Br. J. Pharmac. (1978), **64**, 607–614

COMPARISON OF THE RECEPTOR BINDING CHARACTERISTICS OF OPIATE AGONISTS INTERACTING WITH μ- OR κ-RECEPTORS

H.W. KOSTERLITZ & FRANCES M. LESLIE[1]

Unit for Research on Addictive Drugs, University of Aberdeen, Aberdeen

1 The receptor binding characteristics of various morphine-like and ketazocine-like opiate agonists were measured by inhibition of [^{3}H]-naloxone binding in homogenates of brain and of ileal myenteric plexus-longitudinal muscle of the guinea-pig. No differences were found for the two tissues.

2 The depressant effect of Na^+ on the inhibition of [^{3}H]-naloxone binding by opiate agonists varies widely, giving sodium shifts between 5 and 140. The relationship between Na^+ concentration and inhibition of binding is non-linear, the magnitude of the sodium shift varying directly with the slope of the regression of log IC_{50} on log [NaCl].

3 The sodium shift of ketazocine-like agonists is lower than that of morphine-like agonists but higher than that of opiates with dual agonist and antagonist action. A working hypothesis is proposed which suggests that the κ-receptors for the ketazocine-like drugs are less susceptible to the Na^+ effect than the μ-receptors for the morphine-like drugs.

4 For most of the morphine-like but not the ketazocine-like agonists, a good correlation has been found for the pharmacological activity in the myenteric plexus-longitudinal muscle preparation and the inhibition of binding of [^{3}H]-naloxone at 12 mM Na^+. An exception is fentanyl which has a much greater pharmacological potency than may be expected from its potency in inhibiting [^{3}H]-naloxone binding.

Introduction

In recent years several novel benzomorphans, such as the ketazocines, have been shown to exhibit pharmacological profiles which are significantly different from that of morphine. Unlike other potent narcotic analgesics, these compounds neither precipitate nor suppress withdrawal symptoms in morphine-dependent monkeys (Villarreal & Seevers, 1972; Swain & Seevers, 1974; 1976). *In vitro* studies on guinea-pig isolated ileum and mouse vas deferens preparations have indicated that, although with the exception of ketazocine itself these anomalous benzomorphans are pure opiate agonists, they are considerably less sensitive to the antagonist action of naloxone than are drugs which have a high dependence liability (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975). These findings, together with evidence of non-morphine like effects in chronic spinal dogs, have prompted the suggestion that ketazocine-like drugs interact with an opiate receptor (κ-receptor) other than that specific to morphine (μ-receptor) (Martin, Eades, Thompson, Huppler & Gilbert, 1976).

In the light of this hypothesis, the aim of this paper is to compare the opiate receptor binding characteristics of morphine-like and ketazocine-like agonists in both guinea-pig ileum and brain homogenates. Some of the results have previously been presented to a meeting of the British Pharmacological Society (Kosterlitz & Leslie, 1977).

Methods

Preparation of homogenates

Guinea-pigs of either sex, weighing 400 to 600 g, were killed by cervical dislocation. One or both of the following tissues was then excised and cooled as rapidly as possible.

Ileum. The entire ileum, with the exception of the terminal 10 cm portion, was removed and washed thoroughly with Krebs-Tris solution (0°C, pH 7.4) to remove intestinal debris. Myenteric plexus-longitudinal muscle strips were prepared by a modification (Kosterlitz, Lydon & Watt, 1970) of the method of

[1] Present address: Addiction Research Foundation, 701 Welch Road, Palo Alto, Calif. 94304, U.S.A.

Rang (1964) and placed in ice-cold Tris-HCl buffer (0.05 M, pH 7.4 at 37°C) until required. Typically, muscle strips from 6 to 8 guinea-pigs were pooled to provide sufficient material for a single experiment. The collected strips were blotted lightly on filter paper and transferred to a beaker containing 1 ml of 0.05 M Tris buffer solution for 100 mg tissue. They were then minced finely by repeated cutting with a small pair of scissors, before homogenization with an Ultra Turrax TP-18-10 homogenizer (maximum speed for 2 min). The homogenate was diluted with 2 vol of Tris buffer and centrifuged at 600 *g* for 10 min, after which the supernatant fluid was collected and centrifuged for a further 15 min at 49,000 *g*. The resulting pellets were resuspended in either 0.05 M Tris or in Krebs-Tris buffer (pH 7.4 at 37°C).

Brain. The animal was decapitated and the brain removed immediately to an ice-cold petri dish. The cerebellum, which is devoid of significant receptor binding activity (Pert & Snyder, 1973), was excised, the remaining tissue divided into 3 to 4 portions and transferred to a beaker containing the appropriate volume of ice-cold Tris buffer (1 ml/100 mg tissue). Two brains, each weighing 2.5 to 3.5 g were usually required for a single experiment. Following homogenization with an Ultra Turrax homogenizer at 75% maximum speed for 60 s, the homogenate was diluted with a further 2.5 volumes of ice-cold buffer. The remainder of the preparative procedure was identical to that outlined above for the ileum.

Both ileum and brain receptor preparations could be stored at 0°C for 24 h without significant loss of stereospecific [^{3}H]-naloxone binding.

Inhibition of [^{3}H]-naloxone binding

Opiate receptor affinities were determined by a modification of the method of Pert & Snyder (1973). Triplicate samples were prepared in plastic tubes containing 1.9 ml homogenate, a fixed concentration of [^{3}H]-naloxone (1 nM in brain preparations, 2 nM in ileum) and six concentrations of the unlabelled test drug to give a final volume of 2.0 ml. In experiments in which the effects of various concentrations of Na^+ were investigated, an aliquot of NaCl solution was also added to each tube. Following incubation at 37°C for 20 min, the samples were cooled on ice for 30 to 45 min and then filtered through Whatman GF/B glass fibre filters. The filters were washed twice with 5 ml of ice-cold buffer and transferred to plastic vials containing 1 ml Soluene (Packard Instrument Co.). Proteins were digested by incubation at 50°C for 1 h, after which 10 ml scintillation fluid (Multisol II, Intertechnique) was added and the samples reheated in order to remove chemiluminescence. Radioactivity was measured by liquid scintillation spectrometry at a counting efficiency of approximately 35%.

Stereospecific [^{3}H]-naloxone binding was determined by subtracting the binding in the presence of an excess of inhibiting drug (50 nM Mr 2266) from that in its absence. The (+)-isomer of this opiate antagonist, Mr 2267, did not affect binding in concentrations of up to 50 nM. For each test drug, the concentration required to produce a 50% inhibition of [^{3}H]-naloxone binding (IC_{50}) was calculated by log-probit regression analysis.

Solutions and drugs

The composition of the Krebs-Tris solution was (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.2 and Tris-HCl, 25.

The following drugs were used. (±)-cyclazocine ((±)-α-5,9-dimethyl-2-cyclopropylmethyl-2′-hydroxy-6,7-benzomorphan, Dr N.B. Eddy); (±)-ethylketazocine methanesulphonate ((±)-α-9-methyl-5-ethyl-8-oxo-2-cyclopropylmethyl-2′-hydroxy-6,7-benzomorphan, (Win 35,197-2), Sterling-Winthrop); fentanyl citrate (Janssen Pharmaceuticals); ketobemidone hydrochloride (Boehringer, Ingelheim); (±)-ketazocine methanesulphonate ((±)-α-5,9-dimethyl-8-oxo-2-cyclopropylmethyl-2′-hydroxy-6,7-benzomorphan, Sterling-Winthrop); levorphanol tartrate (Roche Products); morphine hydrochloride (Macfarlan Smith); Mr 1353 as the methanesulphonate ((±)-α-5,9-dimethyl-2-(3-methylfurfuryl)-2′-hydroxy-6,7-benzomorphan); Mr 2034 as the free base ((−)-α-(1R,5R,9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2′-hydroxy-6,7-benzomorphan); Mr 2092 as the hydrochloride ((−)-β-(1R,5R,9S)-5,9-dimethyl-2-(L-tetrahydrofurfuryl-2′-hydroxy-6,7-benzomorphan); Mr 2266 as the free base ((−)-α-5,9-dimethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan) (all Mr compounds, Dr H. Merz, Boehringer-Ingelheim); nalorphine hydrobromide (Burroughs Wellcome); NIH 8152 as the hydrochloride ((±)-β-2,9-dimethyl-5-*n*-propyl-2′-hydroxy-6,7-benzomorphan, Dr E.L. May); normorphine (Burroughs Wellcome); (±)-phenazocine hydrobromide ((±)-α-5,9-dimethyl-2-phenylethyl-2′-hydroxy-6,7-benzomorphan, Dr E.L. May).

[^{3}H]-naloxone (4 Ci/mmol) was obtained from Dr Willette, U.S. National Institute on Drug Abuse.

Results

Comparison of inhibition of [^{3}H]-naloxone binding in ileum and brain

The receptor binding characteristics of five narcotic agonists were compared in homogenates of brain and ileum (Table 1). The potency of each drug in inhibit-

ing [^{3}H]-naloxone binding was assessed in Tris buffer without addition of NaCl and in Krebs-Tris buffer. Three of the drugs had pharmacological profiles similar to morphine, while the remaining two, Mr 2034 and ethylketazocine, were representatives of the ketazocine group (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975; Martin *et al.*, 1976). For both groups of drugs, the IC_{50} values found in the ileum, either in Na^+-free Tris buffer or in Krebs-Tris solution, did not differ significantly from those obtained in the brain. This observation extends the findings of Creese & Snyder (1975) to ketazocine-like drugs.

In Krebs-Tris solution, the potency of the compounds in inhibiting [^{3}H]-naloxone binding to brain and ileum receptors was considerably reduced; control experiments showed that the presence of 118 mM NaCl was solely responsible for this effect. Although none of the agonists shown in Table 1 had any significant antagonist properties (Hutchinson *et al.*, 1975), considerable differences were found in their sensitivities to the inhibitory effect of Na^+. In both brain and ileum homogenates, the receptor affinities of the three morphine-like compounds were more markedly affected by sodium than those of the two ketazocine-like agonists.

Analysis of the sodium effect in brain homogenates

As the binding characteristics of the two groups of compounds to brain and ileum receptors appeared to be identical, brain homogenates were used for the further analysis of the Na^+ effect. The receptor affinities of various opiate agonists were measured in the absence and presence of Na^+ and the resulting sodium shifts, i.e. the ratio of the IC_{50} values in the presence of NaCl to those in its absence, calculated. Eleven agonists were tested, six of which, morphine, normorphine, phenazocine, fentanyl, levorphanol and NIH 8152, were morphine-like in character, while five, Mr 1353, Mr 2034, Mr 2092, ethylketazocine and ketazocine, belonged to the ketazocine group. Also included were nalorphine and cyclazocine which have dual agonist and antagonist activity in the guinea-pig ileum and mouse vas deferens (Kosterlitz & Watt, 1968; Hughes, Kosterlitz & Leslie, 1975). The data presented in Table 2 illustrate the wide spectrum of sodium shifts exhibited by the pure agonists, ranging from 136 for normorphine to 4.7 for Mr 2092; cyclazocine and nalorphine were even less sensitive to the action of Na^+. Despite these marked variations, a clear pattern appeared to emerge. The morphine-like agonists were more sensitive to the inhibitory effect of Na^+ than were the ketazocine-like compounds; these, in turn, had higher sodium shifts than the compounds with dual agonist and antagonist activity.

The mechanisms underlying the sodium shift are complex. It has been shown that binding of [^{3}H]-naloxone (Pert & Snyder, 1974; Lee, Akera & Brody, 1977) or [^{3}H]-naltrexone (Simon, Hillier, Groth & Edelman, 1975) is maximally enhanced by 40 to 50 mM Na^+; in our experiments, the maximal effect was reached at 10 mM, with a slight decline at higher concentrations (Figure 1). The relationship between the activity of agonists in inhibiting [^{3}H]-naloxone binding and the concentration of Na^+, as shown for two compounds with widely differing sodium shifts, Mr 2034 and normorphine, is illustrated in Figure 2. Whereas receptor binding of Mr 2034 is greatly reduced in the presence of low concentrations of Na^+, higher concentrations produce little additional effect. In contrast, normorphine, which has a much larger overall sodium shift than Mr 2034, shows a more linear reduction in binding with increasing Na^+ concentrations.

The relationship between Na^+ concentration and sodium shift was best shown after logarithmic transformation of the data. When log IC_{50} was plotted against Na^+ concentration (Figure 3), linear regres-

Table 1 Comparison of the potencies of narcotic analgesic drugs in inhibiting [^{3}H]-naloxone binding in homogenates of brain and ileum of the guinea-pig suspended in Na^+-free Tris buffer or in Krebs-Tris solution

	Brain			*Ileum*		
Drug	*Tris buffer IC_{50} (nM)*	*Krebs-Tris buffer IC_{50} (nM)*	*Sodium shift*	*Tris buffer IC_{50} (nM)*	*Krebs-Tris buffer IC_{50} (nM)*	*Sodium shift*
Normorphine	6.5 ± 0.6 (7)	930 ± 54 (7)	143	8.3 ± 1.5 (6)	1080 ± 136 (6)	130
Morphine	5.4 ± 0.2 (4)	353 ± 31 (4)	65	7.7 ± 1.2 (3)	380 ± 30 (3)	49
(±)-Phenazocine	0.64 ± 0.02 (3)	68 ± 4.5 (4)	106	0.85 ± 0.18 (3)	68 ± 2.3 (3)	80
Mr 2034	0.28 ± 0.01 (3)	2.6 ± 0.1 (4)	9.3	0.30 ± 0.03 (3)	2.6 ± 0.2 (3)	8.7
(±)-Ethylketazocine	3.9 ± 0.6 (3)	27 ± 3.8 (4)	6.9	4.8 ± 0.7 (3)	32 ± 1.4 (3)	6.7

The IC_{50} values are the means ± s.e.; the number of observations are given in parentheses.

Table 2 The effect of Na^+ on the potencies of narcotic analgesic drugs in inhibiting [^{3}H]-naloxone binding in homogenates of guinea-pig brain: comparison of the potencies in presence of 12 mM Na^+ with those in inhibiting the evoked longitudinal contractions of the guinea-pig isolated ileum

	Inhibition of [^{3}H]-naloxone binding (IC_{50}, nM)			*Ratio of IC_{50} in Na^+-free to IC_{50} in Na^+*		*Inhibition of contraction of guinea-pig ileum*	*IC_{50} contractions/ IC_{50} inhibition of*
Drug	*Tris buffer*	*Krebs-Tris buffer*	*Tris buffer with 12 mM Na^+*	*118 mM*	*12 mM*	*(IC_{50}, nM)*	*binding in 12 mM Na^+*
Normorphine	6.9 ± 0.4	939 ± 89	81 ± 7.0	136	12	73	0.9
Phenazocine	0.64 ± 0.02	68 ± 4.5	8.0 ± 1.0	106	12	11	1.4
Ketobemidone	4.5 ± 0.2	453 ± 9.0	66 ± 2.7	101	15	73	1.1
Levorphanol	0.42 ± 0.04	28 ± 2.5	4.3 ± 0.2	67	10	9.2	2.1
Morphine	5.4 ± 0.2	353 ± 31	65 ± 0.4	65	12	69	1.1
Fentanyl	8.4 ± 1.2	324 ± 36	100 ± 11	39	12	0.92	0.009
NIH 8152	2.2 ± 0.2	41 ± 1.3	11 ± 0.6	19	5.0	27	2.5
Mr 1353	3.6 ± 0.5	57 ± 10	34 ± 4.4	16	9.4	12	0.35
Mr 2034	0.28 ± 0.01	2.6 ± 0.1	0.99 ± 0.02	9.3	3.5	0.59	0.60
Ethylketazocine	3.9 ± 0.08	27 ± 3.8	19 ± 1.5	6.9	4.9	0.47	0.025
Ketazocine	16.0 ± 1.5	96 ± 23	102 ± 36	6.0	6.4	2.1	0.02
Mr 2092	1.0 ± 0.2	4.7 ± 0.3	2.8 ± 0.3	4.7	2.8	0.20	0.07
Cyclazocine	0.54 ± 0.08	1.1 ± 0.2	—	2.0	—	—	—
Nalorphine	2.7 ± 0.5	5.0 ± 0.9	—	1.9	—	—	—

The values are the means ± s.e. of 3 to 4 observations (for normorphine, 14 in Tris and 12 in Krebs-Tris buffer). The values for the inhibition of the contractions of the guinea-pig ileum are from Kosterlitz, Lord & Watt (1972), Kosterlitz, Waterfield & Berthoud (1974), Hutchinson *et al.* (1974), Kosterlitz & Waterfield (1975) and Kosterlitz, Hughes, Lord & Waterfield (1976). The drugs have been arranged in descending order of total sodium shift.

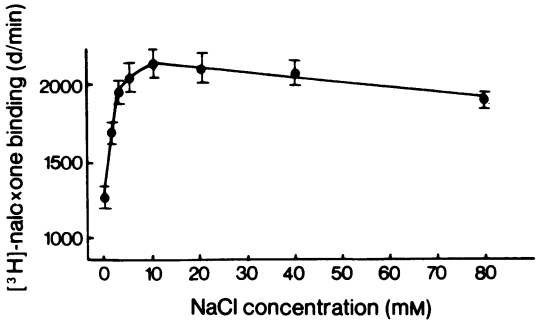

Figure 1 The effect of sodium chloride on [³H]-naloxone binding in guinea-pig brain homogenates. Abscissa scale, concentration of sodium chloride (mM) in the incubation mixture; ordinate scale, stereospecific [³H]-naloxone binding in 1.9 ml of homogenate (d/min). Each result is the mean of 11 observations; vertical lines show s.e. mean.

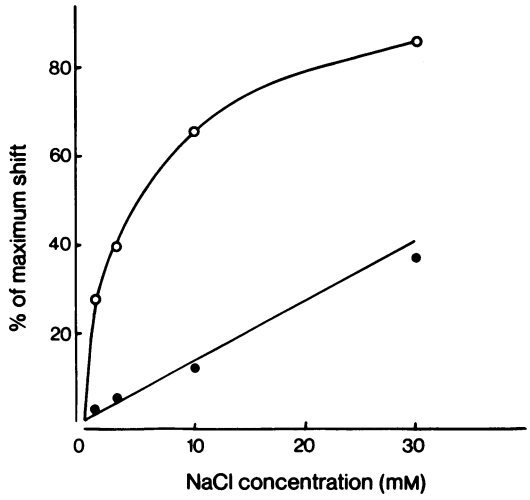

Figure 2 Relationship between agonist receptor affinity and the concentration of sodium chloride. Dose-response curves for inhibition of [³H]-naloxone binding in guinea-pig brain homogenate were constructed in the presence of several concentrations of sodium chloride, and the sodium shifts (i.e. ratio of IC_{50} values in the presence and absence of Na^+) calculated. At each concentration, the sodium shift was expressed as a percentage of the maximum shift, being 6.9 for Mr 2034 (○) and 103 for normorphine (●).

sion lines were obtained with correlation coefficients between 0.939 and 0.996. The slopes of these lines varied from 0.24 for ethylketazocine, which had one of the smallest sodium shifts, to 0.77 for normorphine, which had the largest sodium shift found so far. In each case the slope of the line was significantly different from unity (for $b = 0.77$, $P < 0.005$), indicating a non-linear relationship between Na^+ concentration and agonist affinity for the receptor.

Correlation between receptor affinity and pharmacological activity

It was of interest to compare the receptor affinity of a drug in the absence and the presence of Na^+ with its potency in inhibiting the evoked longitudinal contractions of the guinea-pig ileum. For the majority of the compounds, the IC_{50} values obtained for the longitudinal contractions were intermediate to the IC_{50} values found for inhibition of binding in the presence or the absence of Na^+ (Table 2). The exceptions were fentanyl, the ketazocines and Mr 2092, all of which were more potent in the guinea-pig ileum than would have been expected from their binding affinities, even in the absence of Na^+.

It was decided to examine whether there was a Na^+ concentration below that of Krebs-Tris buffer (118 mM) at which the potencies in inhibiting binding were similar to those required to inhibit the evoked contractions of the guinea-pig ileum suspended in Krebs solution. Since [³H]-naloxone binding was found to be at its maximum at about 12 mM Na^+, this Na^+ concentration was used for binding assays (Table 2). It was found that the first six morphine-like drugs had the largest shifts in the IC_{50} values for inhibition of binding at 12 mM Na^+. The ratio for inhibition of the contractions of the guinea-pig ileum to inhibition of binding at 12 mM Na^+ varied between 0.9 and 2.1, with the exception of fentanyl which, as has already been pointed out, had a much greater activity in the guinea-pig ileum than would be expected from its binding affinity. The four ketazocine-like compounds, the ketazocines, Mr 2034 and Mr 2092 had much smaller IC_{50} shifts at either 118 or 12 mM and the ratio of activity in guinea-pig ileum to binding affinity varied between 0.02 and 0.6. NIH 1852 and Mr 1353 were intermediate between these two groups. The possible significance of these findings is considered in the Discussion.

Discussion

The main purpose of this investigation was a comparison of the binding characteristics of morphine-like drugs, interacting with μ-receptors, with those of ketazocine-like drugs, interacting with κ-receptors. This distinction of receptors is based on the following facts: first, the κ-agonists, the ketazocines and certain *N*-dimethylfuryl or *N*-tetrahydrofurfuryl benzomorphans, represented here by Mr 1353, 2034, 2092, do not substitute readily for morphine in the dependent

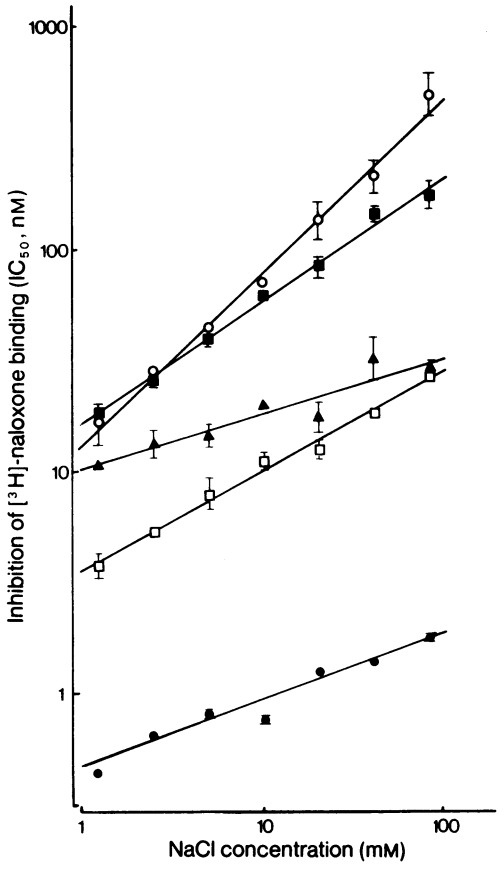

Figure 3 Correlation between sodium concentration and the binding of narcotic agonists to guinea-pig brain homogenates. Abscissa scale, concentration (mM) of sodium chloride; ordinate scale, IC_{50} (nM) for inhibition of stereospecific [^{3}H]-naloxone binding. The values which are the means of 3 observations are plotted on a logarithmic scale. Vertical lines show s.e. means. For the agonists tested, the correlation coefficients and regression equations were: normorphine (○), $r = 0.995$, $y = 0.78x + 1.1$; morphine (■), $r = 0.996$, $y = 0.57x + 1.2$; ethylketazocine (▲), $r = 0.939$, $y = 0.25x + 1.0$; NIH 8152 (□), $r = 0.992$, $y = 0.46x + 0.6$; Mr 2034 (●), $r = 0.974$, $y = 0.30x - 0.3$.

monkey (Villarreal & Seevers, 1972; Swain & Seevers, 1974; 1976); secondly, in the spinal dog they have a pattern of activity different from that of the morphine-like μ-agonists (Martin *et al.*, 1976; Gilbert & Martin, 1976); and finally, in the mouse vas deferens their relative potencies, referred to morphine as standard of unity, are only one-quarter of those obtained in the guinea-pig ileum and, in both preparations, they require about six times more naloxone for antagonism than the μ-agonists (Kosterlitz, Waterfield & Berthoud, 1974; Hutchinson *et al.*, 1975; Kosterlitz & Waterfield, 1975; Kosterlitz, Hughes, Lord & Waterfield, 1976).

In this context, it was important to prove that the binding characteristics of brain receptors were similar to those in homogenates of the myenteric plexus-longitudinal muscle preparation. Such a similarity has now been demonstrated to exist not only for μ-receptors (Creese & Snyder, 1975) but also for κ-receptors.

The decrease by Na^+ of the affinity of agonists for the opiate receptors is not an effect of ionic strength or cations in general; only Li^+ has a similar effect (Simon, Hiller & Edelman, 1973; Pert & Snyder, 1974; Simon *et al.*, 1975). It has been shown by Pert, Snyder & May (1976) that the effect of Na^+ on the IC_{50} values for the inhibition of [^{3}H]-naloxone binding by ketazocines is smaller than that found for morphine-like μ-agonists. Since there is no pharmacological evidence for any antagonist activity of ethylketazocine, and ketazocine has only a weak antagonist component, ethylketazocine was considered by Pert *et al.* to be an exception to the general rule that compounds with low sodium shifts have dual agonist and antagonist activities. The *N*-dimethylfuryl and *N*-tetrahydrofurfuryl benzomorphans, used in the present investigation as further examples of κ-agonists, do not have antagonist activity and yet they have sodium shifts lower than those characteristic for μ-agonists. As far as nalorphine, a compound with dual agonist and antagonist action, is concerned, Martin (1967) proposed that it interacts with μ-receptors only as an antagonist but not as an agonist, a concept which has been extended recently (Martin *et al.*, 1976; Gilbert & Martin, 1976). In support of this view, it has been found (Kosterlitz & Watt, 1968; Kosterlitz, Lord & Watt, 1972) that opiates with dual agonist and antagonist action, e.g. nalorphine, levallorphan and diprenorphine, require more naloxone for the antagonism of their agonist activity than do the μ-type agonists. Moreover, the agonist action of nalorphine and levallorphan is much weaker in the mouse vas deferens than in the guinea-pig ileum (Hughes *et al.*, 1975). These observations are in agreement with the view that the agonist effect of these compounds is not due to an interaction with μ-receptors.

It may be concluded that on the present evidence the low sodium shifts observed for the κ-agonists cannot be explained by an antagonist component and it is uncertain that the even lower sodium shifts of drugs with dual action are caused by their antagonist component. An alternative hypothesis would be based on the assumption that sodium ions have a smaller effect on the interaction of drugs with κ-receptors than with μ-receptors. The examination of this possi-

bility will be possible when tritiated κ-agonists become available.

The fact that the IC_{50} values of μ-agonists for the inhibition of contractions in the guinea-pig ileum correlate better with IC_{50} values for inhibition of [^{3}H]-naloxone binding at 12 mM Na^+ than at 118 mM Na^+, raises the question of the level of the Na^+ concentration at the receptor sites. However, a comparison of the IC_{50} values obtained at 0 and 12 mM Na^+ cannot be used as evidence in view of the enhancement of naloxone binding at 12 mM Na^+ which is probably due to an increase in affinity (Simon *et al.*, 1975; Lee *et al.*, 1977) rather than an increase in the number of binding sites (Pert & Snyder, 1974). Moreover, since the conditions used for assaying inhibition of binding and depression of muscular contractions are different, caution has to be exercised when comparing the IC_{50} values obtained in the two systems.

Finally, attention is drawn to fentanyl which is much more potent in inhibiting the contractions of the guinea-pig ileum than could be expected from its potency in inhibiting [^{3}H]-naloxone binding. There are several possible explanations for this discrepancy. Firstly, in the guinea-pig ileum fentanyl may be converted to a compound with a higher affinity for opiate receptors, a process which may not occur in brain homogenates. Secondly, as a highly lipophilic substance it may be actively concentrated in the biophase of the guinea-pig ileum (Wüster & Herz, 1976) but possibly not the brain homogenate. Lastly, it may interact with receptors other than the μ-receptors. No decision can be taken on the evidence available at present.

Supported by grants from the Medical Research Council, the U.S. National Institute on Drug Abuse (DA 00662) and the U.S. Committee on Problems of Drug Dependence. F.M.L. was a MRC Research Student. Gifts of the drugs mentioned in Methods are gratefully acknowledged.

References

CREESE, I. & SNYDER, S.H. (1975). Receptor binding and pharmacological activity of opiates in the guinea-pig intestine. *J. Pharmac. exp. Ther.*, **194,** 205–219.

GILBERT, P.E. & MARTIN, W.R. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent spinal dog. *J. Pharmac. exp. Ther.*, **198,** 66–82.

HUGHES, J., KOSTERLITZ, H.W. & LESLIE, F.M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmac.*, **53,** 371–381.

HUTCHINSON, M., KOSTERLITZ, H.W., LESLIE, F.M., WATERFIELD, A.A. & TERENIUS, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmac.*, **55,** 541–546.

KOSTERLITZ, H.W., HUGHES, J., LORD, J.A.H. & WATERFIELD, A.A. (1976). *Reported to the 38th Meeting of the Committee on Problems of Drug Dependence.*

KOSTERLITZ, H.W. & LESLIE, F.M. (1977). Inhibition of [^{3}H]-naloxone binding by opiate agonists. *Br. J. Pharmac.*, **59,** 478P.

KOSTERLITZ, H.W., LORD, J.A.H. & WATT, A.J. (1972). Morphine receptor in the myenteric plexus of the guinea-pig ileum. In *Agonist and Antagonist Actions of Narcotic Analgesic Drugs.* ed. Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E. pp. 45–61. London: Macmillan.

KOSTERLITZ, H.W., LYDON, R.J. & WATT, A.J. (1970). The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39,** 398–413.

KOSTERLITZ, H.W. & WATERFIELD, A.A. (1975). In vitro models in the study of structure-activity relationships of narcotic analgesics. *A. Rev. Pharmac.*, **15,** 29–47.

KOSTERLITZ, H.W., WATERFIELD, A.A. & BERTHOUD, V. (1974). Assessment of the agonist and antagonist properties of narcotic analgesic drugs by their actions on the morphine receptor in the guinea-pig ileum. In *Narcotic Antagonists. Advances in Biochemical Psycholpharmacology*, vol. 8, ed. Braude, M.C., Harris, L.S. May, E.L., Smith, J.P. & Villarreal, J.E. pp. 319–334. New York: Raven Press.

KOSTERLITZ, H.W. & WATT, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists, with particular reference to N-allylnoroxymorphone (naloxone). *Br. J. Pharmac. Chemother.*, **33,** 266–276.

LEE, C.-Y., AKERA, T. & BRODY, T.M. (1977). Effects of Na^+, K^+, Mg^{++} and Ca^{++} on the saturable binding of [^{3}H]-dihydromorphine and [^{3}H]-naloxone *in vitro*. *J. Pharmac. exp. Ther.*, **202,** 166–173.

MARTIN, W.R. (1967). Opioid antagonists. *Pharmac. Rev.*, **19,** 463–521.

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 nondependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197,** 517–532.

PERT, C.B. & SNYDER, S.H. (1973). Properties of opiate-receptor binding in rat brain. *Proc. natn. Acad. Sci. U.S.A.*, **70,** 2243–2247.

PERT, C.B. & SNYDER, S.H. (1974). Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmac.*, **10,** 868–879.

PERT, C.B., SNYDER, S.H. & MAY, E.L. (1976). Opiate receptor interactions of benzomorphans in rat brain homogenates. *J. Pharmac. exp. Ther.*, **196,** 316–322.

RANG, H.P. (1964). Stimulant actions of volatile anaesthetics on smooth muscle. *Br. J. Pharmac. Chemother.*, **22,** 356–365.

SIMON, E.J., HILLER, J.M. & EDELMAN, I. (1973). Stereospecific binding of the potent narcotic analgesic

^{3}H-etorphine to rat brain homogenates. *Proc. natn. Acad. Sci. U.S.A.*, **70,** 1947–1949.

SIMON, E.J., HILLIER, J.M., GROTH, J. & EDELMAN, I. (1975). Further properties of stereospecific opiate binding sites in rat brain: on the nature of the sodium effect. *J. Pharmac. exp. Ther.*, **192,** 531–537.

SWAIN, H.H. & SEEVERS, M.H. (1974). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence*, **36,** Addendum 1168–1195.

SWAIN, H.H. & SEEVERS, M.H. (1976). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence*, **38,** Addendum 2, 768–787.

VILLARREAL, J.E. & SEEVERS, M.H. (1972). Evaluation of new compounds for morphine-like physical dependence in the rhesus monkey. *Bull. Problems Drug Dependence*, **34,** Addendum 7, 1040–1053.

WÜSTER, M. & HERZ, A. (1976). Significance of physicochemical properties of opiates for *in vitro* testing. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W. pp. 447–450. Amsterdam: North-Holland Publishing Co.

(*Received May 8, 1978.*
Revised July 3, 1978.)