genate was kept in an ice bath before centrifugation at 12 000 *g* for 5 min. The supernatant fraction was carefully drawn off with a microlitre pipette and assayed for noradrenaline essentially as described by Keller, Arvin & others (1976). Briefly, the method involves adsorption of noradrenaline onto alumina followed by high pressure liquid chromatography with electrochemical detection of the eluted noradrenaline. The high sensitivity of this assay enabled us to measure the small amount of noradrenaline (1–2 ng) present in a single pineal. Data were more consistent when noradrenaline concentration was expressed in ng mg^{-1} protein, though conclusions about drug effects were the same when noradrenaline concentration was expressed as ng per pineal gland. Protein was assayed conveniently on the $HClO_4$ precipitate by dissolving the pellet in 0·5 M NaOH (0·5 ml), adding 0·5 ml 0·1 M tris buffer pH 7, and measuring absorbance at 280 and 260 nm. Protein content was calculated from the data of Warburg & Christian as described by Layne (1957).

Fig. 1 shows the concentration of pineal noradrenaline during a 24 h period after injection of the uptake inhibitors. Noradrenaline was significantly increased within 4 h after desipramine injection and remained so at the end of 24 h. These findings are in agreement with the report of Jaim-Etcheverry & Zieher (1971). Another uptake inhibitor, EXP 561* which inhibits uptake into noradrenaline and 5-HT neurons (Fuller & Wong, 1977), produced a similar increase in pineal noradrenaline concentration. Eight h after EXP 561 (10 mg kg^{-1}, i.p.), pineal noradrenaline concentration was increased from the control value of 11·2 ± 2 to 27·4 ± 4 ng mg^{-1} protein. In contrast, fluoxetine (20 mg kg^{-1}) did not lead to a significant change in noradrenaline concentration at any of the times measured. This dose of fluoxetine is twice that previously shown to inhibit uptake into brain 5-HT neurons for more than 24 h as measured by block of *p*-chloroamphetamine depletion of 5-HT (Fuller, Perry & Molloy, 1975).

In vitro studies with brain synaptosomes and *in vivo* studies evaluating uptake in brain and heart have established that desipramine is a highly selective inhibitor of noradrenaline and not 5-HT uptake, whereas fluoxetine is a highly selective inhibitor of 5-HT uptake (see Fuller & Wong, 1977). Our present results with the pineal gland indicate that desipramine is effective in blocking the uptake of 5-HT into the noradrenaline nerve terminals, whereas fluoxetine is not. These data are interpreted as confirming that the specificity of uptake inhibitors of this kind resides in the uptake pump rather than the substrate. This idea is mechanistically sound, since the interaction of the inhibitors is not thought to involve combination with the substrate molecule, but rather combination with the receptor macromolecule so as to compete with the substrate attachment.

* 4-Phenyl-bicyclo(2,2,2)octan-1-amine HCl monohydrate.

May 20, 1977

REFERENCES

FULLER, R. W., PERRY, K. W. & MOLLOY, B. B. (1975). *J. Pharmac. exp. Ther.*, **193**, 796–803.
FULLER, R. W. & WONG, D. T. (1977). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **36**, 2514–2518.
JAIM-ETCHEVERRY, G. & ZIEHER, L. M. (1968). *Z. Zellforsch. mikrosk. Anat.*, **86**, 393–400.
JAIM-ETCHEVERRY, G. & ZIEHER, L. M. (1971). *J. Pharmac. exp. Ther.*, **178**, 42–48.
KELLER, R., ARVIN, O., MEFFORD, I. & ADAMS, R. N. (1976). *Life Sci.*, **19**, 995–1004.
LAYNE, E. (1957). *Methods in Enzymology*, Vol. III, 451–454. New York: Academic Press.

The opiate anomalies — another possible explanation?

R. H. B. GALT, *Imperial Chemical Industries Limited, Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.*

The opium alkaloid morphine (I), with its complex structure and wide range of pharmacological properties, was the starting point of a vast research effort. As structure-activity data have accumulated through the years, interesting hypothetical models of the opiate receptor have been advanced.

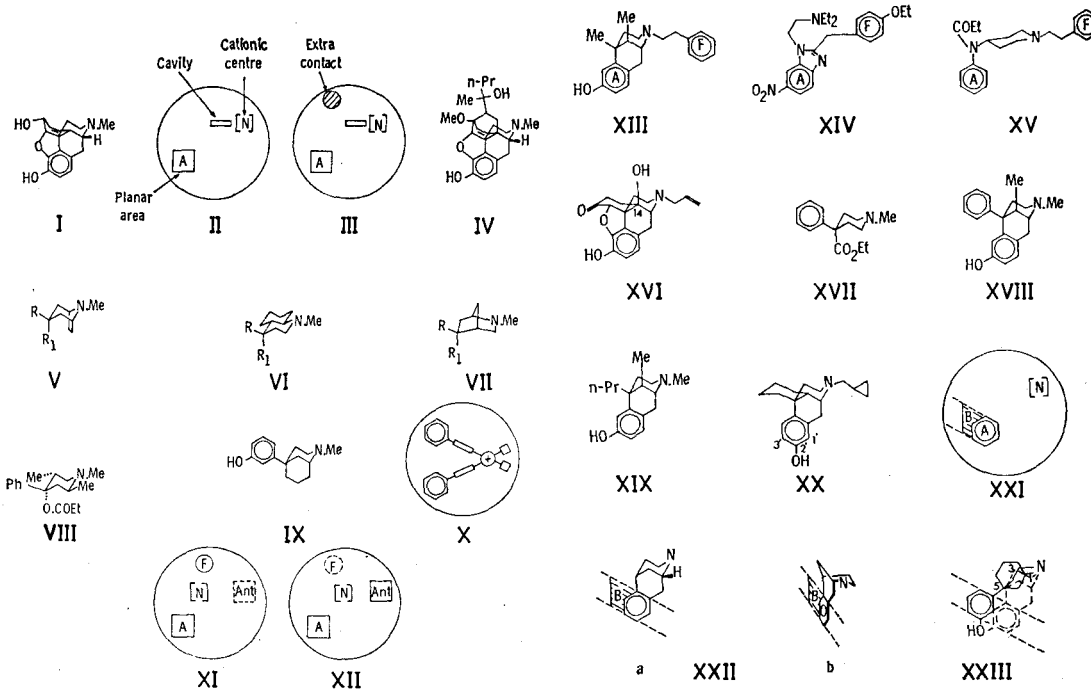
The first (cf. II) was due to Beckett & Casy (1954) who argued that, unlike more flexible molecules, morphine with its rigid structure and defined absolute stereochemistry was surrendering a lot of information about the three-dimensional nature of the receptor at which it exerted its biological effect. This model was extended (cf. III) by Bentley, Cowan & Lewis (1971) to accommodate the very potent Diels-Alder adducts (e.g. IV) derived from thebaine, which were believed to have unearthed another point of receptor contact.

A unique aspect of the structure of morphine (cf. I) is an aromatic ring held rigidly axial to a piperidine ring and this is a key feature in the design of model II. However, biological results on a range of synthetic pairs of isomers, V (Casy & Coates, 1974; Clarke, Kullnig & Martini, 1975), VI (Smissman & Steinman, 1966) and VII (Kupferberg, Mikhail & Portoghese, 1968) in which R = Ph and R′ = CO_2Et (·O·COEt in VI) or vice versa, suggested rather surprisingly that the position of the phenyl group in space with respect to the nitrogen atom

was not critical for activity. Apparently, in phenylpiperidine types, it could be either axial or equatorial. The equatorial phenyl ring possibility gained further support from the trimeperidine series (Fries & Portoghese, 1974) in which the most potent enantiomer (VIII) virtually had the phenyl ring locked in the equatorial position; any 'ring flip' would produce many severe 1,3-diaxial interactions. Finally, the synthesis of IX appeared to put the matter beyond doubt, because the phenyl ring is fixed equatorially and the compound is as active as morphine (Cochran, 1974; May, Oh-ishi & Ong, 1974). Model II could not accommodate this and Portoghese (1965) postulated a swivelling receptor model (X) to explain the two possible positions for the aromatic ring.

Recently an ingenious and more comprehensive model (Creese, Feinberg & Snyder, 1976) was advanced which discussed the structure-activity data in the light of agonist (XI) and antagonist (XII) conformations of the receptor. The agonist conformation (low Na^+ concentration) is an extension of model III and can be stabilized by occupancy of the two lipophilic areas, A and F, neatly explaining the high potency of opiates like phenazocine (XIII), etonitazine (XIV) and fentanyl (XV). In the antagonist conformation (high Na^+), the antagonist area (ANT) comes into play and the F area may not be available. The model also emphasized the tendency towards pure opiate antagonist activity when the antagonist side-chain on the nitrogen atom is sterically maintained in the equatorial position (e.g. by the axial 14-OH of naloxone, XVI) with respect to the piperidine ring. Also, the failure to synthesize opiate antagonists of molecules with flexible or fixed equatorial phenyl groups, e.g. meperidine (XVII) and IX, may be due to a different mode of binding in which the aromatic rings of such compounds make use of the F rather than the A area.

In this article, more structural and stereochemical anomalies in the opiate area are brought to light. Model XI and XII can account for some of these but not others and another possible explanation of these anomalies is suggested. The most puzzling abnormalities are stereochemical and are often overlooked because they do not fit into the nice stereospecific picture of the opiate receptor painted by most of the structure-activity data. For example, *both* optical isomers of IX (with a fixed equatorial phenyl group) show opiate properties. More confusingly, there are also a number of benzomorphans (with rigid axial phenyl rings) where potent opiate effects are seen in *both* optical isomers. The optical isomers of XVIII (GPA 1657 and 1658) are an interesting pair, the former being morphine-like in potency and stereochemistry, the latter being a weaker agonist but capable of supporting morphine dependence at low doses (Block, Clarke & Yokoyama, 1970; Clarke, Hill & others, 1973). Similarly with the benzomorphan (XIX), the isomer with the natural opiate stereochemistry is a potent agonist with low abuse potential in monkeys whilst its mirror image, a weaker analgesic, has a high physical dependence capacity (Ager, Jacobson & May, 1969). The optical isomers of the morphinan (XX), with the phenolic hydroxyl moved from the 3′ to the 2′ position, are also unusual. The one which is

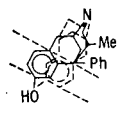


morphine-like in shape is morphine-like in potency; the other is a weak agonist but both are equipotent as opiate antagonists! (Baruth, Leimgruber & others, 1974).

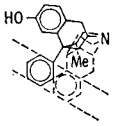
A slight modification of models II or XI in which the planar binding area (A) is extended slightly in a north-westerly direction (the B-area; cf. XXI) helps to explain these anomalies: XXII (a and b) is an attempt to depict this modified model in two different planes using the rigid benzomorphan part of the morphine skeleton. Any molecule, therefore, which has (by rigidity) or can project (by bond rotation, ring flipping etc.) a planar surface and a cationic centre in the same juxtaposition in space as model XXI could have opiate properties.

The most active enantiomer (1*S*, 5*R*) of IX fits the new model as shown in XXIII, projected on top of the dotted benzomorphan skeleton. Interestingly, although the aromatic rings of IX and morphine do not overlap in XXIII, the phenolic hydroxyl groups of both molecules could have the same receptor contact point. The less active enantiomer of IX fits in exactly the same way but has the cyclohexane ring attached to position 3 rather than 1.

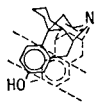
Benzomorphans which have opiate properties despite having the opposite absolute stereochemistry to morphine (e.g. GPA 1658 and the 'unnatural' isomer of XX) fit as in XXIV in which the nitrogen atom and the phenolic hydroxyl are superimposable but the planar surface is again moved slightly to the north-west. In XXIV, there is the implication that all the 'wrong' optical isomers of rigid opiates can bind to the receptor. Since this is not so, one has to argue that the spatial requirements for these isomers are more critical. An alternative possibility for GPA 1658 is depicted in XXV with the non-phenolic ring providing the planar surface. The more active isomer of XX can fit (cf. XXVI) making use of the extended planar area whilst its mirror is an excellent fit on the rigid opiate frame (cf. XXVII).



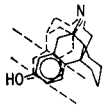
XXIV



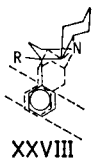
XXV



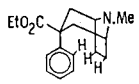
XXVI



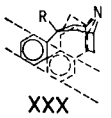
XXVII



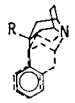
XXVIII



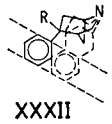
XXIX



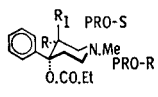
XXX



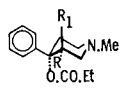
XXXI



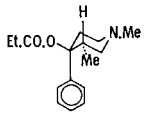
XXXII



XXXIII



XXXIV



XXXV

In XXIV, there is the implication that all the 'wrong' optical isomers of rigid opiates can bind to the receptor. Since this is not so, one has to argue that the spatial requirements for these isomers are more critical. An alternative possibility for GPA 1658 is depicted in XXV with the non-phenolic ring providing the planar surface. The more active isomer of XX can fit (cf. XXVI) making use of the extended planar area whilst its mirror is an excellent fit on the rigid opiate frame (cf. XXVII).

Re-examination of some of the evidence for the apparent lack of specificity for the aromatic ring in synthetic opiates raises some interesting points. For example, both isomers of VI are active. The one with the morphine conformation (VI, R_1 = Ph, R = O·CO·Et) fits model II, but so does the other (R_1 = O·CO·Et, R = Ph) if the piperidine flips to a boat (cf. XXVIII). The latter isomer with its equatorial phenyl ring can also fit model XXI in a similar way to example XXIII. The isomer of V with R_1 = Ph and R = CO_2Et is unlikely to exist in the morphine conformation due to severe steric interactions (cf. XXIX). The aromatic ring will be pushed away from the ethano bridge into a shape like XXX which nicely fits model XXI with the extended planar area. The isomer V (R = Ph, R_1 = CO_2Et) with an equatorial phenyl group can fit as shown in XXIII but it can also be accommodated by the simpler model (II) if the piperidine ring flips to near the boat conformation (cf. XXXI). The more potent isomer of VII has R_1 = Ph and R = CO_2Et and is morphine-like conformationally; the other is not but can fit model XXI as shown in XXXII.

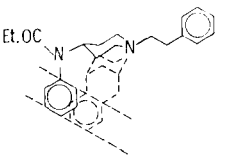
Fries & Portoghese (1976) diagnosed an enantiotopic stereoselectivity in substituted phenyl piperidines. Briefly, analogues of XXXIII, with substituents on the back (PRO-S) edge of the piperidine ring were more active than their optical isomers (XXXIV), neatly demonstrating the 'Ogston Effect' in drug-reception interactions (Ogston, 1948). Sometimes the more active isomer of XXXIII had an axial substituent (e.g. XXXIII; R′ = Me, R = H), sometimes it was equatorial (XXXIII; R = Et or allyl, R′ = H). These important results can be interpreted in slightly different ways. Firstly, it can be argued that, when it is conformationally possible (i.e. not too unfavourable thermodynamically), the aromatic ring in phenyl piperidines has to become axial (like morphine) before or during receptor contact. This is done by inverting the piperidine ring, when XXXIII with an axial methyl at R_1 becomes XXXV with an equatorial methyl on the *front* edge of the piperidine ring. The attraction of this interpretation is that it fits in an absolute sense with the stereochemistry of the natural opiates (cf. I) in which the back edge of the piperidine ring is never substituted. Alternatively, XXXIII and all its relatives with equatorial phenyl groups can be 'upturned' and fitted on model XXI as shown in example XXIII, an inversion

which also brings substituents to the front edge of the piperidine ring. This second alternative seems necessary to explain the trimeperidine (VIII) and others in which the barrier to the phenyl ring becoming axial is prohibitive.

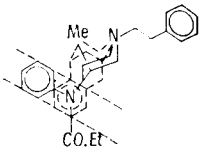
The structurally unusual fentanyl family (Janssen, 1962) can fit model XXI in several ways, for example XXXVI and XXXVII. The very potent 3-methyl analogue is believed to have the 3*S*, 4*R* configuration (Janssen, Niemegeers & Van Bever, 1974) which is best accommodated by XXXVII. Another interesting possibility (Creese & others, 1976) is that the phenyl of the *N*-phenethyl group (present in the most potent members of the series) provides the planar area (A in model XI).

It is interesting that most molecules which hypothetically make use of the extended lipophilic area (B in model XXI) for binding cannot be converted to potent antagonists in the classical way by replacing the *N*-methyl group by, for example, an allyl substituent. Is it because the 'sweep area' of the side-chain is different (cf. XXXVIII and XXXIX)? However antagonist properties are partially recovered in flexible phenyl piperidines when a phenolic hydroxyl is inserted (Langbein, Merz & others, 1974). Does this substituent help to stabilize an axial phenyl ring conformation in these molecules? Another interpretation of the anomalies in opiate antagonists has been suggested by Hite & Tecle (1976).

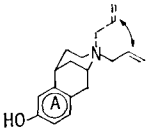
The structural similarity of leu- and met-enkephalin (XXXX) to morphine has been pointed out (e.g. Horn & Rogers, 1976); morphine after all is bio-synthesized in the opium plant from two molecules of tyrosine. In trying to establish likely conformations for these flexible endogenous substances it may not be sufficient to compare them to morphine alone. The opiate receptor can apparently accept a wide variety of shapes and sizes but the modified model (XXI) may represent a minimum requirement for binding.



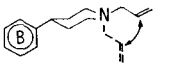
XXXVI



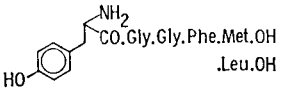
XXXVII



XXXVIII



XXXIX



XXXX

May 20, 1977

REFERENCES

AGER, J. H., JACOBSON, A. E. & MAY, E. L. (1969). *J. medl Chem.*, **12**, 288–289.

BARUTH, H., LEIMGRUBER, W., MOCHASI, E. & RANDALL, L. O. (1974). *Adv. Biochem. Psychopharm.*, **8**, 45.

BECKETT, A. H. & CASY, A. F. (1954). *J. Pharm. Pharmac.*, **6**, 986–1001.

BENTLEY, K. W., COWAN, A. & LEWIS, J. W. (1971). *Ann. Rev. Pharmac.*, **11**, 241–270.

BLOCK, F. B., CLARKE, F. H. & YOKOYAMA, N. (1970). *J. medl Chem.*, **13**, 488–492.

CASY, A. F. & COATES, J. E. (1974). *Org. Magn. Reson.*, **6**, 441.

CLARKE, F. H., HILL, R. T., SAELENS, J. K. & YOKOYAMA, N. (1973). *Adv. Biochem. Psychopharm.*, **8**, 82–84.

CLARKE, R. L., KULLNIG, R. K. & MARTINI, C. M. (1975). *J. medl Chem.*, **18**, 496–501.

COCHRAN, T. G. (1974). *Ibid.*, **17**, 987–989.

CREESE, I., FEINBERG, A. P. & SNYDER, S. H. (1976). *Proc. natn. Acad. Sci. U.S.A.*, **73**, 4215–4219.

FRIES, D. S. & POROTGHESE, P. S. (1976). *J. medl Chem.*, **19**, 1155–1158 and refs. cited therein.

FRIES, D. S. & PORTOGHESE, P. S. (1974). *Ibid.*, **17**, 990–993.

HITE, G. A. & TECLE, H. (1976). *Proceedings of the 38th Annual Scientific Meeting Committee on Problems of Drug Dependence*, 464–470. *Nat. Acad. Sci.*

HORN, A. S. & RODGERS, J. R. (1976). *Nature*, **260**, 795–797.

JANSSEN, P. A. J. (1962). *J. Anaesth.*, **34**, 260.

JANSSEN, P. A. J., NIEMEGEERS, C. J. R. & VAN BEVER, W. F. M. (1974). *J. medl Chem.*, **17**, 1047–1051.

KUPFERBERG, H. J., MIKHAIL, A. A. & PORTOGHESE, P. S. (1968). *Ibid.*, **11**, 219–225.

LANGBEIN, A., MERZ, H., STOCKHAUS, K. & WICK, H. (1974). *Adv. Biochem. Psychopharm.*, **8**, 157.

MAY, E. L., OH-ISHI, T. & ONG, H. H. (1974). *J. medl Chem.*, **17**, 133–134.

OGSTON, A. G. (1948). *Nature*, 162, 963.

PORTOGHESE, P. S. (1965). *J. medl Chem.*, **8**, 609–616.

SMISSMAN, E. E. & STEINMAN, M. (1966). *Ibid.*, **9**, 455–457.