arch. Pharmac.*, **319**, 197–205.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197**, 517–532.

McKAY, J.S., LINAKER, B.D. & TURNBERG, L.A. (1981). Influence of opiates on ion transport across rabbit ileal mucosa. *Gastroenterology*, **80**, 279–284.

MILLER, R.J. & CUATRECASAS, P. (1979). Neurobiology and neuropharmacology of the enkephalins. In *Adv. Biochem. Psychopharmac.*, Vol. 20, pp. 187–225. ed. Loh, H.H. & Ross, D.H. New York: Raven Press.

PIERCEY, M.F. & RUWART, M.J. (1979). Naloxone inhibits the anti-diarrhoeal activity of loperamide. *Br. J. Pharmac.*, **66**, 373–375.

PORRECA, F., COWAN, A., RAFFA, R.B. & TALLARIDA, R.J. (1982). Tolerance and cross-tolerance studies with morphine and ethylketocyclazocine. *J. Pharm. Pharmac.*, **34**, 666–667.

ROEMER, D., BUESCHER, H.H., HILL, R.C., PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSER, D. & HUGUENIN, R. (1977). A synthetic enkephalin analogue with prolonged parenteral and oral analgesic activity. *Nature*, **268**, 547–549.

SANDHU, B.K., TRIPP, J.H., CANDY, D.C.A. & HARRIES, J.T. (1981). Loperamide: Studies on its mechanism of action. *Gut*, **22**, 658–662.

SMITS, S.E. & TAKEMORI, A.E. (1970). Quantitative studies on the antagonism by naloxone of some narcotic and narcotic-antagonist analgesics. *Br. J. Pharmac.*, **39**, 627–638.

SZEKELY, J.I., DUNAI-KOVACS, Z., MIGLECZ, E., RONAI, A.Z. & BAJUSZ, S. (1978). *In vivo* antagonism by naloxone of morphine, β-endorphin and a synthetic enkephalin analog. *J. Pharmac. exp. Ther.*, **207**, 878–883.

TALLARIDA, R.J., HARAKAL, C., MASLOW, J., GELLER, E.B. & ADLER, M.W. (1978). The relationship between pharmacokinetics and pharmacodynamic action as applied to *in vivo* pA_2: application to the analgesic effect of morphine. *J. Pharmac. exp. Ther.*, **206**, 38–45.

TALLARIDA, R.J. & MURRAY, R.B. (1981). *Manual of Pharmacologic Calculations with Computer Programs.* New York: Springer-Verlag.

TYERS, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmac.*, **69**, 503–512.

UPTON, N., SEWELL, R.D.E. & SPENCER, P.S.J. (1982). Differentiation of potent μ- and κ-opiate agonists using heat and pressure antinociceptive profiles and combined potency analysis. *Eur. J. Pharmac.*, **78**, 421–429.

VALIULIS, E. & LONG, J.F. (1973). Effect of drugs on intestinal water secretion following cholera toxin in guinea-pigs and rabbits. *Physiologist*, **16**, 475.

WEI, E.T., TSENG, L.F., LOH, H.H. & LI, C.H. (1977). Comparison of the behavioural effects of β-endorphin and enkephalin analogues. *Life Sci.*, **21**, 321–325.

WOOD, P.L., CHARLESON, S.E., LANE, D. & HUDGIN, R.L. (1981). Multiple opiate receptors: differential binding of μ, κ and δ agonists. *Neuropharmacology*, **20**, 1215–1220.

WOOD, P.L. (1982). Multiple opiate receptors: Support for unique, mu, delta and kappa sites. *Neuropharmacology*, **21**, 487–497.

YAKSH, T.L. & RUDY, T.A. (1977). Studies on the direct spinal action of narcotics in the production of analgesia in the rat. *J. Pharmac. exp. Ther.*, **202**, 411–428.

(Received April 29, 1983.)