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REVIEW

The enkephalins and opiates: structure-activity relations

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The X-ray structures of 9 'opiate' drugs which exhibit a range of pharmacological activity have been examined in detail leading to the theory that one of the reasons why the enkephalins and related peptides possess morphine-like activity is because they have a tyrosine, and hence a 'tyramine', residue at the amino terminal position. This residue or a conformationally similar moiety, can be shown to be present in many opiates and analogues.

The fact that opiate or analgesic drugs, in general, show a high degree of pharmacological stereoselectivity and in some cases stereospecificity has always been taken as evidence that they must be intereacting with specific receptors in nervous tissue. Recently it has been possible to demonstrate directly the presence of these stereospecific opiate receptors by studying the binding of radioactive agonists and antagonists to both central and peripheral nervous tissue. (Goldstein, Lowney & Pal, 1971; Pert & Snyder, 1973; Simon, Hiller & Edelman, 1973; Terenius, 1973; Creese & Snyder, 1975). This technique together with the development of two other *in vitro* methods, namely the stereospecific inhibitory action of opiate agonists on the electrically induced contractions of the longitudinal muscle of the guinea-pig ileum and the mouse vas deferens (Kosterlitz & Waterfield, 1975) has removed many of the problems of structure-activity studies carried out *in vivo* where factors of drug transport and metabolism may complicate the issue. The question why highly specific receptors should exist for opiates, when these alkaloids do not occur naturally in animals or man remained unanswered. Earlier it had been speculated that the brain might contain an endogenous-morphine like factor (Goldstein, 1973) and this has indeed been shown to be the case.

The enkephalins

Hughes (1975) and Hughes, Smith & others (1975), have isolated and determined the structure of a factor having morphine-like agonist activity from the brain of the pig. This compound, enkephalin, was shown to be a mixture of two related pentapeptides whose sequences were Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu. Methionine-enkephalin is 20 times more active than normorphine in the mouse vas deferens and equipotent with normorphine in the guinea-pig ileum (Hughes & others, 1975). Leucine-enkephalin has half the potency of methionine-enkephalin in the vas deferens and 1/5 the potency in the ileum. In these systems, morphine and normorphine are equiactive, but, normorphine has a quicker onset of action and is easily washed out so it is preferred to morphine. The inhibitory effects of both enkephalins could be completely antagonized by naloxone, and methionine-enkephalin was about 3 times more potent than morphine in blocking the stereospecific binding of [^{3}H]naloxone in Na^+-free homogenates of guinea-pig brain (Hughes & others, 1975). Waterfield, Hughes & Kosterlitz (1976) found morphine and methionine-enkephalin to exhibit cross tolerance in morphine-tolerant mice. In studies of central analgesic activity in rats Belluzzi, Grant & others (1976) found both enkephalins to be similar in potency but that each was less potent than morphine with shorter durations of action. Both these effects could be related to their rapid enzymatic degradation.

Simantov & Snyder (1976) have isolated and identified both enkephalins in bovine brain in which there is 4 times more leucine-enkephalin than methionine-enkephalin which is the reverse of the situation in pig brain. It has also been shown that the regional distribution of enkephalin activity, as measured by a displacement of stereospecific [^{3}H]-opiate binding, largely parallels opiate receptor binding but that there are exceptions (Simantov, Kuhar & others, 1976b). The possibility that enkephalin may be a neurotransmitter or neuromodulator is supported by subcellular fractionation studies in rat brain which show it to be located in synaptosomal fractions that are enriched in nerve terminals (Simantov, Snowman & Snyder, 1976c). The phylogenetic distribution of enkephalin activity

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closely resembles the pattern of [^{3}H]naloxone binding (Simantov, Goodman & others, 1976a).

Microiontophoresis studies of methionine-enkephalin on single neurons in the rat brainstem have shown that its predominant action is to suppress the firing rate of spontaneously active neurons (Bradley, Briggs & others, 1976). Similar effects are obtained with morphine and etorphine, these actions and those of methionine-enkephalin could be blocked by iontophoretically applied naloxone. In the cat brainstem, however, although methionine- and leucine-enkephalin have inhibitory actions similar to those of morphine, none of these effects can be blocked by naloxone (Gent & Wolstencroft, 1976). The receptors here must clearly be different from those in the rat brainstem and from those in peripheral systems where naloxone is a potent antagonist. At the biochemical level, *in vitro* studies with slices of the rat corpus striatum have shown that opiates such as morphine and levorphanol produce an increase in cGMP concentrations whilst dextrorphan was inactive, and also that the morphine effect could be blocked by naloxone (Minneman & Iversen, 1976). Both leucine- and methionine-enkephalin in the presence of the peptidase inhibitor bacitracin also increased cGMP concentrations, although at higher concentrations than the opiates, these effects were also blocked by naloxone.

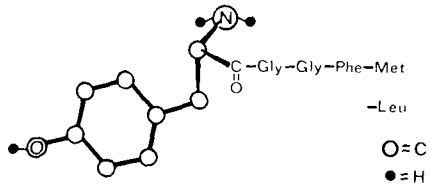
In summary there is a very convincing body of evidence that the enkephalins are endogenous morphine-like factors, this would therefore explain the existence of highly specific 'opiate' receptors in nervous tissue, i.e. they did not occur by chance but are there to interact with an endogenous ligand.

Structural and conformational relations between the opiates and the enkephalins

As the enkephalins and opiate agonists compete for the same receptors and produce the same pharmacological response, it is likely that there are structural and conformational similarities between these two groups of molecules. Superficially, however, this seems unlikely as the enkephalins are peptides and morphine is an alkaloid. However, the discovery that in the primary sequence tyrosine was the residue at the amino terminal position (Hughes & others, 1975) is of considerable significance for understanding why these peptides have morphine-like effects. As tyrosine is in the terminal amino position its nitrogen atom is basic i.e. it can carry a positive charge at physiological pH; if tyrosine were at any other position in the sequence the nitrogen atom would be part of an amide bond and therefore unprotonated at physiological pH. Thus the enkephalins will contain the 'tyramine' moiety of tyrosine as their basic end group and if we make the assumption that this is the primary locus of interaction of these peptides with their receptors this would explain why the 'tyramine' moiety is common to many of the most potent groups of opiate analgesics such as the morphines, morphinans and 6,7-benzomorphans.

In certain cases potent antagonists can be derived from the above groups of agonists by substitution of various bulky groups for the methyl group on the nitrogen atom (Jacobson, May & Sargent, 1970), however, these drugs still retain the 'tyramine' moiety in their structure. Before the amino acid sequence of enkephalin was published, Goldstein, Goldstein & Cox (1975) attempted to deduce by molecular analogy and model building what the primary structure and conformation of a typical opiate-like peptide might be. A linear heptapeptide having the structure Tyr-Gly-Gly-Gly-Lys-Met-Gly was prepared and shown to have a weak morphine-like action. These authors correctly deduced the first three residues from the amino end of the enkephalins but they decided incorrectly that the basic nitrogen atom would be supplied by the lysine residue rather than by the *N*-terminal tyrosine.

Independently of our initial publication (Horn & Rodgers, 1976), and based solely on molecular model building, Bradbury, Smyth & Snell (1976a) have suggested possible similarities between methionine-enkephalin and morphine. However, if one were to base the argument about the similarity of the tyramine moiety in the enkephalins to the equivalent function in morphine (Fig. 1a-c) solely on these two structures it would not be entirely convincing due to the complex nature of the alkaloid morphine; i.e. how could one be sure that the tyramine fragment is probably the important core of the molecule? Additional evidence for this concept is provided by examining synthetic analogues of morphine which have had part of the morphine structure removed yet still retain as much or more morphine-like activity (Horn & Rodgers, 1976). (−)-Levorphanol (Fig. 2a) lacks 2 of the oxygen functions and the isolated double bond of morphine yet it is more potent than morphine in various *in vivo* (Jacobson & others, 1970) and *in vitro* (Pert & Snyder, 1973; Kosterlitz & Waterfield, 1975) test systems, hence these functions are non-essential for opiate-agonist activity. Further simplification of the C-ring of levorphanol by partially replacing it with two methyl groups gives the agonist (−)-metazocine (Fig. 2b) which in *in vivo* animal tests and in man has about the same analgesic



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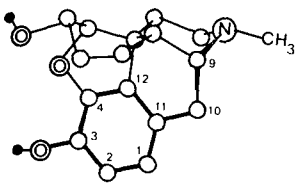

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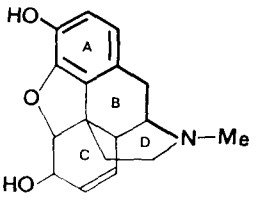

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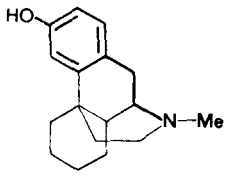
FIG. 1. a. Amino acid sequences of the enkephalin mixture with the 'tyramine' moiety of the tyrosine residue in heavy outline. b. X-ray structure of (−)-morphine (Mackay & Hodgkin, 1955). c. Chemical formula for (−)-morphine.



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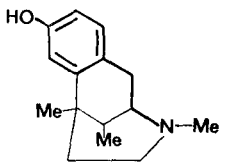

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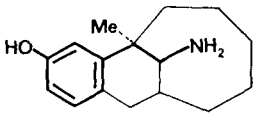

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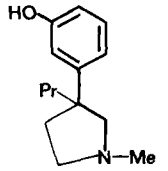

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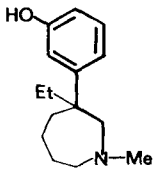

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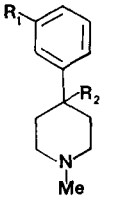

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FIG. 2. a. Levorphanol. b. Metazocine. c. 5,6,7,8,9,-10,11,12,-Octahydro-3-hydroxy-5α-methyl-5,11-methanobenzocyclodecen-13-amine. d. Profadol. e. Meptazinol. f. 4-Phenylpiperidines.

activity as morphine (Jacobson & others, 1970); *in vitro* studies indicate that it is interacting with the same population of receptor sites as the other opiates (Pert, Snyder & May, 1976). Thus it can be concluded that an intact C-ring is not essential for potent agonist activity. Levorphanol (Fig. 2a) and metazocine (Fig. 2b) can readily be seen to have two possible 3-carbon chains between the phenol ring and the nitrogen atom as well as the previously mentioned 'tyramine' moiety. However, preparation of simple 3-carbon amines carrying a *m*-phenolic-OH group yielded drugs with only very weak analgesic activity (Percherer, Sunbury & others, 1968).

One apparent objection to our hypothesis is the fact that the 'tyramine' moiety of the tyrosine residue of the enkephalins contains a primary amino group whereas the morphines, morphinans and 6,7-benzomorphans have a tertiary nitrogen atom. Normorphine, a secondary amine which is less active than morphine in certain *in vivo* tests, is however, as active as morphine in *in vitro* tests and *in vivo* if it is given intracisternally (Kosterlitz & Waterfield, 1976). Although this is true for morphine and normorphine, Kosterlitz & Waterfield (1976) have shown that this is the exception rather than the rule. The most convincing evidence that amino groups other than tertiary ones are compatible with agonist activity comes from the recent work of Freed, Potoski & others (1976 a,b) in which they show that various 1,3-bridged aminotetralins (Fig. 2c) which contain primary amino groups, are as potent or more so than morphine *in vivo*, the drug in Fig. 2c was 8 times as potent as morphine in the D'Amour-Smith rat tail flick test. Thus depending on the nature of the rest of the molecules a drug containing a primary amino group can act as a potent analgesic.

The importance of the phenolic-OH group in these groups of analgesics is well known. Thus the methyl ether of morphine, codeine, has only 0·7% of the potency of morphine in the guinea-pig ileum and a mere 0·05% of its activity in the rat brain homogenate stereospecific binding assay (Kosterlitz & Waterfield, 1975). In man codeine has about 8% of the activity of the parent drug. The des-hydroxy

morphinans are also known to be weaker agonists than the hydroxylated compounds (Jacobson & others, 1970). In the 6,7-benzomorphan series it has been demonstrated that replacement of the 2'-phenolic –OH by $-NO_2$, $-NH_2$, Cl or F groups leads to less active agonists (Jacobson & May, 1965). Bentley & Hardy (1967) have shown that preparation of Diels-Alder adducts and other derivatives of oripavine, i.e. the 6,14-endoethenotetrahydro-oripavine derivatives, produced compounds with much greater activity than morphine as analgesics, one of the best known examples being etorphine which is 1000–80 000 times more potent than morphine when given subcutaneously in various tests (Blane, Boura & others, 1967). In the guinea-pig ileum assay it is 790 times more potent than morphine (Kosterlitz, Lord & Watt, 1972). Part of its increased potency *in vivo* compared to morphine is due to its greater lipid solubility (Kosterlitz & others, 1972). In this series of agonists compounds with a phenolic-OH at the 3 position (oripavine derivatives) are 10–50 times more potent than the corresponding derivatives having a 3-methoxy group (thebaine derivatives) (Lewis, Bentley & Cowan, 1971).

Further simplifications of the morphine skeleton have led to drugs having greater degrees of conformational freedom such as profadol (Fig. 2d) meptazinol (Fig. 2e) and the 4-phenylpiperidines (Fig. 2f) (Cavalla, Bishop & others, 1965; Janssen & Van der Eycken, 1968; Goode & White, 1971). Although in the tyramine fragment of morphine the hydroxyl group is *para*, molecular models of profadol and meptazinol, which have a *meta* hydroxyl group clearly show that the spatial disposition of the nitrogen atom with respect to the benzene ring and hydroxyl group is very similar to that in morphine. The 4-phenylpiperidines (Fig. 2e) are based on the analogous fragment in morphine and it is not surprising that the optimal position for the hydroxyl group is *meta* with respect to the piperidine ring (Janssen & Van der Eycken, 1968). In the case of these drugs however, there are 3 carbon atoms separating the nitrogen atom from the aromatic ring and molecular models and the B distance (Fig. 3) in meperidine (Fig. 2f $R_1=H$, $R_2=COOEt$) (Table 3) indicate that the distances here differ from those found for the more rigid opiates. In various *in vitro* systems bemidone (Fig. 2f $R_1=OH$, $R_2=COOEt$) has 1/12 and (−)-profadol (Fig. 2d) 1/9 of the potency of (−)-morphine (Kosterlitz & others, 1972; Kosterlitz & Waterfield, 1975). As an analgesic in postoperative patients 20–50 mg of profadol are equivalent to 10 mg of morphine (Casy, 1971). It is therefore tempting to ascribe these decreases in potency to the greater degrees of conformational mobility and to the fact that the various moieties may not be held in their optimal positions as in the more rigid opiates. This simple concept is complicated, however, by the fact that ketobemidone (Fig. 2f $R_1=OH$, $R_2=$ –CO–Et) in *in vivo* (Jacobson & others, 1970) and *in vitro* (Kosterlitz & Waterfield, 1975) tests has a similar potency to morphine. Clearly a study of the conformation of this drug in solution and in the solid state could be worthwhile. Thus there seems to be convincing evidence that in opiates of various degrees of structural complexity an important element is the 'tyramine' moiety or other conformationally equivalent structural element.

X-ray crystallographic studies

As the opiates and closely related analogues are fairly rigid molecules information obtained from X-ray analysis of crystals of the drugs will give a reasonably accurate idea of the actual conformation of these drugs at the 'opiate receptor'. It is known from nmr studies in solution that morphine and other Δ^7-morphine type alkaloids, including the 14-hydroxy analogues, possess a similar conformation to one found in the solid state (Okuda, Yamaguchi & others, 1964; Carroll, Moreland & others, 1976). We have therefore computed by standard procedures certain molecular parameters (Fig. 3) for the tyramine fragment in several conformationally restricted opiate agonists and antagonists based on published X-ray data. We felt it reasonable to include data from both opiate agonists and antago-

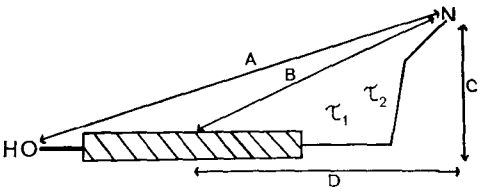

FIG. 3. Diagrammatic presentation of a side-on view of the tyramine moiety, the rectangle representing the benzene ring. A = distance of the nitrogen atom from the oxygen atom. B = distance of the nitrogen atom from the centre of the aromatic ring. C = Perpendicular distance of the nitrogen atom above the plane of the benzene ring. D = distance of the centre of the aromatic ring from the point of intersection of the plane of the benzene ring and the perpendicular distance C. The torsion angle τ_1 is defined as the angle between the planes of the atoms C_1–C_{11}–C_{10} and C_{11}–C_{10}–C_9, i.e. for rotation about the C_{11}–C_{10} bond Torsion angle τ_2 is the angle between the planes of the atoms C_{11}–C_{10}–C_9 and C_{10}–C_9–N, i.e. for rotation about the C_{10}–C_9 bond.

nists because not only are they structurally very similar, but there is now evidence that they are combining with the same or very similar receptor sites (Kosterlitz & others, 1972; Pert & Snyder, 1973; Kosterlitz & Waterfield, 1975; Schulz & Goldstein, 1975; Simon, Hiller & others, 1975). The drugs we have examined are the agonists morphine, codeine, azidomorphine and 3-*O*-methyletorphine, the antagonists naloxone, *N*-allylnormetazocine and 7-hydroxy levallorphan and the agonist-antagonists cyclazocine and nalbuphine (Fig. 4 and Table 1). Their pharmacological activities are shown in Table 2. We find the following mean values for the various molecular parameters (Fig. 3) are A = 7·0 Å B = 4·4 Å C = 1·1 Å D = 4·3 Å $\tau_1 = 173°$ $\tau_2 = -89°$.

It is possible that a combination of some of these or closely similar values may correspond to the optimal opiate receptor site conformation for the 'tyramine' fragment of the tyrosine residue in the opiate-like peptides. However, the values we have suggested might correspond to the preferred ones for the 'tyramine' residue of enkephalin at its receptor site may not be the ones found by X-ray crystallography or theoretical calculations because these pentapeptides are flexible molecules, unlike the opiates and close analogues which are fairly rigid. Although the X-ray structure of the potent opiate agonist etorphine has not been determined it can be assumed that the molecular parameters of the rigid ring system will be very similar to that found for its *O*-methyl ether (Table 1) this idea is supported by the fact that morphine and its *O*-methyl ether, codeine, have similar values for these parameters. The determination of these values for the agonists, antagonists and agonists-antagonists groups of analgesics clearly shows that the tyramine moiety is essentially the same even though the complexity of the overall ring systems vary; this is obviously a reflection of the quite rigid nature of this part of the molecular framework. The introduction of large groups (allyl, methyl, cyclopropyl) onto the nitrogen atom, resulting in opiate antagonists (Jacobson & others, 1970), does not appear to bring about significant conformational changes in the tyramine moiety in the solid state. In the case of the opiate antagonists it seems likely that the bulky *N*-substituents bring about their effects predominantly at the receptor level rather than by affecting both the conformation of the drug and its receptor, as is probably the case in more flexible molecules. It would be of interest to know what effect the introduction of allylic and other substituents onto the nitrogen atom of the tyrosine fragment of the enkephalins and other opiate-like peptides produced.

S/A relations for peptides with opiate-like actions

Since our original publication (Horn & Rodgers, 1976) new work has produced a better understanding of the structural features associated with opiate-like activity in these peptides. Bradbury, Smyth & Snell (1976) have shown that methionine-enkephalin is contained within the structure of the polypeptide β-lipotropin, which has 91 residues, as the sequence 61–65, and it has been suggested that β-lipotropin may be the precurors of methionine-enkephalin. A larger fragment of β-lipotropin the so-called C-

Table 1. *Molecular parameters for various opiate agonists and antagonists.* The molecular parameters are defined in the legend to Fig. 3. Values A, B, C, τ_1 and τ_2 were all calculated from the published atomic coordinates, the values for D were obtained by simple geometry.

Agonists	A (Å)	B (Å)	C (Å)	D (Å)	τ_1 (degrees)	τ_2 (degrees)
(−)-Morphine HI[a]	7·08	4·55	0·81	4·47	164·7	−94·9
(−)-Codeine HBr[b]	7·06	4·54	0·88	4·45	171·9	−91·4
(−)-Azidomorphine[c]	7·00	4·49	1·29	4·30	181·8	−87·3
(−)-3-*O*-Methyletorphine HBr[d]	7·14	4·55	0·74	4·48	167·7	−93·1
Antagonists						
(−)-Naloxone HCl[e]	6·96	4·32	1·27	4·12	180·7	−87·3
(±)-2-Allyl 5,9-dimethyl-2-hydroxy 6,7-benzomorphan HBr (*N*-Allylnormetazocine HBr)[f]	6·89	4·42	1·74	4·06	174·2	−81·3
(−)-6-Hydroxy-levallorphan[g]	6·96	4·43	1·58	4·13	172·0	−81·6
Agonist—antagonists						
(−)-Cyclazocine HBr[h]	7·08	4·55	1·23	4·38	182·6	−94·2
(−)-Nalbuphine HCl[i]	7·14	4·61	0·71	4·54	169·2	−98·1

a Mackay & Hodgkin (1955). b Kartha, Ahmed & Barnes (1962). c Sasvari, Simon & others (1974). d Van den Hende & Nelson (1967). e Sime, Forehand & Sime (1975). f Fedeli, Giacomello & others (1970). g Blount, Mohacsi & others (1973). h Karle, Gilardi & others (1969). i Sime, Dobler & Sime (1976).

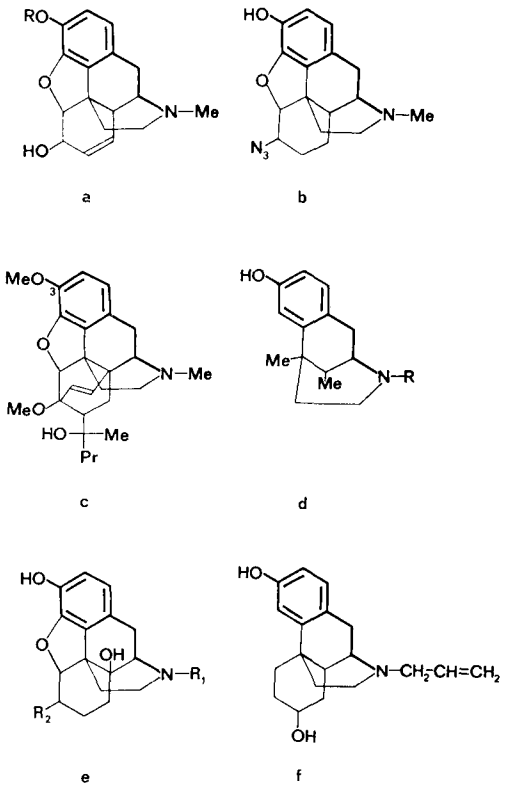

FIG. 4. Chemical formulae for opiate agonists, antagonists and agonist-antagonists whose crystal structures have been determined by X-ray analysis. The 'tyramine' fragment is in heavy outline. a R = H, morphine; R = CH_3 codeine. b Azidomorphine. c 3-O-Methyletorphine. d R = $-CH_2.CH=CH_2$, *N*-allylnormetazocine; R = $-CH_2-\triangleleft$ cyclazocine. e R_1 = $-CH_2-CH=CH_2$, R_2=O naloxone, R_1 = $-CH_2-\square$ R_2=OH Nalbuphine. f 6-Hydroxylevallorphan.

fragment, residues 61–91, has also been isolated from the pituitary gland and has been shown by Bradbury, Smyth & others (1976) to be much more potent than methionine-enkephalin both in *in vivo* and *in vitro* tests of opiate-like activity. These authors have examined a series of β-lipotropin fragments for their ability to displace the stereospecific binding of [^{3}H]naloxone and [^{3}H]dihydromorphine. The following six fragments were found to be active in the above *in vitro* tests, 61–91 (C fragment), 61–89, 61–87 (C′ fragment), 61–69, 61–68, 61–65 (methionine-enkephalin); the first two fragments were the more potent ones in the group. Removal of the methionine residue giving the sequence 61–64 led to a large fall in activity. β-Lipotropin itself and fragments 1–58, 1–38, 41–58 and 70–79 were more or less inactive. Replacing the 2-glycine moiety in the C fragment of

Table 2. *Agonist and antagonist activities of various opiates and analogues.* The tests used are shown in brackets.

Relative agonist activity (Morphine = 1·0)			
(−)-Morphine		1·0[a]	(hot-plate)
(−)-Codeine		0·14[a]	(hot-plate)
(−)-Azidomorphine		293·7[b]	(hot-plate)
(−)-3-*O*-Methyletorphine		96[c]	(tail pressure)
Relative antagonists activity (Nalorphine = 1·0)			
(−)-Naloxone		32·5[d]	(tail-flick blockade)
(−)-6-Hydroxylevallorphan		1·45[e]	(tail-flick blockade)
(±)-*N*-Allylnormetazocine		2·0[f]	(Abstinence precipitation)
Relative agonist-antagonist activity			
		Agonist (Morphine = 1·0)	Antagonist (Nalorphine = 1·0)
(−)-Cyclazocine	9·58[f]	(hot plate)	1·12[f] (tail-flick blockade)
(−)-Nalbuphine	4–5[g]	(Phenylquinone writhing test)	0·012[g] (Abstinence precipitation)
	'weak'	(hot plate)	

a—Jacobson & others (1970). b—Knoll, Furst & Kelemen (1973). c—Bentley, Hardy & Meek (1967). d—Aceto, McKeen & Pearl (1969). e—Blount & others (1973). f—Pert & others (1976). g—Jasinski & Mansky (1972).

β-lipotropin by either L-proline or L-alanine reduces activity markedly (Birdsall, Bradbury & others, 1976). From the porcine neurohypophysis-hypothalamus, Guillemin, Ling & Burgus (1976) have isolated the fragment 61–76 of β-lipotropin and have shown that it is slightly less active than methionine-enkephalin. The lack of opioid activity of β-lipotropin and the effectiveness of the 61–91 fragment has been confirmed by Cox, Goldstein & Li (1976). Preparation of the amide form of the free acid of methionine-enkephalin resulted in a compound with 5 times the potency of the parent compound and a longer duration of action in the guinea-pig ileum assay, the methionine sulphoxide, however, has only 1/2 the potency of the free acid (Lazarus, Ling & Guillemin, 1976). These workers also showed that β-lipotropin and fragment 61–63 were inactive in the guinea-pig ileum and stereospecific opiate binding tests; fragment 61–64 has about 1/100 activity of methionine-enkephalin, a similar result has been reported by Bradbury & others (1976). Seidah, Lis & others (1976) have isolated the fragment 61–82 of β-lipotropin after trypsin cleavage and have demonstrated its enkephalin-like activity, these workers also showed that the fragment 66–91 was inactive. A synthetic peptide consisting of the first three residues of the enkephalins i.e. Tyr-Gly-Gly, has been shown to be inactive both *in vivo* and *in vitro* (Büscher, Hill & others, 1976). The conclusion to be drawn from the work is the importance of the methionine-enkephalin fragment for the opiate-like activity of all the β-lipotropin fragments.

Recent reports can be interpreted as providing direct experimental evidence for our hypothesis of the

crucial importance of the tyrosine residue in the enkephalins and analogues (Horn & Rodgers, 1976). Büscher & others (1976) and Morgan, Smith & others (1976) have shown that desamino-Tyr-leucine and methionine-enkephalins are both inactive. Cabamylation of the *N*-atom of the tyrosine residue of β-lipotropin C fragment, i.e. removal of the basic nature of this residue, greatly reduces activity in the stereospecific opiate binding assay (Birdsall & others, 1976). Removal of the hydroxyl group of the tyrosine residue in methionine-enkephalin, or its benzylation in the C fragment, both have pronounced adverse effects on activity (Birdsall & others, 1976; Morgan & others, 1976). Structure activity studies by Chang, Fong & others (1976) also stress the importance of the terminal tyrosine residue and in particular the need for the aromatic hydroxyl group of tyrosine. In this context it is most interesting that the method of deactivation of the enkephalins in rat and human plasma and in rat brain is by enzymatic cleavage of the Tyr-Gly amide bond (Hambrook, Morgan & others, 1976). Additional evidence for our suggestion of a conformational similarity between the 'tyramine' moieties in the enkephalins and morphine has been provided by nmr studies (Bleich, Cutnell & others, 1976; Jones, Gibbons & Garsky, 1976; Roques, Garbay-Jaureguiberry & others, 1976).

Possible mechanisms of binding of the enkephalins and opiates

Because the *N*-terminal tyrosine residue seems to be of such central importance for the production of opiate-like activity it is likely that these molecules may bind to their receptor via the so called 'Zipper' mechanism (Burgen, Roberts & Feeney, 1975; Jones & others, 1976). The tyramine residue or part of it could bind first to a receptor subsite and this could be followed by a series of conformational changes of the partly bound ligand ultimately leading to the binding of the rest of the molecule to its respective subsites. This is to be contrasted with the 'lock and key' model in which the ligand binds directly to its receptor site in the correct conformation. The opiates being fairly rigid molecules, *will* bind predominantly via the 'lock and key' model.

It has been suggested that it is the positively charged form of the opiates nitrogen atom that interacts with its receptor rather than the free base (Beckett & Casy, 1954; Portoghese, 1966) and at a physiological pH of 7·4 this seems probable. Belleau & Morgan (1974), however, have suggested it is the free lone pair of electrons on the nitrogen atom that interacts with the opiate receptor. Studies by Opheim & Cox (1976) with the quaternary levorphanol methiodide, which carries a permanent positive charge, tend to support the idea that the active species at the opiate receptor is the charged form.

Conformationally flexible analgesics

The fact that some of the endogeneous opiate-like ligands are flexible molecules that may bind via a 'Zipper' mechanism may also help to explain the potency of other flexible synthetic analgesics, such as methadone and dextromoramide (Fig. 5) which are structurally distinct from the opiate-like peptides and opiates themselves. Although methadone and dextromoramide do not contain a 'tyramine' moiety there is good evidence that they are acting at the same receptor site as the opiates (Cox & Weinstock, 1964; Kosterlitz & others, 1972; Kosterlitz & Waterfield, 1975). They do however, contain a phenyl

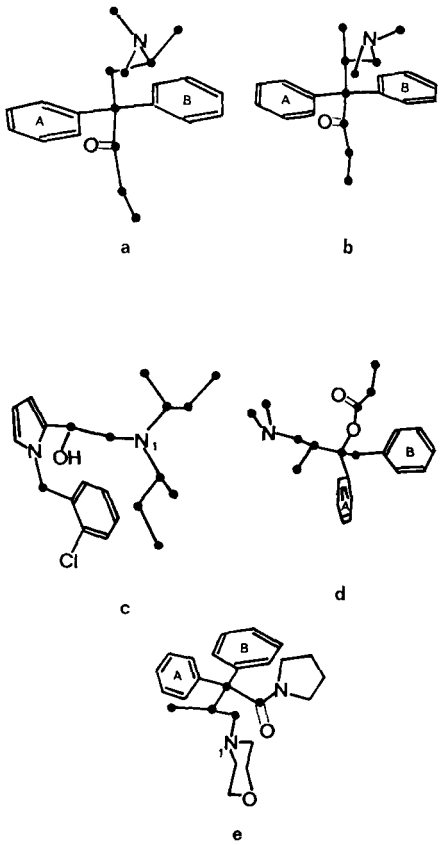

FIG. 5. Chemical formulae for various conformationally flexible analgesics. The formulae shown are approximations of the crystal conformations. a Methadone HBr. b Isomethadone HCl. c Viminol *p*-hydroxybenzoate. d Dextropropoxyphene HCl. e Dextromoramide bitartrate.

ring and a basic nitrogen atom (Fig. 5) and it is possible that these groups could position themselves on the opiate receptor in a similar fashion to the equivalent functions in the opiates and the 'tyramine' residue of the enkephalins. The rest of the molecule could then provide the necessary extra binding sites for the other subsites of the opiate receptor. Although there have been several X-ray studies on these flexible analgesics (Fig. 5 and Table 3) it is difficult to relate the results to a possible receptor site conformation, this can be readily seen by the range of values found for the distance of the nitrogen atom from the centre of the aromatic ring(s) (Table 3).

Table 3. *Molecular parameters for various flexible analgesics.* The parameter 'Ph–N' is the distance of the centre of the phenyl ring from the nitrogen atom. Where there is more than one phenyl ring or nitrogen atom these are designated Ph_A, Ph_B N_1, respectively.

Meperidine HBr[a]	Ph –N = 5·71 Å	
Viminol *p*-hydroxybenzoate[b]	Ph $-N_1$ = 5·12 Å	
Methadone HBr[c]	Ph_A – N = 6·28 Å	Ph_B – N = 5·33 Å
Isomethadone HCl[d]	Ph_A – N = 6·30 Å	Ph_B – N = 5·36 Å
Dextropropoxyphene HCl[e]	Ph_A – N = 5·30 Å	Ph_B – N = 7·68 Å
Dextromoramide bitartrate[f]	Ph_A – N_1 = 6·22 Å	Ph_B – N_1 = 5·06 Å

a—Van Koningsveld (1970). b—Silverton & Lloyd (1975). c—Hanson & Ahmed (1958). d—Shefter (1974). e—Bye (1973) f—Bye (1975).

In conclusion, there is evidence to support the hypothesis that the presence of a tyrosine residue (and hence a 'tyramine' moiety) in the terminal amino position of these peptides is the key factor in explaining why they possess opiate-like activity. The main support for this idea is the fact that in several classes of opiate drugs and analogues the 'tyramine' fragment or a conformationally equivalent moiety seems to be a crucial element and secondly that structure-activity studies with these peptides show the importance of the *N*-terminal tyrosine residue. It must be stressed, however, that although the presence of the 'tyramine' fragment may be of importance in explaining the action of several groups of agonists and antagonists the stereospecificity/selectivity factor and the contribution of additional modes of binding from other portions of the molecule are obviously also critical (Portoghese, 1965). This is evident from the reduction or loss in activity on removing one or more residues from the C terminal position of the enkephalins and also from the fact that drugs not possessing a 'tyramine' moiety such as dextromoramide and methadone are active agonists at opiate receptors.

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