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The contribution of intrinsic activity to the action of opioids *in vitro*

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1 The effects of opioids were compared in five field-stimulated isolated tissue models, the guinea-pig ileum and vasa deferentia from rat, rabbit and mice of the Alderley Park and C57BL/6 strains.

2 Although the μ-receptor agonist [D-Ala^2, MePhe^4, Gly-ol^5] enkephalin appeared to act at similar receptors in the guinea-pig ileum, rat vas deferens, mouse vas deferens and C57BL/6 mouse vas deferens preparations, its potency varied considerably between these preparations. Similar potency differences were also observed with the κ-agonist, ethylketocyclazocine.

3 It is proposed that these variations in potency reflect differences in the number of spare receptors present in each model. The finding that some drugs which have agonist activity in the more sensitive preparations behave as antagonists in the less sensitive tissues supports this proposal and highlights the importance of intrinsic activity in determining the action of opioids.

4 Many of the prototypic opioid agonists were found to be either partial agonists (eg. morphine and bremazocine) or to possess affinity for more than one receptor type (eg. ethylketocyclazocine, Mr 2034).

Introduction

Opioids inhibit the electrically-evoked contractions of a range of isolated tissue preparations. There are, however, considerable differences between the sensitivities of the various preparations to standard opioid agents. The majority of results can be attributed to the existence of different receptor populations in each tissue. Thus the mouse vas deferens possesses μ, δ and κ-receptors (Hutchinson *et al.*, 1975; Lord *et al.*, 1976; 1977) whilst the guinea-pig ileum is sensitive only to μ- and κ-agonists. Some tissues appear to possess only a single type of receptor. The rabbit vas deferens has been reported to respond only to κ-agonists (Oka *et al.*, 1980) whilst the hamster vas deferens is selectively sensitive to δ-agonists (McKnight *et al.*, 1984). However, not all results can be explained solely in terms of different receptor populations. Several recent studies have demonstrated that morphine, traditionally regarded as a μ-receptor agonist, behaves as an antagonist in vasa deferentia from the C57BL/6 mouse (Miller & Shaw, 1984) and the rat (Liao *et al.*, 1981; Henderson *et al.*, 1982). Whilst it is tempting to rationalise such findings by suggesting the existence of novel types of receptor in these tissues, this is not supported by experimental data. For example, the affinities of antagonists for the receptors in the rat vas deferens are similar to their affinities for μ-receptors in the mouse vas deferens (Smith & Rance, 1983) and in the rat brain (Carroll *et al.*, 1984). It is thus necessary to seek alternative explanations for these apparent anomalies. It has been proposed by Liao *et al.* (1981) and by Smith & Rance (1983) that morphine is a partial agonist at the μ-receptor. Since morphine behaves as a full agonist in the guinea-pig ileum and mouse vas deferens preparations (Hutchinson *et al.*, 1975) it follows that these tissues must have spare receptors. However, the finding that morphine is a pure antagonist in the rat vas deferens requires not only that this tissue has few, if any spare receptors, but also that the low level of receptor activation produced by morphine fails to elicit any detectable agonist response: i.e. the tissue exhibits a threshold effect (Ariëns *et al.*, 1964). Thus it is implicit in this explanation that the response of a tissue to an opioid depends on a variety of factors including not only the drug's affinity and receptor selectivity but also its intrinsic activity and the receptor reserve of the assay system.

The present study was designed to establish the relative importance of these factors in determining the actions of opioids in isolated tissue systems. Five

tissues were studied, the guinea-pig ileum, and vasa deferentia from rat, rabbit and mice of the Alderley Park and C57BL/6 strains. Since the number of spare receptors should influence the sensitivity of a tissue to agonists, the potencies of the standard μ, δ and κ agents [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGO) (Handa *et al.*, 1981), [D-Thr^2, Leu^5] enkephalyl-Thr (DTLET) (Zajac *et al.*, 1983) and ethylketocyclazocine (EKC) (Hutchinson *et al.*, 1975) were determined.

Having characterized the assay systems it was then possible to examine a wider range of opioids representing μ and κ-agonists, 'agonist-antagonist analgesics' (Martin, 1979) and narcotic antagonists. By comparing the effects of these compounds in models possessing different receptor populations and different degrees of receptor reserve an attempt has been made to classify the drugs in terms of both their receptor selectivity and their intrinsic activity.

Methods

Guinea-pig ileum

Guinea-pig ileum myenteric plexus longitudinal muscle strips were prepared according to the method of Paton & Zar (1968). These tissues were mounted in 5 ml organ baths containing Krebs solution of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.18, CaCl 2.52 and glucose 11.1. A mixture of 95% O_2/5% CO_2 was used for aeration.

The upper end of the tissue was attached to an isotonic transducer and the resting tension was adjusted to 0.2 g. Contractions were elicited by passing pulses (1 ms duration, 0.1 Hz at supramaximal voltage) between ring electrodes positioned above and below the tissue.

Mouse vas deferens

Vasa deferentia were removed from mice of the Alderley Park and C57BL/6J strains weighing between 22 and 28 g. The tissues were mounted as described for guinea-pig ileum, but with two modifications: Mg^{2+} was omitted from the Krebs solution and stimulation was with trains of pulses (1 ms pulses at 50 Hz for 100 ms repeated at 10 s intervals).

Rat and rabbit vas deferens

Tissues were removed from Alderley Park rats weighing 200 to 250 g and from New Zealand White rabbits weighing 2.5 to 3 kg. The vasa deferentia were mounted and stimulated as described for guinea-pig ileum except that the resting tension was increased to 0.4 g.

General

After setting up, all tissues were allowed to equilibrate for 60 min before beginning the experiment. Agonist dose-response curves were obtained by the cumulative method, and the tissues were then washed and allowed to regain their pre-drug twitch height before subsequent drug additions.

Antagonists, when used, were allowed to equilibrate for 30 min before repeating the agonist dose-response.

In all tissues the appropriate standard agonists (EKC, DAGO and/or DTLET) were also tested, but since the purpose of this study was to measure differences in the sensitivity of the models, results are expressed as IC_{50} concentrations rather than as ratios to the standard agonist.

Antagonist affinities (Ke values) were calculated as: Ke = [antagonist]/dose ratio -1.

Drugs

Drugs were obtained from the following sources: ethylketocyclazocine, cyclazocine, pentazocine and Win44441 (3(2-α, 6-α, 11S)-(−)-1-cyclopentyl-5-(1,2,3,4,5,6- hexahydro- 8- hydroxy- 3,6,11- trimethyl-2,6-methano-3-benzazocin 11-yl)-3-pentanone methane sulphonate) from Sterling Winthrop: bremazocine and tifluadom, from Sandoz; naloxone, naltrexone, nalbuphine, DuPont; butorphanol, Bristol Laboratories; diprenorphine, Reckitt and Colman; nalorphine, Wellcome; Mr2266 ((−)-2-(3-furylmethyl)- 5,9- diethyl-2′-hydroxy- 6,7- benzomorphan) and MR2034 ((−)-α- (IR, 5R, 9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6, 7-benzomorphan), Boehringer Ingelheim; U-50488H (*trans*-3,4-dichloro-*N*-methyl-*N*- [2-(1-pyrrolidinyl)cyclohexyl]- benzeneacetamide methane sulphonate), Upjohn; morphine and methadone, McFarlan Smith; levallorphan-Roche; [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin, (D-Thr^2, Leu^5]enkephalyl-Thr and ICI 174864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu), Chemistry Department II, ICI Pharmaceuticals. Drugs were dissolved in distilled water with the addition of a few drops of 0.1N HCL where necessary.Subsequent dilutions were made in Krebs solution.

Results

Tissue characterization

Table 1 illustrates that the μ-agonist DAGO was able to inhibit the electrically-evoked contractions of the guinea-pig ileum, mouse vas deferens, C57BL/6 mouse vas deferens and rat vas deferens, but not the rabbit vas deferens. Furthermore, the naloxone Ke values against DAGO were all within the range 1.3–4.4 nM

Table 1 Agonist potency (IC_{50}) and antagonist potency of naloxone (Ke value) in five isolated tissue preparations

	RVD		*C57*		*MVD*		*LVD*		*GPI*	
	IC_{50}	*Ke*	IC_{50}	*Ke*	IC_{50}	*Ke*	IC_{50}	*Ke*	IC_{50}	*Ke*
μ-Agonists										
DAGO	2640 ± 410	3.9 ± 1.4	2411 ± 325	4.4 ± 2.2	311 ± 26	3.7 ± 0.4	—	—	14.3 ± 1.2	1.3 ± 0.2
Morphine	—	—	—	—	478 ± 287	4.2 ± 2.0	—	—	28.4 ± 2.9	1.8 ± 0.1
Methadone	—	—	—	—	681 ± 298	3.1 ± 0.5	—	—	31.2 ± 6.1	2.6 ± 0.1
κ-Agonists										
EKC	—	—	221 ± 53.5	17.9 ± 4.2	64.2 ± 6.6	15.8 ± 1.6	39.9 ± 23.6	18.8 ± 2.2	1.51 ± 0.16	15.4 ± 4.5
Tifluadom	—	—	68 ± 26.1	13.9 ± 1.8	14.8 ± 3.5	14.2 ± 3.4	5.7 ± 0.1	11.9 ± 2.1	0.39 ± 0.04	15.1 ± 6.3
Ketocyclazocine	—	—	786 ± 321	32.3 ± 5.5	166 ± 40.0	26.4 ± 2.9	85.8 ± 17.8	27.6 ± 3.2	19.9 ± 5.8	28.3 ± 3.3
U-50488H	—	—	640 ± 351	19.0 ± 3.2	438 ± 159	19.4 ± 1.6	2300 ± 100	34.5 ± 3.9	84.4 ± 43.0	10.0 ± 1.1
Mr2034	—	—	867 ± 289	21.1 ± 4.1	188 ± 70.2	11.4 ± 0.6	95.7 ± 35.9	31.6 ± 12.7	1.35 ± 0.8	16.6 ± 2.3
Bremazocine	—	—	—	—	7.4 ± 2.1	11.6 ± 2.1	4.2 ± 0.5	18.2 ± 5.9	0.60 ± 0.17	28.5 ± 3.4
δ-Agonist										
DTLET	7043 ± 2401	ND	1.41 ± 0.1	22.1 ± 3.5	0.46 ± 0.02	29.9 ± 4.1	—	—	82.6 ± 8.6	4.1 ± 1.7
Agonist/antagonists										
Pentazocine	—	—	—	—	—	—	—	—	1412 ± 556	27.3 ± 2.4
Butorphanol	—	—	—	—	—	—	—	—	4.9 ± 1.6	8.6 ± 1.1
Cyclazocine	—	—	—	—	—	—	—	—	3.2 ± 1.3	16.5 ± 1.1
Nalbuphine	—	—	—	—	—	—	—	—	138 ± 59.6	3.4 ± 0.5
Diprenorphine	—	—	—	—	—	—	—	—	3.4 ± 1.2	24.8 ± 2.8
Levallorphan	—	—	—	—	—	—	—	—	2.4 ± 1.0	18.5 ± 8.4
Nalorphine	—	—	—	—	—	—	—	—	308 ± 151	13.5 ± 2.3

All values are means ± s.e.mean of between 4 and 10 determinations and are expressed as nanomolar concentrations.
—: Not active; ND: Not determined; RVD: rat vas deferens; C57: C57BL/6 mouse vas deferens; MVD: mouse vas deferens; LVD: rabbit vas deferens; GPI: guinea-pig ileum. DAGO:[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; EKC: ethylketocyclazocine; DTLET:[D-Thr^2,Leu^5]enkephalin.

confirming that the effects of this agonist were μ-receptor-mediated in all cases. However, the tissues varied considerably in their sensitivity to the μ-agonist, with IC_{50} concentrations ranging from 14.3 nM in the guinea-pig ileum to 2.64 μM in the rat vas deferens.

The κ-agonist ethylketocyclazocine was effective in all tissues except the rat vas deferens. Naloxone antagonism studies yielded similar Ke values in all tissues (range 15.4–18.8 nM) indicating that ethylketocyclazocine acts at κ-receptors in the mouse vas deferens, C57BL/6 mouse vas deferens, guinea-pig ileum and rabbit vas deferens. As in the case of DAGO, the sensitivity of tissues to ethylketocyclazocine varied considerably. IC_{50} values ranged from 1.5 nM in the guinea-pig ileum to 221 nM in the C57BL/6 mouse vas deferens.

Results with the δ-agonist DTLET were less conclusive. Although this peptide exhibited agonist activity in the guinea-pig ileum, mouse vas deferens and C57BL/6 mouse vas deferens, only in the two mouse preparations was the naloxone Ke value sufficiently high to be consistent with an action at the δ-receptor. The much lower Ke value in the guinea-pig ileum (4.3 nM) is similar to that obtained with the μ-agonist DAGO and suggests that the action of this agent in the guinea-pig ileum (a tissue devoid of δ-receptors) is mediated by the μ-receptor. Although DTLET was without effect in the rat vas deferens, in the presence of a mixture of peptidase inhibitors (leucyl-leucine, 2×10^{-3} M; bestatin, 3×10^{-5} M; thiorphan, 3×10^{-7} M and captopril 10^{-5} M) (McKnight *et al.*, 1983), a weak agonist response was detected suggesting that this peptide is susceptible to enzymatic inactivation in the rat vas deferens. However, the low potency of DTLET in this preparation prevented the determination of a naloxone Ke value.

Agonist effects of opioids

The effects of three standard μ-agonists morphine, methadone and DAGO were compared in the five isolated tissue models (Table 2). None produced an effect in the κ-receptor model, the rabbit vas deferens. However, in two of the tissues shown to possess μ-receptors, the rat vas deferens and C57BL/6 mouse vas deferens, only DAGO was effective, whilst in the guinea-pig ileum and mouse vas deferens all three agonists elicited a response. In all cases where effects were observed, the naloxone Ke values were consistent with an action at the μ-receptor. In common with DAGO, both morphine and methadone were approximately 20 times less active in the mouse vas deferens than in the guinea-pig ileum.

The effects of the κ-agonists were similar to those of ethylketocyclazocine. Thus tifluadom, ketocyclazocine, U50488 and Mr 2034 consistently inhibited the contractions of the guinea-pig ileum, mouse vas deferens, rabbit vas deferens and C57BL/6 mouse vas deferens but were inactive in the rat vas deferens. An exception, however, was bremazocine which, although active in the guinea-pig ileum and rabbit vas deferens, produced shallow dose-responses in the mouse vas deferens and failed to elicit a response in the C57BL/6 mouse vas deferens. In all cases the naloxone Ke values against these agonists were within a factor of two of the values against ethylketocyclazocine, indicating that all these agents act predominantly at the κ-receptor. The potencies of the κ-agonists followed the same pattern as ethylketocyclazocine. Thus the lowest IC_{50} values were observed in the guinea-pig ileum and the highest values occurred in the C57BL/6 mouse vas deferens.

The compounds of the agonist-antagonist group were active only in the guinea-pig ileum. High naloxone Ke values were obtained against pentazocine, cyclazocine, diprenorphine, levallorphan and nalorphine, indicating that the agonist effects of these agents are mediated via the κ-receptor. In contrast, nalbuphine proved more sensitive to naloxone (Ke = 3.4 nM) indicating an action at the μ-receptor, and butorphanol yielded an intermediate Ke value (8.6 nM) possibly suggesting an action at both μ and κ-sites.

None of the opioid antagonists depressed the contractions of any of the tissues at concentrations up to 1 μM (5 μM in the case of ICI 174864).

Antagonist activity

It is clear from Table 1 that many compounds failed to elicit a response in one or more of the isolated tissue models. This lack of agonist activity could indicate that the compounds lack affinity for the opioid receptors in a particular tissue. Alternatively, the intrinsic activity of the compounds may be insufficient to generate a response. In this latter case it should be possible to demonstrate antagonist activity.

Table 2 reveals that both morphine and methadone were able to antagonize DAGO in the rat vas deferens and C57BL/6 mouse vas deferens, confirming that these compounds possess affinity for the μ-receptors in these tissues. In contrast, at the κ-receptors of the rabbit vas deferens and C57BL/6 mouse vas deferens, and the δ-receptor of the C57BL/6 mouse vas deferens, no significant affinity could be demonstrated.

Amongst the κ-agonists, only U-50488H failed to antagonize DAGO in the rat vas deferens, indicating that the remaining compounds in this group all possess significant affinity for the μ-receptor. The δ-affinity of most of these compounds could not be established because of their agonist effects in the mouse vas deferens and C57BL/6 mouse vas deferens. However, bremazocine, which proved inactive as an agonist in

Table 2 Antagonist affinities (Ke values) against DAGO (μ-receptor), ethylketocyclazocine (κ-receptor) and DTLET (δ-receptor) in mouse, rat and rabbit vas deferens

	DAGO		*Ethylketocyclazocine*		*DTLET*
	MVD	*RVD*	*MVD*	*LVD*	*MVD*
μ-Agonists					
Morphine	1700[1] ± 370	1500 ± 230.0	> 5000[1]	> 6000	> 5000[1]
Methadone	475[1] ± 117	230 ± 14.5	2850[1] ± 1060	> 5000	> 5000[1]
DAGO	—	—	—	> 3500	—
κ-Agonists					
EKC	—	278 ± 49.0	—	—	—
Tifluadom	—	1084 ± 116.0	—	—	—
Ketocyclazocine	—	191 ± 30.3	—	—	—
U50488	—	> 5000	—	—	—
MR2034	—	27.8 ± 3.2	—	—	—
MR1353	—	349 ± 80.0	—	—	—
Bremazocine	1.5[1] ± 0.3	4.5 ± 0.6	3.9[1] ± 1.1	—	24[1] ± 6.2
δ-Agonist					
DTLET	—	—	—	—	—
Agonist-antagonists					
Pentazocine	576.4 ± 106.2	628.3 ± 233.0	301 ± 130	373 ± 80	3050 ± 481
Nalorphine	49.7 ± 16.9	27.0 ± 6.9	284 ± 82.7	146 ± 43	353 ± 82
Levallorphan	9.8 ± 1.0	10.9 ± 3.4	12.4 ± 4.0	22.3 ± 6.2	22.1 ± 4.3
Butorphanol	7.8 ± 2.1	26.2 ± 4.9	27.6 ± 1.9	29.0 ± 3.4	112 ± 34.8
Nalbuphine	48.9 ± 9.8	57.0 ± 5.1	300 ± 150	147 ± 3.1	1395 ± 155
Diprenorphine	0.4 ± 0.1	0.2 ± 0.04	0.5 ± 0.1	3.4 ± 1.4	3.6 ± 1.6
Cyclazocine	12.1 ± 3.4	16.9 ± 6.1	7.7 ± 1.2	28.4 ± 2.8	26.3 ± 7.6
Narcotic antagonists					
MR2266	3.7 ± 0.7	3.5 ± 0.9	5.5 ± 0.1	14.5 ± 1.4	13.4 ± 3.8
Naloxone	3.9 ± 0.9	3.9 ± 1.4	17.0 ± 7.9	18.8 ± 2.2	30.1 ± 3.7
WIN44441	1.4 ± 0.3	0.5 ± 0.1	5.5 ± 2.1	14.1 ± 2.7	15.2 ± 4.8
Naltrexone	1.2 ± 0.2	3.1 ± 0.9	4.4 ± 0.4	9.0 ± 2.5	12.3 ± 1.7
ICI174864	> 5000		> 5000	> 10000	32.9 ± 4.7

All figures are Ke values (nM) ± s.e.mean and are the mean of between 4 and 8 determinations.
[1]: Value obtained in C57BL/6 mouse vas deferens. —: Agonist activity prevented the determinatioon of a Ke value. MVD: mouse vas deferens. C57: C57BL/6 mouse vas deferens. RVD: rat vas deferens. LVD: rabbit vas deferens. GPI: guinea-pig ileum. EKC: ethylketocyclazocine; DTLET: [D-Thr^2,Leu^5]enkephalin; DAGO: [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin.

the C57BL/6 mouse vas deferens, was a potent antagonist at all three receptor types in this tissue.

All of the drugs belonging to the agonist/antagonist group displayed significant μ, δ and κ antagonist activity, as did the narcotic antagonists. It was thus possible to compare the affinities of these compounds for the μ-receptors of the rat vas deferens and the mouse vas deferens. Figure 1 reveals a highly significant correlation between these two measures of μ-affinity ($r = 0.96$, slope = 0.95) and demonstrates that the μ-receptor populations in these tissues could not be distinguished by the drugs employed in this study.

Discussion

Our results concerning the receptor populations present in each model differ from earlier literature in two respects. Waterfield *et al.* (1978) observed that the C57BL/6 mouse vas deferens was particularly insensitive to μ-agonists such as normorphine. In contrast we find that agents acting at both δ and κ-receptors are also less effective in this preparation. Nevertheless, the C57BL/6 preparation is capable of responding to all three classes of agonist.

In the rat vas deferens our conclusions differ

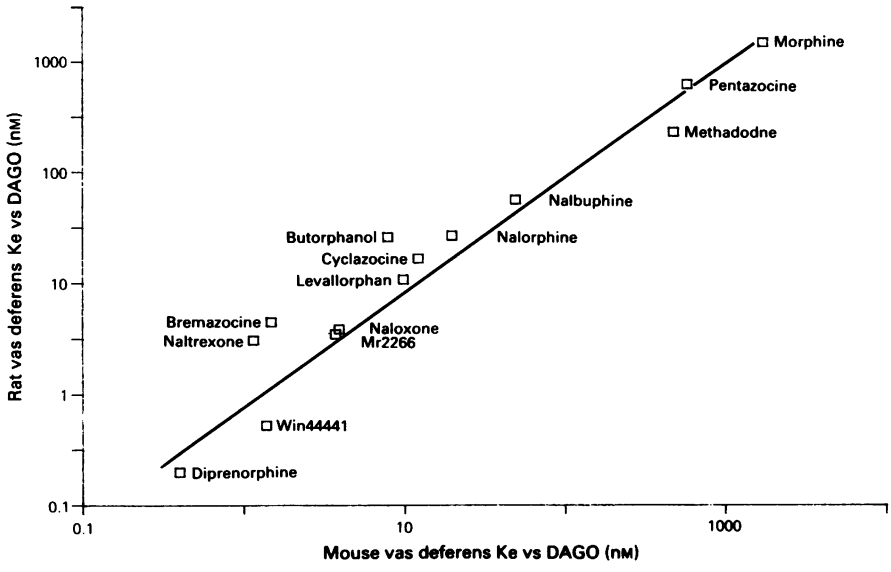

Figure 1 Correlation between μ-affinity (Ke vs DAGO) determined on the mouse vas deferens and rat vas deferens preparations. Slope = 0.996; $r = 0.944$. Ke: antagonist affinities. DAGO: [D-Ala^2, MePhe^4,Gly-ol^5]enkephalin.

somewhat from those of Schulz *et al.* (1979). These authors noted that morphine is without effect in the rat vas deferens and postulated that the receptors in this tissue are of a novel type, termed the ε-receptor. The present study confirms morphine's lack of agonist activity but, in common with Henderson *et al.* (1982) and Smith & Rance (1983) we find morphine to be an antagonist. Thus the alkaloid does not lack affinity for the receptors of the rat vas deferens. Furthermore, the strong correlation between the affinities of drugs for the μ-receptor of the mouse vas deferens and their antagonist activity in the rat preparation indicates that the two receptor populations are similar, if not identical.

The receptor selectivities of the standard agents yielded several unexpected findings. Whilst the μ-agonists morphine, methadone and DAGO appear to be relatively free from affinity for other sites, the δ-agonist [D-Thr^2,Leu^5]enkephalyl-Thr clearly acts at μ-receptors in the guinea-pig ileum preparation. Of greater significance however, is the lack of selectivity of the compounds generally considered to be κ-agonists. Of these drugs only U-50488H fails to exhibit μ-antagonist activity on the rat vas deferens preparation. Particularly potent were Mr2034, which also antagonizes the analgesic action of morphine (Rourke & Shaw, 1984) and bremazocine, which has high affinity for all three types of opioid receptor.

The differences in the IC_{50} concentrations of the μ and κ-agonists are consistent with each tissue having a different number of spare receptors. Thus for the μ-site the size of receptor reserve is: guinea-pig ileum > mouse vas deferens > C57BL/6 mouse vas deferens > rat vas deferens. For the κ-receptor the order is: guinea-pig ileum > rabbit vas deferens > mouse vas deferens > C57BL/6 mouse vas deferens. However, differences in receptor reserve do not, at first sight account for the observation that many compounds (especially those belonging to the agonist-antagonist group) behave as agonists in one tissue and antagonists in another. Although classical receptor theory (Ariëns *et al.*, 1964) predicts that partial agonists should elicit a maximal response in models with a high receptor reserve, it also predicts that in systems with few spare receptors such drugs should still generate a response, albeit with a reduced maximum. However, these theories are based largely on data from tissues in which responses are mediated by receptors sited directly on smooth muscle. In contrast, the opioids act by inhibiting the electrically-evoked release of neurotransmitters, and many processes intervene between the occupation of a receptor by an opioid and the measured response. There is thus no *a priori* reason to assume that under these circumstances the measured effect is linearly related to receptor activation.

The recent finding by Smith (1984) that increasing the stimulus intensity in the mouse vas deferens abolishes the agonist effect of morphine, but not of the full agonist DAGO, demonstrates that under some circumstances partial agonists do not produce any

measurable response in systems that are still sensitive to full agonists, i.e. tissues can exhibit threshold phenomena.

It may thus be concluded that in an isolated tissue model, two factors – the intrinsic activity of the drug and the receptor reserve of the tissue – determine whether the affinity of an opioid is expressed as agonist or antagonist activity.

The finding that drugs that possess significant intrinsic activity need not necessarily generate any response in some tissues is of some importance. It illustrates the need to examine drugs in more than one tissue since in preparations such as the rat vas deferens or C57BL/6 mouse vas deferens partial agonists are indistinguishable from pure antagonists, whilst the guinea-pig ileum cannot discriminate between partial and full agonists.

A broader implication of this study concerns the interpretation of all studies using opioids. Morphine and methadone are widely used as prototypic μ-agonists and any effects produced by these agents are attributed to activation of μ-receptors. Similarly, the failure of morphine to elicit a response in an assay system is interpreted as indicating a lack of functional μ-receptors. Since morphine is capable of behaving as an antagonist in tissues with a low receptor reserve, it is conceivable that some systems that possess μ-receptors may not respond to this agent, whereas a full agonist such as DAGO may be effective. Furthermore, it is possible that some of the effects of morphine may be a result of its antagonist rather than its agonist properties.

Still greater caution is required with agents such as ethylketocyclazocine, Mr2034 and bremazocine which are frequently employed as κ-agonists. All of these drugs are able to act as μ-antagonists, and bremazocine may also behave as a δ or κ-antagonist. In the absence of a selective κ-antagonist it is extremely difficult to ascribe positively the effects of any of these agents to a particular type of receptor.

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