JOURNAL OF PHARMACEUTICAL SCIENCES

JUNE 1984
VOLUME 73 NUMBER 6

RESEARCH ARTICLES

New Insights in the Clastic Binding Hypothesis for Opiate–Receptor Interactions I: Electron-Transfer Mechanism

VERA M. KOLB

Received August 9, 1982, from the *Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901.* Accepted for publication January 19, 1983.

Abstract □ A clastic binding hypothesis for opiate–receptor interactions, in which the key step in eliciting the opiate response is an electron transfer from the opiate nitrogen to the receptor, is analyzed from a chemical point of view and is found to be chemically feasible. An extended form of this hypothesis is proposed in order to accommodate recent observations about the N-demethylation of morphines in the brain. An *in vitro* model system for studying the electron-transfer reactions of morphines is proposed, and the initial experiments in this system are reported.

Keyphrases □ Clastic binding hypothesis—electron-transfer mechanism, opiate receptor interactions, *in vitro* model system □ Opiate-receptor interactions—electron-transfer mechanism, extended clastic binding hypothesis, *in vitro* model system □ Electron transfer—mechanism, extended clastic binding hypothesis, opiate-receptor interactions, *in vitro* model system

Belleau *et al.* (1, 2) presented experimental evidence that the directionality of the lone electron pair on nitrogen (N lone pair) of morphine-type opiates is directly related to their opiate activity. On this basis Belleau and Morgan (3) proposed the clastic binding hypothesis for opiate–receptor interactions. According to this hypothesis, morphine-type opiates achieve productive binding with the opiate receptor *via* a clastic binding process that includes a stereospecific electron transfer from the lone electron pair of opiates to some electrophilic site at the receptor. Such clastic binding is tantamount to injecting electrons at the receptor level. The electron transfer from nitrogen to the receptor was proposed to lead to the oxidation of the *N*-methyl substituent. Such oxidation may lead to a subsequent *N*-demethylation. Contrary to the *N*-demethylation hypothesis of Beckett *et al.* (4) in which the *N*-demethylation is an extrinsic preliminary step in the mechanism of opiate action, in the clastic binding the *N*-demethylation process participates *in conjunction with the opiate receptor* in eliciting the opiate response. According to Belleau and Morgan "the opiate receptor complex would possess an intrinsic and specific oxidative *N*-demethylase activity."

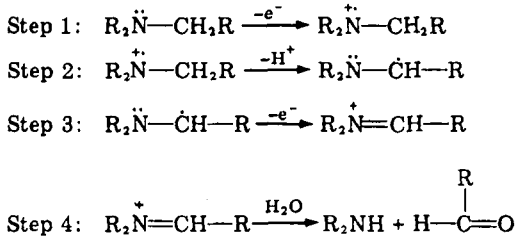
The aforementioned concept obviously requires that the location of the sites of *N*-demethylation within the CNS is proximal to or coincident with that of the opiate receptors. This was indeed found to be the case by Fishman *et al.* (5–7). They have demonstrated that the *N*-demethylation of morphine in rat brain is localized in sites with high opiate-receptor content. Moreover, the *N*-demethylation in the brain was found to proceed only when pharmacological concentrations of the substrate were present: the fact which indicated the biological significance of the reaction. This brain dealkylase was found to be different from that responsible for the same transformation in the liver (6). The brain demethylation of morphine was decreased by tolerance and by opiate antagonists such as naloxone, which itself was not dealkylated in the brain (7). These results provided strong support for the concept that *N*-dealkylation of narcotics in their CNS target sites is intimately involved in the expression of their action.

The *N*-dealkylation reaction occurring in the enzymatic process described above may follow the same mechanistic path as the same reaction occurring in the "test-tube" with the "regular" chemical reagents, or it may follow a different path. We were interested in exploring the former possibility. Specifically, we wanted to find out if the currently available chemical data on electron transfers from the N lone pair of morphines or model compounds, such as tertiary aliphatic amines, to electron acceptors are compatible with the hypothesis of Belleau and Morgan (3) and the findings of Fishman *et al.* (5–7).

THEORETICAL

Electron-Transfer Reactions and *N*-Dealkylations of Morphines—A possible mechanism by which the electron transfer from the N lone pair of the tertiary aliphatic amines to various electron acceptors leads to the *N*-dealkylation is presented in Scheme I. This mechanism has been found to be valid

0022-3549/84/0600-0715$01.00/0



Scheme I—Possible mechanism of the oxidative N*-dealkylation of tertiary aliphatic amines.*

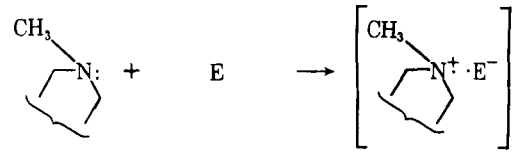
for the electrochemical (anodic) oxidations and dealkylations of tertiary aliphatic amines (8, 9) as well as for their reactions with various chemical agents, such as chlorine dioxide, *N*-bromosuccinimide, manganese dioxide, and ozone (10). Such a mechanism has been recently proposed also for the mitochondrial monoamine oxidase-catalyzed amine oxidation (11).

The first step of the mechanism shown in Scheme I is a one-electron transfer from the nitrogen of a tertiary amine to a suitable (redox-matched) electron acceptor (not shown). This slow step was found to be rate determining in the chemical and electrochemical reactions (8–10). The radical cation produced in the first step undergoes a loss of proton from an α-position yielding, after the rearrangement of electrons, an α-amino radical; this is shown in the second step. The α-amino radical species undergoes a one-electron transfer from nitrogen to give an immonium species, as shown in the third step. The immonium ion is readily hydrolyzed into a secondary (*N*-dealkylated) amine and an aldehyde, as shown in the fourth step.

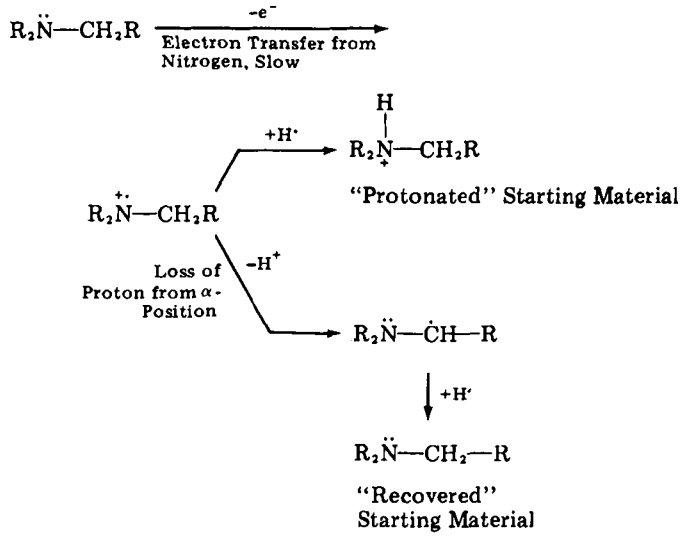
The mechanism presented in Scheme I may be proposed to operate in the cases when the *N*-demethylation of morphine occurs in the brain, namely when the concentration of morphine is at its pharmacological level (5). From this mechanism, however, it is not obvious why the *N*-demethylation does not occur at concentrations of morphine lower than the pharmacological concentration (5). The latter observation may be accommodated, however, by the following hypothesis. The electron transfer from the N lone pair of the morphine nitrogen to the electron acceptor (E) at the receptor may result in the formation of a stable caged radical-anion–radical-cation (RARC[1]) pair (Scheme II) between morphine and a hypothetical electron acceptor at the receptor. No bonds are made or broken in this process[2].

The formation of a RARC (charge-transfer) complex may be the most frequently occurring mode by which the morphine nitrogen interacts with the electron acceptor at the receptor. This mode of interaction, however, would not elicit the biological response according to the clastic binding hypothesis, since the electron transferred from the N lone pair is *not free* to be injected into the receptor, but is confined within the RARC cage. The formation of the RARC complex may be responsible for the affinity of nitrogen to the "amine-binding site" of the receptor (14), which would contribute to the overall affinity of morphine to the receptor, while the injection of the electron from the morphine nitrogen to the receptor would be responsible for the intrinsic activity (efficacy) (15) of morphine.

Only as a rare event would the electron from the N lone pair be injected to the receptor and, thus, be lost from nitrogen. The latter species would become a *free* radical cation which then could further react, as shown in Scheme I, to ultimately lead to *N*-dealkylation. Thus, according to this concept, the pharmacological concentration of morphine would be some critical concentration at which these rare events of the injecting the N lone pair electrons to the receptor cumulate and become "visible." A necessary condition for



Scheme II



Scheme III

inducing analgesia would be the injection of the N lone pair electrons to the receptor, and the *N*-dealkylation would normally accompany this reaction.

From the chemical point of view, however, one would predict that the injection of the N lone pair electron to the receptor does not necessarily have to be accompanied by *N*-dealkylation, since the nitrogen radical cation may react by a pathway different than that shown in Scheme I. Two such possible pathways are shown in Scheme III. According to this Scheme the nitrogen radical cation, formed by the loss of one electron from the N lone pair, may give either "protonated" or "recovered" starting material. The upper arrow in Scheme III shows that the nitrogen radical cation may pick up a hydrogen atom from the receptor (probably in a random fashion), giving material that is the same as the protonated starting material, but is obviously not obtained by protonation of the starting material. (Hence the word protonated in Scheme III is in quotations.) The lower arrow in Scheme III shows a pathway where the nitrogen radical cation loses an α-proton (the same as the step 2 in Scheme I) and the resulting α-amino radical picks up a hydrogen atom from the receptor, giving material that is the same as the starting material, but is not the recovered starting material since the α-hydrogen was exchanged. (Hence the word recovered in Scheme III is in quotations.) This pathway has been observed to occur during photooxidations of tertiary amines[3].

If the morphine radical cation at the receptor follows the pathway(s) shown in Scheme III, even as a rare event, it is conceivable that the abstraction of H· from the receptor would be random and, thus, may cause damage of the receptor site normally involved in the *N*-demethylation, as well as to the electron acceptor at the receptor. The damage of the receptor may become permanent and irreversible in the cases of prolonged use or abuse of morphines. The extent of the damage to the electron-acceptor site does not have to be the same as that to the *N*-dealkylation site. The opiate response still may be achieved if the latter site is damaged. The observed decrease of the brain *N*-demethylation of morphine by tolerance (7) may be caused by the damage of the *N*-dealkylation sites.

In summary, our view of the clastic binding process is the following. The attraction of morphine to its amine-binding site at the receptor is proposed to be achieved *via* formation of a RARC (charge-transfer) complex (Scheme II), an event which is *not* accompanied by the opiate response. Only occasionally a true electron transfer, *i.e.*, the electron injection, to the receptor occurs, leading to the opiate response. Normally, this electron injection is accompanied by *N*-demethylation (Scheme I), but it does not have to be, since other chemically feasible pathways are available, leading to "recovered" or "protonated" morphine, for example (Scheme III). These pathways may normally operate for various *N*-alkyl opiates which may not be dealkylated in the brain and, in the case of tolerance, when the *N*-demethylation is decreased.

Chemical considerations presented above in conjunction with the pharmacological observations on the *N*-demethylation of morphine in the brain suggest that the extended version of the clastic binding hypothesis is feasible both chemically and pharmacologically. Therefore, a detailed study of the electron-transfer reactions of morphines seems worthwhile.

Study of Electron-Transfer Reactions of Morphines in an *In Vitro* Model System—Despite a very extensive research in the field of opiate receptors, relatively little is known about these receptors because they are quite labile

[1] We are suggesting the term RARC pair for the caged radical-anion–radical-cation pair in accordance with the nomenclature introduced by Meyers *et al.* (12) who named the caged radical-anion–radical pair RARP.

[2] The RARC pair is an outer-sphere electron-transfer complex (13). In the latter complexes no bonds are made or broken during the course of the reaction (13). The immediate effect of the outer-sphere electron transfer is the change in the charge on the reactants (13). In the example shown in Scheme II the electron donor (nitrogen) which was neutral became positive, and the electron acceptor (E) which was neutral became negative. These complexes are also called charge-transfer complexes.

[3] Private communication by Prof. Fred D. Lewis, to whom thanks are expressed.

and easily denatured (16). The true nature of these receptors has not as yet being elucidated; after years of careful research by independent investigators, there still exists appreciable controversy over whether the receptor is a protein, a lipid, or both (16, 17).

The question if the protein or the protein part of the opiate receptor can function as an electron-transfer protein, as required by the clastic binding hypothesis, cannot be answered at this time since so little is known about the opiate receptor. If the opiate receptor protein can indeed function as an electron-transfer protein, one may visualize it, in general terms, as shown in Scheme IV (18):

Source of Electron ⟶

Way In ⟶ Trap ⟶ Way Out ⟶ Sink

Scheme IV

Scheme IV represents a directed electron transfer from a source of electron, morphine in this case, to the electron-transfer protein, in this case the opiate receptor (the enclosed block). In this model, the electron-transfer protein has the capability of controlling the electron transfer by a mechanism which "gates" either the "entering" or "leaving" channel. The movement of an electron through the protein may be realized, as suggested previously (18), *via* a localized hop mechanism and electron tunnelling.

We shall concentration only on the "way in" channel of the opiate receptor protein (Scheme IV), since the clastic binding hypothesis deals thus far only with this part of the opiate receptor. It is our desire to model this "way in" channel in such a way as to fulfill the chemical requirements of the proposed extended form of the clastic binding hypothesis, and then to study the chemistry of the electron transfer from morphines in such a model.

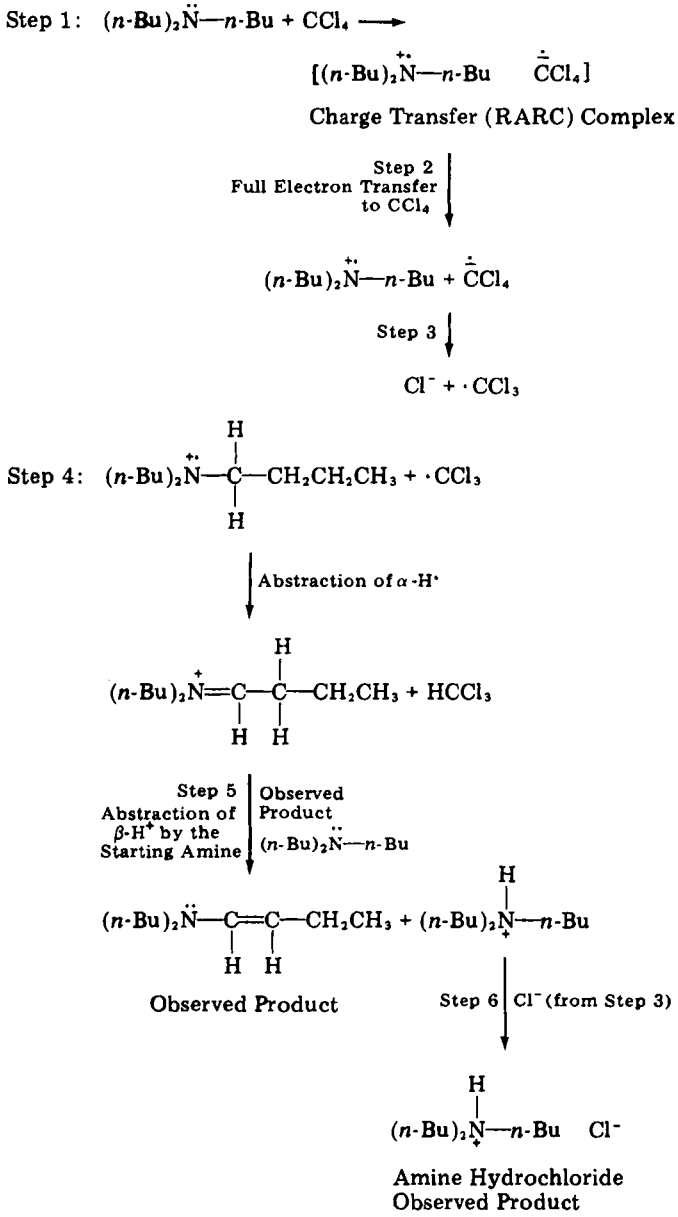
The "way in" channel in Scheme IV is actually the point of injection of the morphine N lone pair to the receptor, and it already has been discussed as "the electron acceptor at the receptor" (E) (Scheme II; see previous section). The chemical requirement for E was that morphine nitrogen can form a persistent RARC or charge-transfer complex with E (Scheme II), which only rarely leads to the actual loss of the N lone pair electron to the electron acceptor, *i.e.*, to the full electron transfer (Schemes I and III).

We decided to explore a possibility that perhalomethanes (CX_4, X = Cl or Br) may represent such models, since we were familiar with their exceptionally good electron-accepting ability (12, 19). In this paper we explore the behavior of carbon tetrachloride (CCl_4) in its reactions with amines in general and with a series of morphines. Work with CCl_3Br, which is a better electron acceptor than CCl_4, is in progress.

The reaction of amines with CCl_4 has been studied extensively (20-30). It is a well-established fact that amines—primary, secondary, and tertiary aliphatic, as well as aromatic—form charge-transfer complexes with CCl_4 (20, 25-27, 30). For example, amines such as triethylamine, di-*n*-propylamine, and tri-*n*-propylamine have been found spectrometrically to form 1:1 charge-transfer complexes with halomethanes (25). The K_f value for the *n*-butylamine-CCl_4 complex of 0.032 M^{-1} indicates a weak bond between the two species, as expected in a charge-transfer complex (27). Since these charge-transfer complexes are outer-sphere electron-transfer complexes in which no bonds are made or broken, their persistency shows as a lack of the actual chemical reaction.

When the N lone pair electron is fully transferred to CCl_4, a series of chemical reactions are initiated which ultimately lead to amine hydrochloride and other products (Scheme V). Such reactions in the case of morphines must be very slow if CCl_4 is to be used as a model for the electron acceptor at the opiate receptor, since the actual loss (injection) of the N lone pair electron to the receptor is proposed to be a rare event. Since the ultimate product of the reaction of an amine with CCl_4 is the amine hydrochloride, as shown in Scheme V and amply demonstrated in other examples (20-29), the speed and the extent of this reaction can be determined by measuring, respectively, the rate of appearance and the amount of amine hydrochloride, or just the halide ion.

The literature data on the rates of the reactions of amines with CCl_4 could not be extrapolated with confidence to the case of morphines, since they are somewhat contradictory. Thus, reports exist stating that amine hydrochloride precipitates were formed rather rapidly after dissolving the amines in CCl_4 (25, 27) and also that the formation of such precipitates is extremely slow (21). These discrepancies may be due to the uncontrolled presence of catalysts, such as UV light, oxygen, Cu^+, Cu^{2+}, Fe^{2+}, brass, and even traces of metal impurities from a stirring bar, all of which have been found to influence the reaction rate. However, the influence of these catalysts is not clear in all the cases. Thus, one report states that the reaction is prevented if light or other catalysts are excluded (26), but another report describes that the reaction proceeds in dark without a catalyst (28).



Scheme V—Chemical reactions leading to the amine hydrochloride (based on Ref. 28).

Therefore, since there were no satisfactory literature data that we could extrapolate to the case of morphines, we undertook the study of the reactions of morphine, oxymorphone, and naloxone with CCl_4.

EXPERIMENTAL

Melting points were determined on a capillary melting-point apparatus[4] and were not corrected. The NMR spectra were taken on a 90-MHz spectrometer[5], and the IR spectra were recorded on a spectrophotometer[6]. TLC was done on aluminum sheets precoated with 0.2 mm of silica gel[7]; the eluant was chloroform–methanol–concentrated ammonium hydroxide (132:12:0.9 by volume). The spots were observed in a UV chamber at the wavelength of 254 nm. The carbon tetrachloride used was an ACS-analyzed reagent[8], containing only 0.5 ppm of chloride ion and 0.02 ppm of heavy metals. Oxymorphone hydrochloride[9], naloxone hydrochloride[9], and morphine[10] were from outside sources.

Conversion of Naloxone Hydrochloride to Naloxone—A solution of nal-

[4] Thomas-Hoover.
[5] Perkin-Elmer R 32.
[6] Beckman IR-10 and Perkin-Elmer 297 spectrophotometers.
[7] Silica gel 60, F_{254}; EM Reagents.
[8] MCB.
[9] ENDO Laboratories.
[10] Scripps Clinic and Research Foundation.

Table I—Reaction of Selected Morphines and Other Amines with Carbon Tetrachloride

Amine	Reaction Time, h	Isolated Product(s)	Yield, %	Reference
Naloxone	3	Trace of amine hydrochloride was presumably formed[a]	trace of Cl^- was observed	this work
Morphine	20	—	—	this work
Oxymorphone	30	Trace of amine hydrochloride was presumably formed[a]	trace of Cl^- was observed	this work
Triethylamine	72	Amine hydrochloride	2.2	21
2-Diethylaminoethanol	72	Amine hydrochloride	24.4	21
Piperidine	24	—	—	22
	72	Amine hydrochloride	2.6	22

[a] See *Experimental*.

oxone hydrochloride (0.70 g, 0.0019 mol) in ethanol and water was poured into an aqueous solution of borax[11] (sodium tetraborate, $Na_2B_4O_7 \cdot 10H_2O$; certified ACS). The resulting solution was extracted three times with chloroform (ACS quality, distilled). The combined chloroform extracts were washed with borax and water, dried (anhydrous magnesium sulfate), and evaporated leaving naloxone as 0.56 g of a white solid (90% yield), mp (chloroform–petroleum ether) 177–178.5°C, (ethyl acetate) 177–178.5°C [lit. (31) mp (ethyl acetate) 177–178°C, 184°C]. The crude as well as the recrystallized material was TLC pure, R_f 0.65.

Treatment of Naloxone with Carbon Tetrachloride—Naloxone (0.35 g, 1.1×10^{-3} mol) was mixed with carbon tetrachloride (30 mL, ~300 times molar excess over naloxone) and the mixture was refluxed to achieve dissolution. After the dissolution was complete, the reflux was continued for 1.5 h under the laboratory fluorescent lights. Air was not excluded, as the reflux was performed in a reflux apparatus equipped with a $CaCl_2$ drying tube on top of the reflux condenser. The solution was then stirred at room temperature for the 1.5-h period. After that time, the solvent was evaporated on a rotary evaporator, and the white solid residue was dried in a vacuum oven, giving 0.34 g of the material (97% recovery).

The IR spectrum of this material was essentially identical with that of the starting material. The C=O stretch was at 1728 cm^{-1} (nujol) as in the starting material, and no peak was observed at 1713 cm^{-1}, which corresponds to the C=O stretch of naloxone hydrochloride (nujol). The NMR spectrum ($CDCl_3$) of the recovered material was essentially identical with that of the starting material. TLC showed only one spot at R_f 0.65, corresponding to naloxone (the eluant would convert any naloxone hydrochloride to naloxone), indicating that no oxidative-degradation products were present.

A test for the presence of trace amounts of naloxone hydrochloride was performed as follows. The recovered material was dissolved in ether (ACS quality, distilled). The ethereal solution was washed three times with distilled water, the combined aqueous layers were acidified with 6 M HNO_3, and an aqueous solution of $AgNO_3$ was added. At first no cloudiness appeared, and only after ~5 min of standing a barely visible cloudiness developed, but no visible precipitate was formed. The theoretical yield of AgCl is 0.154 g. We have found that the AgCl test described above gave an immediate precipitate when applied to only a few milligrams of oxymorphone hydrochloride. This AgCl test gave us >99% accuracy when applied to the determination of chloride ion in a small (0.4×10^{-3} mol) sample of pure thymoxyethyl diethylamine hydrochloride (theoretical yield of AgCl, 0.058 g).

The ether which was used for the extraction, when treated as above (washed with water, the aqueous layer acidified with 6 M HNO_3, and aqueous $AgNO_3$ added), gave a completely clear solution which remained clear even after prolonged standing, thus indicating the absence of chloride ion as determined by the AgCl test. An experiment (blank) was also performed, in which 50 mL of CCl_4 was refluxed under laboratory lights for 13 h and stirred at room temperature in the dark for 17 h, and then worked up in the identical manner as the sample containing naloxone. No trace of chloride ion was observed in the AgCl test.

Conversion of Oxymorphone Hydrochloride to Oxymorphone—Oxymorphone hydrochloride (0.86 g, 0.025 mol) dissolved in water was converted into oxymorphone in the same fashion as the naloxone hydrochloride conversion into naloxone, yielding 0.48 g of oxymorphone (62%), mp (chloroform–petroleum ether) 249–250°C (dec.) [lit. (31) mp. (boiling ethanol, ethyl acetate, or benzene) 248–249°C (dec.)]. The crude as well as the recrystallized material were TLC pure, R_f 0.55.

Treatment of Oxymorphone with Carbon Tetrachloride—Oxymorphone (0.14 g, 0.47×10^{-3} mol) was treated with carbon tetrachloride (50 mL; ~1100 molar excess over oxymorphone) in the same manner as naloxone (*vide supra*), except that the reaction time was 10 times longer in the oxymorphone case. Thus, oxymorphone and CCl_4 were refluxed for 15 h under the laboratory fluorescent lights and stirred at room temperature for 15 h in the dark. The same work-up as for naloxone provided a white fine solid, which represented essentially quantitatively recovered starting material. The IR and NMR spectra of this material did not reveal the presence of oxymorphone hydrochloride and were identical to the corresponding spectra of the starting material. The AgCl test did not provide a visible precipitate, but the cloudiness observed was much more pronounced than in the case of the naloxone reaction; the theoretical yield of AgCl is 0.068 g. TLC showed the presence of only one spot, R_f 0.55, corresponding to oxymorphone, which indicated that no other compounds, such as degradation or oxidation products, were present.

Treatment of Morphine with Carbon Tetrachloride—Morphine (0.0965 g, 0.318×10^{-3} mol), mp 248–250°C (dec.) [lit. (31) mp for the monohydrate 254–256°C (dec.)], was treated with carbon tetrachloride (55 mL; ~1800 molar excess over morphine) under reflux and laboratory fluorescent lights for 5 h and at room temperature in the dark for 15 h. Again, as in the previous experiments, air was not excluded. After the above period, the solvent was removed by evaporation and the residual white solid was dried in a vacuum oven. The IR of this solid was identical, peak by peak, to the IR of the starting material.

This solid was suspended in water in a separatory funnel and shaken with ether, and the precipitate was removed by filtration. The white solid thus obtained showed only one spot on TLC (R_f 0.16) at the same place as morphine, thus indicating that no oxidative-degradation products were present. The aqueous and ether layers were separated. No material was recovered from the ether layer. The aqueous layer was acidified with 6 M HNO_3, and an aqueous solution of $AgNO_3$ was added. No precipitate or cloudiness was observed even after several hours. Only when the solution was looked at against a black background was a slight bluish-gray opaqueness, characteristic of AgCl, observed. After 10 d, a trace of precipitate (AgCl) was observed on the bottom of the flask.

RESULTS AND DISCUSSION

Morphine, oxymorphone, and naloxone, as freshly prepared free bases, were treated with CCl_4 open to air, under the laboratory fluorescent lights, and under reflux. A new polytef-coated stirring bar was used each time to avoid metal contamination, which is possible with used stirring bars. The carbon tetrachloride used was of analyzed-reagent quality and contained only 0.02 ppm of heavy metals. We used the above morphines, with an unprotected phenol moiety, so that the free phenol group could act as a built-in inhibitor (19) for free radicals: this narrowed the number of possible products. The extent of the reaction was followed by measuring the amount of chloride ion, which is formed by the decomposition of $CCl_4^{\cdot-}$ (step 3 in Scheme V). The recovered morphines were inspected by NMR and IR to see if there were any CCl_3-adducts in the phenol ring, since the latter ring would act as scavenger for the CCl_3 radicals formed simultaneously with the chloride ion (step 3).

We have found that the morphines studied react extremely slowly with CCl_4 (Table I and *Experimental Section*). For example, oxymorphone after 30 h of treatment with CCl_4 was completely recovered, with no spectrometric evidence of CCl_3- or other adducts, and only traces of chloride ion were detected.

Table I illustrates, for comparison, the literature results for some other amines of interest: triethylamine, as an example of tertiary amine; 2-diethylaminoethanol, as an example of a hydroxy amine in which, like in oxymorphone and naloxone, the hydroxyl group is in a close proximity to the amino group; and piperidine, a cyclic amine which is found as part of all the morphines. While the results for triethylamine and piperidine show slow reactions similar to our results for the morphines, the reaction of 2-diethylaminoethanol seems to be much faster. This point deserves further investigation. Further studies and evaluations of CCl_4 as an *in vitro* model for an electron acceptor at the receptor are in progress.

REFERENCES

(1) B. Belleau, T. Conway, F. R. Ahmed, and A. D. Hardy, *J. Med. Chem.*, **17**, 907 (1974).

[11] Fisher Scientific Co.

(2) J. Dimaio, F. R. Ahmed, P. Schiller, and B. Belleau, in "Recent Advances in Receptor Chemistry," F. Gualtieri, M. Giannella, and C. Melchiorre, Eds., Elsevier/North-Holland Biomedical, 1979, pp. 221-234.
(3) B. Belleau and P. Morgan, *J. Med. Chem.*, **17**, 908 (1974).
(4) A. H. Beckett, A. F. Casy, and N. J. Harper, *J. Pharm. Pharmacol.*, **6**, 874 (1956).
(5) J. Fishman, E. F. Hahn, and B. I. Norton, *Nature (London)*, **261**, 64 (1976).
(6) E. F. Hahn, B. I. Norton, and J. Fishman, *Biochem. Biophys. Res. Comm.*, **89**, 233 (1979).
(7) E. F. Hahn, B. I. Norton, and J. Fishman, in "Endogenous and Exogenous Opiate Agonists and Antagonists," E. L. Way, Ed., Pergamon, New York, N.Y., 1980, pp. 529-532.
(8) P. J. Smith and C. K. Mann, *J. Org. Chem.*, **34**, 1821 (1969), and references therein.
(9) L. C. Portis, V. V. Bhat, and C. K. Mann, *J. Org. Chem.*, **35**, 2175 (1970).
(10) F. D. Lewis and T.-I. Ho, *J. Am. Chem. Soc.*, **102**, 1751 (1980), and references therein.
(11) R. B. Silverman, S. J. Hofman, and W. B. Catus III, *J. Am. Chem. Soc.*, **102**, 7126 (1980).
(12) C. Y. Meyers, W. S. Matthews, L. L. Ho, V. M. Kolb, and T. E. Parady, in "Catalysis in Organic Synthesis," G. V. Smith, Ed., Academic, New York, N.Y., 1977, pp. 217, 260-278.
(13) N. Sutin in "Oxidases and Related Redox Systems," Vol. I, T. E. King, H. S. Mason, and M. Morrison, Eds., Wiley, New York, N.Y., 1965, pp. 37-50.
(14) V. M. Kolb, *J. Pharm. Sci.*, **67**, 999 (1978).
(15) A. Korolkovas, in "Essentials of Molecular Pharmacology," Wiley-Interscience, New York, N.Y., 1970, pp. 265-288, and references therein.
(16) N. M. Lee and A. P. Smith, *Life Sci.*, **26**, 1459 (1980), and references therein.
(17) T. M. Cho, P. Y. Law, and H. H. Loh, in "Neurochemical Mechanisms of Opiates and Endorphins, Advance Biochemical Phychopharmacology," Vol. 20, H. H. Loh, and D. H. Ross, Eds., Raven, New York, N.Y., 1979, pp. 69-101, and references therein.
(18) G. R. Moore and R. J. P. Williams, *Coordination Chem. Rev.*, **18**, 125 (1976), and references therein.
(19) C. Y. Meyers and V. M. Kolb, *J. Org. Chem.*, **43**, 1985 (1978).
(20) R. Foster, "Organic Charge-Transfer Complexes," Academic, New York, N.Y., 1969, pp. 278-280, 300, and references cited therein.
(21) R. F. Collins, *Chem. Ind. (London)*, **1957**, 704.
(22) N. H. Cromwell, P. W. Foster, and M. M. Wheeler, *Chem. Ind. (London)*, **1959**, 228.
(23) R. Foster, *Chem. Ind. (London)*, (**1960**) 1354.
(24) G. J. Beichl, J. E. Colwell, and J. G. Miller, *Chem. Ind. (London)*, **1960**, 203.
(25) D. P. Stevenson and G. M. Coppinger, *J. Am. Chem. Soc.*, **84**, 149 (1962).
(26) W. J. Lautenberger, E. N. Jones, and J. G. Miller, *J. Am. Chem. Soc.*, **90**, 1100 (1968).
(27) C. J. Biaselle and J. G. Miller, *J. Am. Chem. Soc.*, **96**, 3813 (1974).
(28) D. N. Kender, *Diss. Abstr. Int. B*, **37**, 1689 (1976).
(29) S. A. Markarian and H. Fisher, *J. Chem. Soc. Chem. Commun.*, **1979**, 1055.
(30) K. M. C. Davis and M. F. Farmer, *J. Chem. Soc. B*, **1967**, 28.
(31) The Merck Index, 9th ed., Merck and Co., Rahway, N.J., 1976.

ACKNOWLEDGMENTS

Presented at the National Meeting of the American Chemical Society, Seattle, Washington, March 1983, in the Medicinal Chemistry Section, Abstract 22 (Electron-Transfer Mechanism).

This work was supported in part by the University Research Foundation, La Jolla, Calif. Discussions of the electron-transfer reactions of amines with carbon tetrachloride with Prof. Cal Y. Meyers are gratefully acknowledged.

New Insights in the Clastic Binding Hypothesis for Opiate–Receptor Interactions II: Proton-Transfer Mechanism

VERA M. KOLB ˣ and STEVE SCHEINER

Received June 22, 1983, from the *Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901.* Accepted for publication August 12, 1983.

Abstract □ *Ab initio* (4-31G) molecular orbital calculations were performed on model systems to investigate the proton-transfer version of the clastic binding hypothesis for opiate-receptor interactions. Ammonia was chosen as the model for the nitrogen-containing portion of the opiate molecule, while ammonia and water were chosen as models for the proton acceptor at the receptor. The equilibrium position of a proton situated between the two molecules is found to be determined primarily by the orientation of the proton-donor molecule with some influence also from the other molecule. Misalignments of the lone pairs can significantly alter equilibrium populations when the proton affinities of the two molecules are similar.

Keyphrases □ Clastic binding hypothesis—proton-transfer mechanism, opiate-receptor interactions, *ab initio* molecular orbital calculations □ Opiate-receptor interactions—proton-transfer mechanism, clastic binding hypothesis, *ab initio* molecular orbital calculations □ Proton transfer—mechanism, clastic binding hypothesis, opiate-receptor interactions, *ab initio* molecular orbital calculations

Belleau *et al.* (1, 2) have presented concrete evidence that the relative spatial orientation of the lone electron pair on nitrogen (N lone pair) or morphine-type opiates is crucial for their opiate activity. On this basis they proposed the clastic binding hypothesis for opiate-receptor interactions (3). According to this hypothesis, morphine-type opiates achieve productive binding with the opiate receptor through a clastic binding process involving a stereospecific electron transfer from the nitrogen lone electron pair of the opiate to some electrophilic site at the opiate receptor. This hypothesis was recently analyzed from a chemical point of view and was found to be chemically feasible (4). An extended form of this hypothesis was proposed (4) to accommodate recent observations by Fishman *et al.* concerning the *N*-demethylation of morphines in the brain (5-7).

Belleau and co-worker (2) later proposed another version of the clastic binding hypothesis according to which the N lone pair of opiates is involved in a stereospecific proton transfer to the receptor leading to strong analgesia. For optimal analgesia, a morphine should have the N lone pair oriented properly for a facile proton transfer. This version of the clastic binding