IN VITRO MODELS IN THE STUDY OF STRUCTURE-ACTIVITY RELATIONSHIPS OF NARCOTIC ANALGESICS

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INTRODUCTION

The structure-activity relationships of narcotic analgesics have attracted a great deal of attention over many years and there have been a number of reviews (1–8) on this subject. The parameter used in most investigations has been the analgesic or antinociceptive effects of the narcotic analgesics. Whereas drugs with mainly agonist action can be readily assessed by any of the many tests used, this is not the case for drugs with dual agonist and antagonist activities. These latter drugs show little or no antinociceptive activity in the mouse hot-plate and the rat tail-pressure tests and give inconsistent results in the mouse and rat tail-flick tests. The electric tail-shock test in the mouse and the writhing tests respond to analgesics with dual agonist and antagonist actions but the latter are prone to give "false positives" (9). The antagonist potencies of pure antagonists or drugs with dual action are readily determined in rodents or in morphine-dependent monkeys (8).

In view of the complexities of the problem, it appeared desirable to develop in vitro models in which the pharmacological actions of these drugs could be studied. The brain and spinal cord seemed too complex for this purpose, a view recently borne out by receptor-binding studies which showed that only a relatively small number of neurons in the central nervous system are affected by morphine-like drugs and that these morphine-sensitive neurons are very unevenly distributed (10).

An examination of the action of morphine on neuronal junctions outside the central nervous system has shown that it depresses impulse transmission at certain junctions of the autonomic nervous system; these sites are the exception rather than the rule and they are not characteristic of either species or organ or tissue. The

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earliest observation was that by Trendelenburg (11) in 1917, who showed that the peristaltic reflex elicited by distention of the lumen of the isolated ileum of the guinea pig is inhibited by low concentrations of morphine; in the rabbit, however, this reflex is not inhibited by morphine (12). Depression of impulse transmission in the electrically stimulated ileum is due to a reduction in acetylcholine release (13); in the rabbit, morphine does not reduce transmitter release (14). Other morphine-sensitive cholinergic junctions are those that mediate bradycardia caused by vagal stimulation in the rat and rabbit; they are unaffected by morphine in the guinea pig and cat (15).

Two morphine-sensitive adrenergic junctions have been found, namely in the nictitating membrane of the cat (16, 17) and in the vas deferens of the mouse but not in the rat, guinea pig, or rabbit (18). The adrenergic junctions in the sinoauricular node of the cat (15) and the myenteric plexus of the guinea pig are not affected by morphine (19).

The value of a model for research into the mode of action of morphine-like drugs at a cellular and molecular level can be assessed from its ability to predict the potencies of these compounds. Because many of these drugs have agonist and antagonist properties, a prediction of the relative agonist and antagonist activities is also required. In the whole animal or in man the agonist activities become evident as analgesia, depression of respiration, euphoria, and the other clinical manifestations observed after administration. Antagonist activities are measured in the whole animal or man by the reversal of the action of morphine-like drugs or by the induction of withdrawal when there is chronic dependence on morphine-like drugs. At present, the most promising models are, first, the coaxially stimulated ileum of the guinea pig, second, the electrically stimulated preparation of the mouse vas deferens, and, third, the stereospecific binding capacity of brain homogenates.

This review falls into two parts. The first deals with the validity of the selected models, and the second with the results obtained regarding structure-activity relationships.

PREDICTIVE VALUE OF IN VITRO MODELS

The Myenteric Plexus of Guinea Pig Ileum

The cholinergic junctions of the myenteric plexus of guinea pig ileum are very suitable for the assay of the agonist and antagonist properties of narcotic analgesic drugs (20–22). Agonist activities are measured by the depression of the contraction of the longitudinal muscle evoked by coaxial electrical stimulation; the relative potencies are compared to the available clinical data on analgesia in man. Antagonist activities are estimated by the antagonist effects on the depression of the longitudinal contraction by standard concentrations of morphine or normorphine; the antagonist potencies are compared to the potencies of the compounds to cause withdrawal symptoms in the morphine-dependent monkey as reported by the Pharmacology Department of the University of Michigan.

Narcotic analgesic drugs can be grouped as compounds with mainly agonist action, as compounds with mainly antagonist action, and as compounds with dual

agonist, and antagonist, actions. For the purpose of a comparison between potency in human analgesia and agonist potency in guinea pig ileum, drugs with agonist or with dual action belonging to different chemical classes and of widely differing potency have been used.

AGONIST POTENCIES When the relative potencies for human analgesia are plotted against the relative agonist potencies in guinea pig ileum, a linear relationship is obtained (Figure 1). For the calculation of the correlation coefficient and the regression equation, the value for codeine has been omitted because most of its action in man may be due to biotransformation to morphine (33). The predictive value of guinea pig ileum is obviously very high over the whole potency spectrum, which comprises five orders of magnitude. This finding is the more remarkable because there are considerable uncertainties in the assessment of analgesia in human patients in whom variations in distribution, metabolism, and excretion may have a modifying influence. These factors may be a possible explanation for the deviation of the slope of the regression line from unity.

It is of interest that the relative agonist potencies of compounds with dual agonist, and antagonist actions are also highly correlated with the analgesic potencies in man, although it cannot be excluded that such compounds may have a greater agonist activity in the guinea pig ileum than in human analgesia. The only compound that is considerably more potent in guinea pig ileum than in man is levallorphan; it should be noted, however, that estimates of the analgesic action of this drug in man have not been very satisfactory.

ANTAGONIST POTENCIES For the comparison of the antagonist effects, compounds with dual agonist and antagonist actions and antagonists with only negligible agonist component (naloxone, naltrexone, GPA 2163, and Mr 1256) have been used. The correlation between the relative antagonist potencies in guinea pig ileum and those in the morphine-dependent monkey is very close, and the slope of the regression line does not differ from unity (Figure 2).

Correlation between Affinity in Brain Homogenates and Potency in Guinea Pig Ileum

From the results reported so far it would appear that the morphine-receptor in guinea pig ileum is very similar to the receptor that mediates analgesia in man. This view is supported by the good correlation between the potencies of antagonists to reduce stereospecific naloxone binding in brain homogenates (ED_{50}) obtained by Pert, Pasternak & Snyder (42) in the presence of 100 mM NaCl and the dissociation equilibrium constants (K_e) which are the reciprocals of the affinity constants and measure the antagonist potencies in the myenteric plexus of guinea pig ileum (20, 21) (Figure 3). Because with agonist drugs the dissociation equilibrium constants cannot be used, the relative potencies have been calculated from the concentrations giving a 50% depression of the evoked twitch of the longitudinal muscle of guinea pig ileum (ID_{50}) and the values causing a 50% reduction in stereospecific naloxone binding (ED_{50}) and compared with each other (Figure 4). Although the latter

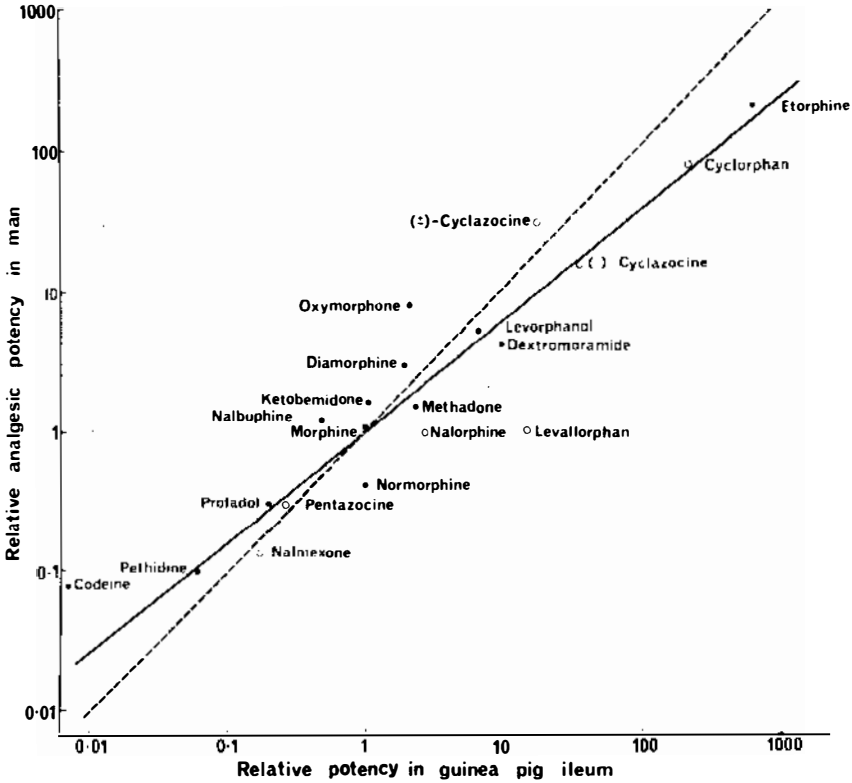


Figure 1 Correlation between the relative agonist potencies of narcotic analgesics in guinea pig ileum and in analgesia in man (morphine = 1). The effects in segments of guinea pig ileum are measured by the depression of the isometric contraction of the longitudinal muscle evoked by coaxial stimulation (0.1 Hz, 0.5 ms, supramaximal voltage). The values are plotted on a logarithmic scale. Correlation coefficient without codeine, $r = 0.926$ ($n = 19$). The solid line has been drawn from $\log y = 0.79 \log x - 0.03$, the slope being different from unity ($P < 0.02$); the interrupted line has a slope of 1. The slopes for the compounds with (○) and without (●) antagonist activity do not differ, and the apparent shift of the values of the drugs with antagonist activity is not statistically significant. Sources of values: references 20–32. Nalmexone is N-dimethylallyl-7,8-dihydro-14-hydroxymorphinone and nalbuphine N-cyclobutylmethyl-7,8-dihydro-14-hydroxymorphine.

values were obtained by Pert & Snyder (43) in the absence of NaCl and therefore are uniformly too high (42), the correlation between the relative values obtained by the two methods is surprisingly good in view of the fact that the values have been obtained from different species, different tissues, and by completely different experimental procedures. In particular, the low affinity for codeine (0.035% of morphine) is of interest; in guinea pig ileum, codeine has only 0.7% of the potency of

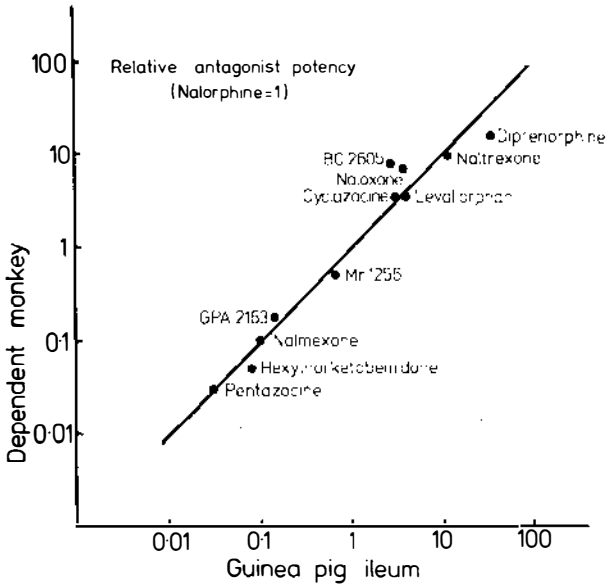


Figure 2 Correlation between the relative antagonist potencies of narcotic analgesics in guinea pig ileum and morphine-dependent monkey (nalorphine = 1). The effects in guinea pig ileum are measured by their antagonism of the depressant action of morphine or normorphine on the contraction of the longitudinal muscle evoked by coaxial stimulation as in Figure 1. The values in the monkey are the amounts causing withdrawal (Department of Pharmacology, University of Michigan). The values are plotted on a logarithmic scale. Correlation coefficient, $r = 0.974$ ($n = 11$). The line has been drawn from $\log y = 0.96 \log x + 0.03$. Sources of values: references 20–22, 31, 34–41. GPA 2163 is (–)-β-2-propargyl-5-phenyl-9-methyl-2'-hydroxy-6,7-benzomorphan, Mr 1256 (±)-α-2-(3-furylmethyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan and BC 2605, or oxilorphan, (±)-N-cyclopropylmethyl-3,14-dihydroxymorphinan.

morphine, although in human analgesia the corresponding value is 8%. The affinity of levallorphan is 7 times higher than that of morphine and, in guinea pig ileum, levallorphan is 16 times more potent than morphine; in human analgesia, however, levallorphan and morphine appear to be equipotent. It is tempting to suggest that, for these two drugs, the values obtained in man are modified by factors that are independent of their action on the receptor. In the case of codeine, this modification may be due to biotransformation of codeine to morphine (33) but there is no explanation for the discrepancy obtained with levallorphan.

The Mouse Vas Deferens

Another recently developed model is the mouse vas deferens (18). As far as agonists without significant antagonist properties are concerned, the relative agonist potencies show over a wide range a good correlation with the values obtained on guinea pig ileum (44).

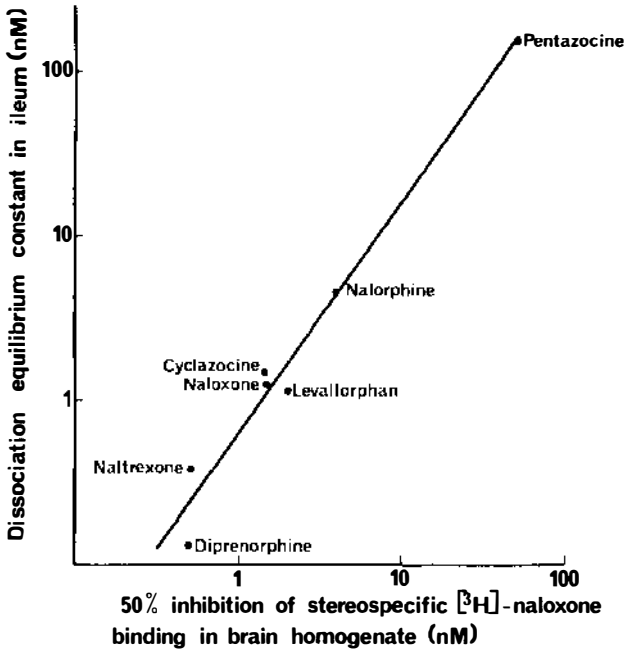


Figure 3 Correlation between the potencies to reduce stereospecific naloxone binding in brain homogenates (42) and the dissociation equilibrium constants measuring antagonist potency in guinea pig ileum (20, 21). Abscissa, concentration (ED_{50}) required to cause 50% inhibition in stereospecific binding (x); ordinate, dissociation equilibrium constant (K_e), i.e. concentration producing a dose-ratio of 2 (y). The values (nM) are plotted on a logarithmic scale. Correlation coefficient, $r = 0.986$ ($n = 7$). The line has been drawn from $\log y = 1.41 \log x - 0.21$.

Drugs with dual agonist, and antagonist action, such as nalorphine and levallorphan, can be readily assessed for their agonist action in guinea pig ileum. Provided the interval between exposure to the different concentrations of these drugs is long enough, dose-response curves with slopes similar to that of morphine are obtained (45). On the other hand, in the mouse vas deferens, dose-response curves for nalorphine or levallorphan are parallel to the abscissa or concave downwards, even when the interval between exposures is prolonged (44). For the determination of the agonist action of drugs with dual action, the lowest concentration that gives a depression of the electrically evoked response, has to be used for the calculation of the agonist potency to obtain a good correlation with the values found in guinea pig ileum.

In the assay of antagonist potencies of compounds with dual action in guinea pig ileum the limiting factor is the ratio of agonist to antagonist activity. If this ratio

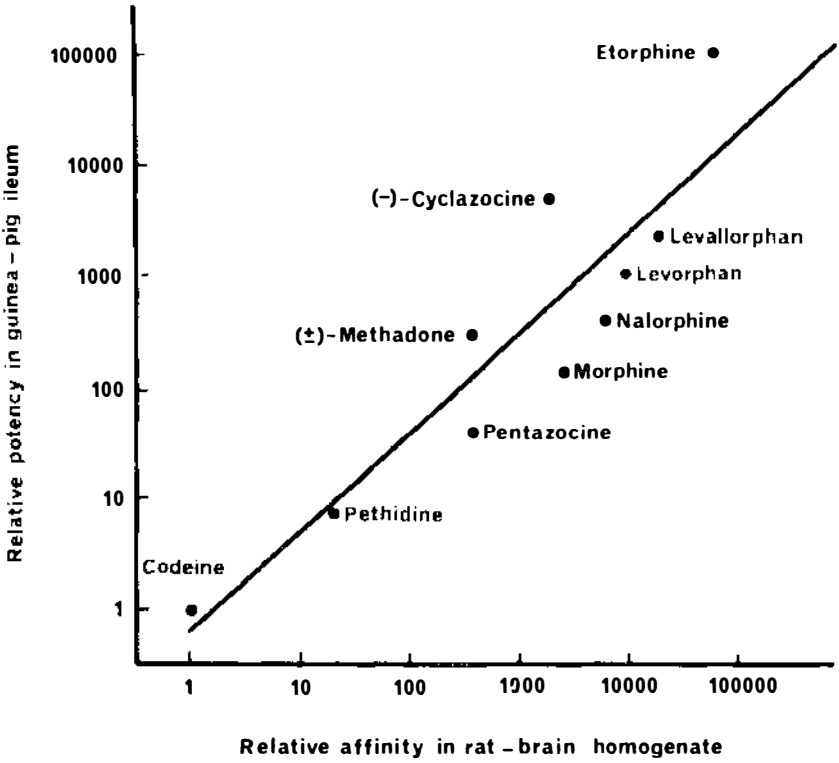


Figure 4 Correlation between the relative potencies to reduce stereospecific naloxone binding in brain homogenate (43) and the relative agonist potencies in guinea pig ileum (codeine = 1). The values are plotted on a logarithmic scale. Correlation coefficient, $r = 0.905$ ($n = 10$). The line has been drawn from $\log y = 0.89 \log x - 0.20$, the slope not being significantly different from 1.

is large, the assessment of the antagonist potency may become impossible because the contraction of the longitudinal muscle is severely depressed before antagonist activity can be discerned. Because of the very flat dose-response curve of such compounds in the mouse vas deferens, much higher concentrations can be used than in guinea pig ileum and an assessment of the antagonist potency be made. For this reason, the antagonist potency of cyclorphan can be determined only in the mouse vas deferens where it was found to be a more potent antagonist than nalorphine in agreement with the tail-flick test of the rat (46).

The mouse vas deferens has not been used extensively to date but promises to be particularly useful for studies of structure-activity relationships of compounds that are antagonists with a strong agonist component.

STRUCTURE-ACTIVITY RELATIONSHIPS IN GUINEA PIG ILEUM

In view of the very good correlation between values obtained in guinea pig ileum and values found in man, the morphine-dependent monkey, and in affinity studies, reference to data from tests performed in vivo are made only in exceptional circumstances.

Effects of Changing the Tertiary N to Secondary or Quaternary N

NOR-COMPOUNDS In general, changing the tertiary N atom with a methyl side chain to a secondary N atom reduces the agonist potency by 80% or more. This has been shown for phenylpiperidines (meperidine or pethidine, bemidone, ketobemidone) and for the benzomorphan, metazocine (31, 47). On the other hand, norcodeine is equipotent with codeine (31), and it has been shown that normorphine is equipotent with morphine in both the guinea pig ileum (21) and the mouse vas deferens (44). The relative agonist potencies of pairs of compounds with tertiary and secondary N atoms are shown in Table 1. The results are in general agreement with the findings obtained in tests in vivo (1–8).

With regard to normorphine, the situation is complicated by the fact that the diffusion barriers found in vivo appear to exist to a very much smaller extent in vitro. When normorphine is injected intravenously in mice, it has only 10% of the an-

Table 1 Relative agonist potencies of nor-compounds in the myenteric plexus of guinea pig ileum (21, 31, 47)

Compound	Number of observations	Relative agonist potency (normorphine or morphine = 1)[a]
Normorphine	6	1.00 ± 0.10
Codeine	5	0.0091 ± 0.0007
Norcodeine[b]	5	0.0088 ± 0.009
Pethidine	4	0.059 ± 0.008
Norpethidine[b]	4	0.0070 ± 0.0007
(±)-Metazocine[b]	5	0.40 ± 0.05
(±)-Normetazocine[c]	5	0
Bemidone[d]	3	0.079 ± 0.009
Norbemidone[d]	3	too low to assay
Ketobemidone[d]	4	1.04 ± 0.10
Norketobemidone[d]	4	0.19 ± 0.03

[a] The values are the means and their standard errors. (-)-Metazocine (α-2,5,9-trimethyl-2'-hydroxy-6,7-benzomorphan) is twice as potent as the racemate, the (+)-isomer being inactive. Some of the nor-compounds have weak antagonist activity: normetazocine 2.3%, norbemidone 0.6%, and norketobemidone 1.9% of the activity of nalorphine.

[b] Dr. E. L. May.

[c] Sterling-Winthrop.

[d] C. H. Boehringer Sohn, Ingelheim.

tinociceptive activity of morphine; after intracisternal injection, however, normorphine is rather more active than morphine (48). It would appear therefore that the decreased activity of normorphine is due to interference with the passage across the blood-brain barrier. As far as interaction at the receptor site is concerned, the presence of a tertiary N atom is not an absolute requirement; under certain yet undefined circumstances, a secondary N atom can replace it.

QUATERNARY COMPOUNDS The quaternary analogs of morphine and nalorphine lose their agonist activity (21, 47). On the other hand, the antagonist potency is not reduced to the same extent; for instance, 12% of that of nalorphine is retained (47). Thus, the effective antagonist potency (P_a) of the quaternary compound is much higher than that of the tertiary parent compound (Table 2).

Importance of Lipophilic Properties

The reduction in agonist potency caused by quaternization is at least partly due to the decrease in the lipophilic property of such compounds. On the other hand, a high degree of lipid solubility is of lesser importance in in vitro models than in tests on the whole animal where the blood-brain barrier has to be passed. In support of this argument, it has been found that the ratio of the potency of lipophilic to that of hydrophilic compounds is higher in the whole animal than in guinea pig ileum (21).

Stereospecificity

As in the whole animal, the morphine receptors in guinea pig ileum exhibit strict stereospecificity. For instance, the agonist and antagonist potencies of α-5,9-dimethyl-2'-hydroxy-2-cyclopropylmethyl-6,7-benzomorphan and of its β-diastereoisomer reside in the (–)-isomers of each pair. The same is true for the α- and β-diastereoisomers of 5,9-diethyl-2'-hydroxy-2-methyl-6,7-benzomorphan (21). A similar finding has been obtained for the ketocyclazocines (22).

Diastereoisomers have been studied in the 4-phenylpiperidine and in the benzomorphan series. In general, good agreement has been obtained between the results on the guinea pig and tests on the whole animal (21).

STRUCTURE IN RELATION TO AGONIST AND ANTAGONIST ACTIVITIES

Unsaturated Three-Carbon and Cyclic Side Chains at the N Atom

MORPHINES, MORPHINANS, AND BENZOMORPHANS In the morphine and morphinan series, replacement of the N-methyl by an N-allyl group produces compounds in which both agonist and antagonist activities are increased, the latter more than the former. This results in a compound with dual agonist and antagonist actions (Table 3).

The agonist and antagonist potencies of a series of derivatives of β-5-phenyl-2'-hydroxy-6,7-benzomorphans are given in Table 4. When the compounds having a methyl group at C_9 are compared, the replacement of the methyl group at the N atom by the unsaturated three-carbon chains, propargyl, chloropropenyl, or allyl

Table 2 Effects of quaternization on agonist and antagonist activities of morphine and nalorphine (21, 47)

Compound	Number of observations	ID_{50}[a] (n*M*)	K_e[a] (n*M*)	P_a (ID_{50}/K_e)	Relative agonist potency (morphine = 1)	Relative antagonist potency (nalorphine = 1)
Morphine hydrochloride	6	68.2 ± 15.0	87.5 ± 18.1	0.8	1	-
Dimethylnormorphinium chloride	4	2430 ± 227	1823 ± 189	1.3	0.028	-
Nalorphine hydrobromide	6	24.3 ± 1.3	4.47 ± 0.59	5.3	2.8	1
Diallylnormorphinium iodide[b]	4	3188 ± 1240[c]	37.2 ± 3.2	86	0.021	0.12

[a]The values are the means and their standard errors. ID_{50} is a measure of agonist activity and K_e of antagonist activity (20).

[b]FR 13-J (C. H. Boehringer Sohn, Ingelheim).

[c]The slope of the dose-response curve is shallow.

Table 3 The effects of replacement of N-methyl by N-allyl in morphine and morphinan (Reproduced with permission from reference 20).

	ID_{50}[a] (n*M*)	K_e[a] (n*M*)	P_a (ID_{50}/K_e)
Morphine	68.2 ± 15.0	87.5 ± 18.1	0.8
Nalorphine	24.3 ± 1.3	4.47 ± 0.59	5.4
Levorphanol	9.18 ± 1.05	7.04 ± 1.48	1.3
Levallorphan	4.28 ± 1.64	1.12 ± 0.23	3.8

[a]The values are the means ± S.E. of 6 observations.

yields compounds that have no significant agonist activity; at the same time, the dissociation equilibrium constant, K_e, which is the reciprocal of the affinity constant and measures antagonist activity, is affected only to a minor degree. These modifications at the N atom therefore abolish agonist activity without changing the affinity to the receptor, thus yielding more or less pure antagonists. Substitution of the methyl group or of the unsaturated three-carbon chains at the N atom by cyclopropylmethyl, and to a lesser extent by cyclobutylmethyl, decreases the value of K_e and therefore increases the affinity to the receptor. The agonist activity, however, increases with cyclopropylmethyl and even more so with cyclobutylmethyl.

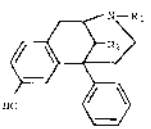
It is of interest that the presence of a methyl group at C_9 in the β-configuration (*trans* phenyl at C_5/methyl at C_9) reduces agonist activity compared to compounds without substitution at C_9. The significance of this observation is discussed later.

Similar findings have been obtained in the 14-hydroxydihydromorphinone series (21). Replacement of N-methyl by allyl causes an almost complete loss of agonist

Table 4 The effects of changes in the N-alkyl and the C_9 groups in β-5-phenyl-9-R_2-2-R_1-2'-hydroxy-6,7-benzomorphans[a] (Reproduced with permission from reference 21)

R_1	R_2	Isomer	Number of observations	ID_{50}[b] (n*M*)	K_e[b] (n*M*)	P_a (ID_{50}/K_e)
Methyl	CH_3	-	6	57.5 ± 1.4	29.2 ± 6.7	2
Propargyl	CH_3	-	5	infinite	31.3 ± 5.4	infinite
Propargyl	H	±	6	590 ± 136	29.6 ± 2.4	20
3-Chloro-2-propenyl	CH_3	-	6	infinite	26.3 ± 4.3	infinite
3-Chloro-2-propenyl	H	+	9	75.3 ± 11.8	25.3 ± 5.8	3
Allyl	CH_3	-	8	infinite	19.8 ± 3.9	infinite
Cyclopropylmethyl	CH_3	-	6	64.1 ± 15.3	2.67 ± 0.63	24
Cyclopropylmethyl	H	-	6	8.3 ± 1.2	4.11 ± 0.80	2
Cyclobutylmethyl	CH_3	-	5	15.5 ± 2.7	5.50 ± 0.89	3

[a]Geigy Pharmaceuticals. Structure:



[b]The values are the means ± S.E.

Table 5 Effects of substitution of the N-methyl group in N-R-14-hydroxy-7,8-dihydromorphinone[a] (Reproduced with permission from reference 21)

R	Number of observations	ID_{50}[b] (n*M*)	K_e[b] (n*M*)	P_a (ID_{50}/K_e)
Methyl[c] (oxymorphone)	4	12.1 ± 2.4	11.2 ± 2.5	1.1
Allyl (naloxone)	6	infinite	1.22 ± 0.03	infinite
Cyclopropylmethyl (naltrexone)	6	d	0.38 ± 0.07	very large
Cyclobutylmethyl	6	60.0 ± 17.2	3.0 ± 0.45	20

[a]Endo Laboratories.
[b]The values are the means ± S.E.
[c]Unpublished results on authentic sample (Dr. E. L. May) which supersede reference 21.
[d]Maximum inhibition, 25%.

activity with a decrease in K_e and thus an increase in the affinity to the receptor. Cyclopropylmethyl at the N atom causes an even greater increase in affinity but there is a slight return of agonist activity. The cyclobutylmethyl homologue has marked agonist activity (Table 5).

PYRROLIDINES In *m*-(N-R-3-isobutyl-3-pyrrolidinyl) phenols the N-cyclopropylmethyl homologue has more agonist activity than the N-allyl compound (31). This is in agreement with the findings in the benzomorphan, morphinan, and morphinone series. The K_e values, however, are not affected in the pyrrolidinyl compounds whereas in the other series the cyclopropylmethyl homologues show a greater affinity to the morphine receptor than the allyl homologues. These observations are confirmed by tests in rodents (49, 50).

4-PHENYLPIPERIDINES It is well known that introduction of allyl groups in 4-phenylpiperidine derivatives does not convey antagonist activity. Recently, the allyl derivatives of alphaprodine and betaprodine (N-methyl-3 (α or β)-methyl-4-phenyl-4-propionyloxy piperidine) have become available (47). None of them has antagonist activity. The relative agonist potencies for morphine = 1 are the following: alphaprodine 0.12, allyl homologue 0.15, betaprodine 0.51, allyl homologue 0.35. Thus, replacement of methyl by allyl has little effect in this series.

Importance of Substitution at C_{14} in Morphines and Morphinans

The allyl homologue of the oxymorphone series, naloxone, has no significant agonist activity, whereas the analogous compound of the morphine series, nalorphine, shows both agonist and antagonist activities. Notwithstanding the presence of an oxo-group at C_6 and the saturation of the C_7–C_8 double bond in naloxone, the more important difference between naloxone and nalorphine is the OH group attached to C_{14}. In this series, C_{14} corresponds to C_9 in in the benzomorphan series where it has been shown above that methyl substitution is important for the suppression of agonist activity.

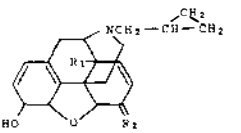
This view is supported by a comparison of N-cyclopropylmethyl morphine derivatives (31), which are shown in Table 6. The agonist activity (ID_{50}) of the cyclopropylmethyl homologue is very much greater than that of morphine itself; it overshadows its antagonist properties (K_e). Conversion of the –OH at C_6 to =O and saturation of the C_7-C_8 bond increases both agonist and antagonist activities, the latter somewhat more than the former. If now an OH group is introduced at C_{14} to give naltrexone, the agonist properties are almost abolished, whereas there is only a small increase in K_e indicating a small loss in affinity. These findings suggest that replacement of N-methyl by N-cyclopropylmethyl causes a decrease in K_e from 68 to 0.8, but this very considerable increase in the affinity to the receptor does not yield by itself a potent antagonist without marked agonist activity. The important change in the molecule occurs when –OH is introduced at C_{14}. The relationship between N-cyclopropylmethylnormorphine and naltrexone is mirrored by the allyl analogs, nalorphine and naloxone.

In the morphinan series, introduction of an OH group at C_{14} reduces the agonist activity of cyclorphan by 89%, thus converting it to a compound (BC 2605) that

Table 6 Assessment of derivatives of N-cyclopropylmethylnormorphine (31)[a]

R_1	R_2	Number of observations	ID_{50}[b] (n*M*)	K_e[b] (n*M*)	P_a (ID_{50}/K_e)	Agonist potency (normorphine = 1)	Antagonist potency (nalorphine = 1)
H		4	2.16 ± 0.14	0.80 ± 0.11	2.7	34	5.6
H	=O	4	1.13 ± 0.15	0.21 ± 0.01	5.5	59	21
OH	=O	6	>850	0.38 ± 0.07	>2200	<0.08	11.8

[a]Structure:

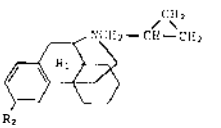


[b]The values are the means and their standard errors. The first two compounds were supplied by Dr. M. D. Gates and the third by Endo Laboratories (naltrexone); the last two compounds are 7,8-dihydro derivatives.

Table 7 The effect of an OH group at C_{14} (R_1) in (–)-N-cyclopropylmethyl-3-hydroxy-(R_2)-morphinan (31, 51)[a]

R_1	Number of observations	ID_{50}[b] (n*M*)	K_e[b] (n*M*)	P_a (ID_{50}/K_e)	Agonist potency (morphine = 1)	Antagonist potency (nalorphine = 1)
H (cyclorphan	4	0.28 ± 0.04	0.61 ± 0.33	0.5	240	-
OH (BC 2605)	11	2.53 ± 0.65	0.86 ± 0.13	3.0	33	5.2

[a]Structure:



[b]The values are the means ± S.E. Cyclorphan was provided by Dr. M. D. Gates and BC 2605 (oxilorphan) by Bristol Laboratories.

possesses effective antagonist potency (Table 7). Esterification of the phenolic 3-OH group with nicotinic acid (BC 2888) reduces the agonist potency by a further 4%, so that only 6.5% of the agonist activity of cyclorphan is retained; the value of K_e, and therefore the affinity to the receptor, remains unchanged: 0.61 nM for cyclorphan, 0.86 nM for BC 2605, and 0.71 nM for BC 2888 (31). As in the benzomorphan and morphinone series, replacement of cyclopropylmethyl by cyclobutylmethyl to give butorphanol (BC 2627) increases the K_e value, i.e. decreases affinity to the receptor (31).

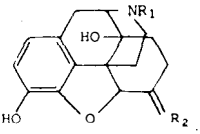
Changes in the Naloxone and Naltrexone Structures

Changes at C_6 influence the K_e values and therefore the affinities of naloxone and naltrexone without introducing agonist activity (Table 8). A change from =O to $=CH_2$ has little effect but the change to $<\frac{CH_3}{OH}$ reduces affinity very much. It is of interest that, so far, the affinity of naloxone has not been increased by changes in the molecule other than at the N atom.

Replacement of N-allyl by N-3-furylmethyl (C. H. Boehringer Sohn, Ingelheim, Mr 1767-Ms) reduces the antagonist potency of naloxone by about 50% without introducing agonist activity (31). As shown below, this particular side chain produces potent antagonists with little agonist activity also in the benzomorphan series.

Table 8 Effects of changes at C_6 (R_2) on the antagonist activities of naloxone and naltrexone derivatives (31, 47)[a]

R_1	R_2	Number of observations	K_e[b] (nM)	Antagonist potency (naloxone = 1)
allyl	=O (naloxone)	6	1.22 ± 0.03	1
allyl	$=CH_2$	4	3.00 ± 0.46	0.41
allyl	$<\frac{CH_3}{OH}$	5	21.1 ± 3.7	0.06
allyl		3	57.1 ± 6.4	0.02
cyclopropylmethyl	=O (naltrexone)	6	0.38 ± 0.07	3.2
cyclopropylmethyl	$=CH_2$	3	0.37 ± 0.02	3.3
cyclopropylmethyl	$<\frac{CH_3}{OH}$	5	1.73 ± 0.28	0.7

[a]Structure: 

[b]The values are the means ± S.E. Compounds supplied by Dr. J. Fishman.

Table 9 Agonist and antagonist properties of (±)-α-5,9-dimethyl-2-furyl (R)-2'-hydroxy-6,7-benzomorphans (31)

R	Number of observations	ID_{50}[a] (n*M*)	K_e[a] (n*M*)	Relative agonist potency (normorphine = 1)
	6	90–570[b]	6.83 ± 0.84	–
	10	10.7 ± 1.0[c]	10.4 ± 1.8	6.2 ± 0.5
	6	50.8 ± 9.2	–	1.50 ± 0.15
	6	11.8 ± 1.2	–	5.6 ± 0.5

[a]The values are the means and their standard errors. The number of observations are given in brackets. The code numbers of the compounds supplied by C. H. Boehringer Sohn, Ingelheim, are from above downwards Mr 1256-Ms, Mr 1029-Ms, Mr 1268-Ms, Mr 1353-Ms. The dose ratios for normorphine concentrations causing inhibitions of the twitch by 30–50% were as follows: Mr 1029, 2.00 ± 0.34; Mr 1268, 1.02 ± 0.12; and Mr 1353, 0.94 ± 0.05.

[b]Shallow dose-response curves: ID_{50} correlated to concentration: Mr 1256, $r = 0.905$ (maximum inhibition, 30%).

[c]Variable slopes of dose-response curves.

N-Furylmethyl Benzomorphans

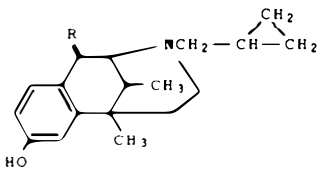
As far as antagonist action is concerned, the N-furylmethyl analogs are the most interesting (Table 9) (31). They have a little residual agonist activity but the dose-response curves are shallow. The homologue (±)-α-5,9-dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan (Mr 1256-Ms) has an antagonist potency of 18% of that of naloxone; 95% of the activity resides in the (–)-isomer. When in the racemate the 5,9-dimethyl is replaced by diethyl (Mr 1302-Cl), the antagonist value becomes 42% of that of naloxone (47). The dose-response curve for agonist activity is shallow and does not reach 50% inhibition. At present, the diethyl derivative is the most potent synthetic antagonist devoid of significant agonist activity. In contrast to these compounds (±)-α-5,9-dimethyl-2-furfuryl-2'-hydroxy-6,7-benzomorphan (Mr 1029-Ms) is an agonist with variable slopes of the dose-response curves; it has only little antagonist activity (31).

The dimethylfuryl homologues, N-(2-methyl-3-furylmethyl) (Mr 1268-Ms) and N-(3-methylfurfuryl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphans are pure agonists of considerable potency (Table 9). These are interesting compounds in that in the morphine-dependent monkey, they neither substitute for morphine nor do they cause a withdrawal syndrome (40, 52).

Table 10 The effects on agonist activity of changes at C_8 in (–)-α-2-cyclopropylmethyl-5-methyl-or-ethyl-9-methyl-8-R-2'-hydroxy-6,7-benzomorphan (Reproduced with permission from references 21, 22)[a]

R	C_5	Number of observations	ID_{50}[b] (n*M*)	Agonist potency (morphine = 1)
$=H_2$ (cyclazocine)	CH_3	6	1.96 ± 0.45	35
=O	CH_3	9	0.77 ± 0.06	89
=O	C_2H_5	7	0.18 ± 0.02	380

[a]Structure:



[b]The values are the means ± S.E. The compounds with an oxo group at C_8 have no antagonist activity. Supplied by Sterling-Winthrop.

Introduction of an =O Group at C_8 in Benzomorphans

α-2-Cyclopropylmethyl-8-oxo-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan, a derivative of cyclazocine, has no antagonist activity in guinea pig ileum; the agonist potency is 2.5 times greater than that of cyclazocine (Table 10). Replacement of CH_3 at C_5 by C_2H_5 causes a further increase in potency, which is now 11 times greater than that of cyclazocine. Neither compound has antagonist activity in the mouse vas deferens (F. M. Leslie, unpublished).

These compounds require about 5 times more naloxone for reversal of their agonist effects than is needed for the reversal of the agonist effects of normorphine (22), an observation similar to that found for the N-dimethylfuryl compounds. As these latter compounds, the 8-oxocyclazocines do not substitute for morphine in the morphine-dependent monkey nor do they cause a withdrawal syndrome (52).

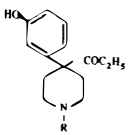
N-Substituted Ketobemidones

The complete series of ketobemidones from N-nor-4-(*m*-hydroxyphenyl)-4-propionylpiperidine to the N-decyl homologue shows two peaks of agonist activity (Table 11), namely when the side chain at the N atom is either methyl or amyl. The nor, butyl, hexyl, and heptyl homologues have an agonist potency of between 10 and 20% of that of ketobemidone ($N–CH_3$). Some of the compounds have weak antagonist activity. The highest effective antagonist potency is exhibited by the hexyl homologue ($P_a = 9.4$) because its agonist potency is very much lower than that of the amyl derivative. These findings are in good agreement with those found in antinociceptive tests in mice and in tests in the morphine-dependent monkey (53). In the series of α-5,9-dimethyl-2-R-2'-hydroxy-6,7-benzomorphans similar peaks of

Table 11 Assessment of N-substituted ketobemidones (47, 47a)[a]

Substitution at N	ID_{50}[b] (nM)	K_e[b] (nM)	P_a[b] (ID_{50}/K_e)	Agonist potency (normorphine = 1)	Antagonist potency (nalorphine = 1)
H (4)	491 ± 173	230 ± 44	2.1	0.19 ± 0.03	0.02
CH_3 (6)	73.3 ± 9.5	115 ± 33	0.6	0.92 ± 0.10	-
C_2H_5 (4)	3650 ± 758	infinite	-	0.016 ± 0.003	
C_3H_7 (4)	953 ± 148	4380 (2)	0.2	0.065 ± 0.01	-
C_4H_9 (5)	416 ± 131	440 ± 60 (3)	0.9	0.19 ± 0.01	-
C_5H_{11} (7)	56.0 ± 13.5	40.8 ± 7.7	1.4[c]	1.24 ± 0.12	?
C_6H_{13} (5)	516 ± 66	54.9 ± 6.6	9.4	0.12 ± 0.02	0.08
C_7H_{15} (5)	518 ± 83	109 ± 22	4.7	0.17 ± 0.02	0.04
C_8H_{17} (4)	950 ± 234	349 ± 59	2.7	0.078 ± 0.009	0.01
C_9H_{19} (3)	3240 ± 1670	3220 ± 1270	1	0.024 ± 0.014	-
$C_{10}H_{21}$ (2)	infinite	infinite	-	-	-

[a]Structure:



[b]The values are the means and their standard errors. The number of observations are given in brackets. The norcompound and ketobemidone were supplied by C. H. Boehringer Sohn, Ingelheim, and the other compounds by Dr. E. L. May.

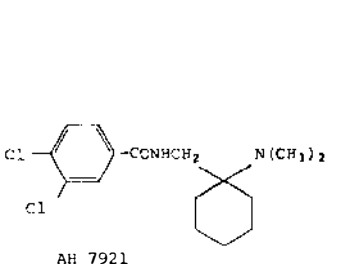
[c]The dose-ratio produced by C_5H_{11} was 2.53 ± 0.53.

agonist activity occur when R is methyl or amyl (54). In guinea pig ileum, the potency of the (±)-methyl homologue of this benzomorphan is 40% and that of the (±)-amyl homologue 48% of morphine (M. Hutchinson, A. A. Waterfield, unpublished). In N-substituted morphines the amyl homologue has an antinociceptive potency similar to that of morphine, whereas the propyl and butyl homologues have very much reduced activity (55). In the guinea pig ileum, the (–)-amyl homologue of the morphinan series is 9 times more potent than morphine (Hutchinson, Waterfield, unpublished), whereas the (–)-methyl homologue, levorphanol, is 7 times more potent (20).

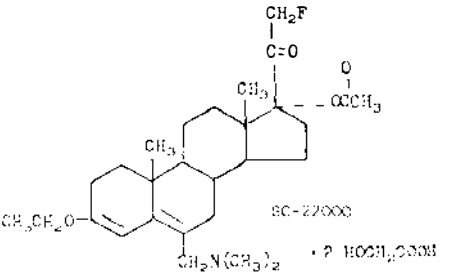
Two Unusual Narcotic Analgesics

In guinea pig ileum, the first one, an N-dimethylamino cyclohexylmethylbenzamide has a potency similar to that of morphine and is devoid of antagonist activity (47; AH 7921, Figure 5*a*). The concentration of naloxone required to antagonize its action is higher than that required for morphine or normorphine. These findings agree with those obtained in rodents, dogs, and monkeys (56).

The second compound is a steroid, 6-dimethylaminomethyl-3-ethoxy-21-fluoro-3,5-pregnadiene-20-one-17α-ol acetate (47; SC-22000, Searle Laboratories; NIH 8607 Figure 5*b*). In guinea pig ileum, its agonist potency is about half that of morphine and readily reversed by naloxone. It has no antagonist activity. These results are in fair agreement with the results obtained with the hot-plate test in which it has one quarter of the potency of morphine, and the observations in the dependent monkey in which it completely suppresses morphine abstinence (57). It is of special interest in that the onset of agonist action is slow (half time of 7 min) and the recovery even slower (half time of 50 min).



(a)



(b)

Figure 5a 3,4-dichloro-(N-dimethylamino)-cyclohexylmethylbenzamide; *b* 6-dimethylaminomethyl-3-ethoxy-21-fluoro-3,5-pregnadiene-20-one-17α-ol acetate.

SUMMARY

Evidence has been adduced for the view that the morphine receptor or receptors in the myenteric plexus of guinea pig ileum are very similar to the brain receptors that mediate the analgesic action of the narcotic analgesics.

Such an in vitro model is very suitable for the study of structure-activity relationships because the agonist and antagonist activities of compounds can readily be assessed. One example is the investigation of the effects of changes at the N atom. Another point of interest is the role of substitutions at the C_{14} atom in morphines and morphinans. Such alterations in structure may have a profound effect on the relative agonist and antagonist activities and may convert a drug with dual agonist and antagonist actions into an antagonist devoid of agonist action.

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