(ii) pseudoplatelets and vacuoles containing bacteria in the same macrophage. Pseudoplatelet formation increases the surface area of neutrophils and the contact of their MPO with bacteria. Phagocytosis of pseudoplatelets by macrophages could be involved in their processing of bacterial antigens and in their complex interactions with lymphocytes and plasma cells.

The role of pseudoplatelets in inflammation, however, may not be strictly constructive or reconstructive. Their presence in synovial fluid in arthritic joints (*8*) could contribute to trauma as well as healing by facilitating the distribution of lysosomal hydrolase, neutral protease, and MPO. Indeed, differences in size and possibly chemotactic responses between pseudoplatelets and neutrophils could result in important differences in their distribution during inflammation. Neutrophils perform their most important functions at extravascular sites. The mature neutrophil, with its lobulate nucleus, is generally considered to be an end-stage cell. This nuclear segmentation may facilitate the cell's deformability and passage through the walls of blood vessels. Nuclear hypersegmentation was prominent in neutrophils in our pus samples, which contained many bacteria and pseudoplatelets with nuclear fragments. This suggests that pseudoplatelet formation is a natural consequence of the nuclear lobulation in the neutrophil that augments its role in acute inflammation. Moreover, the small size of the pseudoplatelet apparently permits its deployment to areas relatively inaccessible to the larger neutrophil.

JACOB S. HANKER
Laboratory of Cytostructure and Cytochemistry, Dental Research Center and *School of Dentistry, University of North Carolina, Chapel Hill 27514*
BEVERLY L. GIAMMARA*
Department of Anatomy, Health Sciences Center, University of Louisville, Louisville, Kentucky 40292

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* Present address: Microelectronics Center of North Carolina, Research Triangle Park, 27709.

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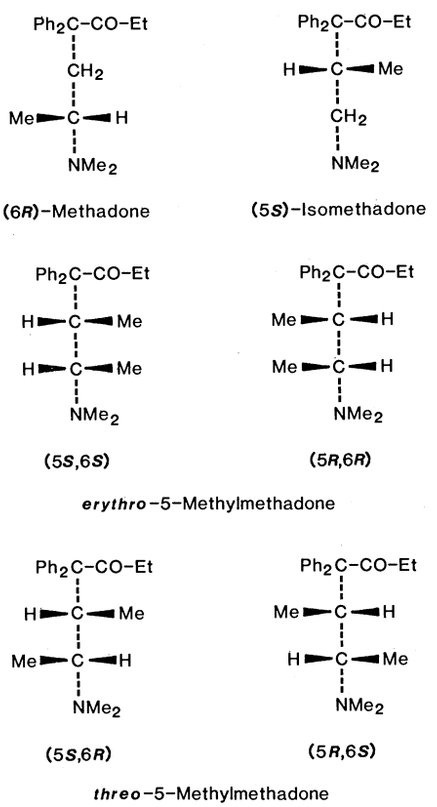
Methadone Conformation and Opioid Activity

Abstract. *The inactive methadone analog* threo-*5-methylmethadone has a solid-state conformation in which the nitrogen is antiperiplanar to the tertiary carbon C(4). Since* threo-*5-methylmethadone exhibits no opioid agonism either in vivo or in vitro, methadone analogs probably do not have this conformation when bound to an opioid receptor. The potent agonist (−)-*erythro-*5-methylmethadone has a solid-state conformation in which the nitrogen atom is rotated back toward the phenyl rings on the quarternary carbon, suggesting that this unusual conformation is the active one.*

The analgesic activity of methadone and some of its derivatives is attributed to its direct binding to opioid receptors (*1*, *2*). There is ample evidence that stereochemistry plays a dominant role in determining opioid receptor affinity (*3*, *4*). However, the inherent flexibility of the methadone molecule has made it difficult to identify unambiguously the molecular conformation that is responsible for receptor binding. The principal source of conformational flexibility in the methadone molecule is the presumed rotation about bonds linking the nitrogen atom and the quarternary carbon atom (Fig. 1A). Crystallographic and spectral analysis of methadone and isomethadone indicated that an antiperiplanar (*5*) arrangement of the charged nitrogen atom and the phenyl-substituted carbon atom may be one of the pharmacophoric conformations (*6*).



Addition of a 5-methyl substituent to methadone altered its activity. In three standard tests the (−) enantiomer of *erythro*-5-methylmethadone was at least five times as potent as (−)-methadone and (−)-isomethadone, whereas the *threo* racemate was totally inactive (*7*–*9*). Inspection of space-filling models showed that the flexibility of methadone is greatly restricted by 5-methyl substitution. The conformation of (−)-*erythro*-5-methylmethadone (Fig. 1C) in the solid state (*7*) is one in which the nitrogen atom is +clinal ($\tau = 97°$) to the phenyl-substituted carbon atom. Molecular mechanics calculations indicate that this is the minimum energy conformation (*10*). Hence the +clinal conformation may be more important than the antiperiplanar arrangement for receptor interaction of methadone and its derivatives. Analysis of the crystal structure of inactive *threo*-5-methylmethadone was undertaken to provide further information about conformational requirements for activity.

threo-5-Methylmethadone has the extended conformation (Fig. 1D). Molecular mechanics calculations indicate that this conformation of the *threo* isomer is

within 1 kcal/mole of the global minimum. However, by these same calculations, the *threo* isomer in the +clinal conformation, observed in the *erythro* compound, is 10 kcal/mole higher than it is in the extended form (*10*). If (−)-methadone and (−)-isomethadone in the extended conformation bind to the receptor responsible for analgesic activity (*11*), there should be nothing to prevent the *threo* isomer from binding in the same conformation. However, the total absence of agonist activity in tests in vivo and in vitro suggests that this conformation is not the one responsible for analgesic activity. The possibility cannot be ruled out that only one of the two compounds, (−)-methadone or (−)-isomethadone, binds in an extended conformation to the opioid receptor and that the additional methyl group on the *threo* isomer interacts unfavorably in that conformation with the receptor.

The exclusion of the extended conformation as an active pharmacophore lends further support to the hypothesis that the +clinal conformation observed for the highly active (−)-*erythro*-5-methylmethadone in the solid state (*7*) is a major contributor to receptor binding resulting in analgesic activity.

The reduced activities of (−)-methadone and (−)-isomethadone relative to (−)-*erythro*-5-methylmethadone are also compatible with their solid-state conformations. Crystallographically observed conformations are usually very near to the local, if not the global, minimum in the energy surface (*12*). The data suggest that for (−)-methadone and (−)-isomethadone the lowest energy conformation of the protonated molecule is one in which the nitrogen is in the inactive extended conformation but that in solution some fraction of the population of molecules is in the active +clinal conformation. Apparently 5-methyl addition to methadone removes this flexibility, fixing the *threo* and *erythro* isomers in the inactive and active conformations, respectively. Inspection of the observed structures suggests that the energetically favored conformations are those in which both of the nonhydrogen substituents on C(6) are *gauche* to the C(5) methyl, thus stabilizing an extended nitrogen conformation in the *threo* isomer and a +clinal nitrogen conformation in the *erythro* isomer.

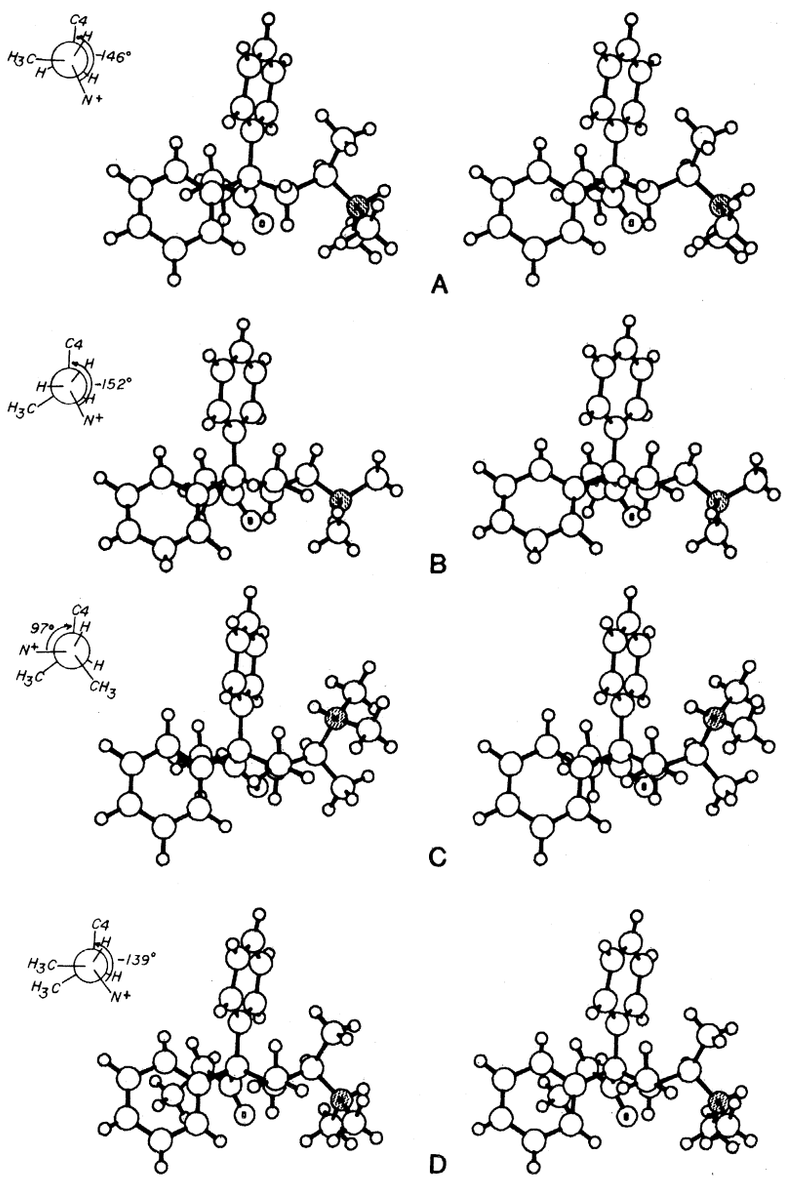


Fig. 1. Stereo ORTEPS of the crystallographically observed conformations of (A) (−)-methadone (*13*), (B) (−)-isomethadone (*14*), (C) (−)-*erythro*-5-methylmethadone (*7*) and (D) (5*S*,6*R*)*threo*-5-methylmethadone. Newman projections of [C(6)→C(5)] are inset for each structure. The orientation of the phenyl rings and C(2) through C(6) are similar in all four compounds. The most active compound, (−)-*erythro*-5-methylmethadone, differs in conformation from the less active (−)-methadone, (−)-isomethadone, and the totally inactive (5*S*,6*R*)*threo*-5-methylmethadone in the orientation of the nitrogen. Because (5*S*,6*R*)*threo*-5-methylmethadone, for which the extended conformation is preferred, exhibits no agonism whatsoever, (−)-methadone and (−)-isomethadone are probably not in the extended conformation when bound to an opioid receptor.

WILLIAM L. DUAX
G. DAVID SMITH
JANE F. GRIFFIN
Molecular Biophysics Department, Medical Foundation of Buffalo, Buffalo, New York 14203
P. S. PORTOGHESE
Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis 55455

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