Relationships between Opiate Receptor Binding and Analgetic Properties of Prodine-Type Compounds

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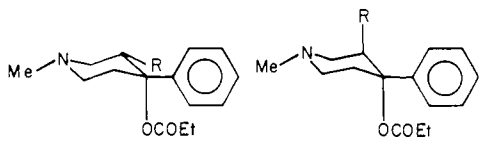
The receptor binding affinities of some diastereoisomeric prodine analogues have been compared with their analgetic activities determined by the hot-plate test in mice. The close correlation found between these potencies indicates that the relative analgetic activities of the α/β isomers are determined primarily by the appropriate association to the receptor sites.

In the course of our studies on the influence of geometrical isomerism upon analgetic activity of α- and β-prodine-type compounds, it has been demonstrated recently that, except for the pair with a C-3 Me substituent, the trans 3-R/4-Ph geometry rather than the cis geometry leads to more active isomers.[1]

Assignment of the configuration trans to α and cis to β isomers was based on chemical studies and the ^{1}H NMR characteristics of isomeric esters.[1-3]

The greater analgetic activity of the α form when R $\neq$ Me, could be related to events at the receptor sites or to differences in transport and distribution of the compounds. In the present study we have investigated the affinity of binding of some isomeric pairs (1–4) to opiate receptors from rat brain homogenates.



α form β form

1, R = Me
2, R = Et
3, R = allyl
4, R = hexyl

The affinities of α- and β-prodine-type compounds for the opiate receptor, compared with their analgetic activities in vivo, are summarized in Table I. The K values represent the concentration required to displace 50% of the specifically bound [^{3}H]dihydromorphine and it is an estimate of the apparent dissociation constant of the analgesic–receptor complex under the experimental conditions used.[4] By plotting the log of ED_{50} (nM/kg) against the corresponding log of K (nM), all the points (except β-**4**) were on a straight line, whose slope (s = 1.16) and linear correlation coefficient (r = 0.99; n = 7), calculated by the method of least squares, were close to unity (Figure 1).

These results indicate that there is almost complete correspondence of analgetic potency with receptor affinity in this class of piperidines. Further this relationship reveals that differences due to absorption, distribution, and metabolism should be minimal in these isomeric pairs.

The high receptor affinity found for the allyl isomer α-**3** emphasizes the association of the equatorial allyl group to an additional binding site of the receptor.[2,5] The enhanced affinity displayed by the more potent enantiomer (+)-α-**3** confirms the stereoselectivity of the opiate receptors.[6] The hexyl derivative β-**4** showed a very weak analgetic activity, poorly dose-related (Table I), and its relatively high affinity for the receptor may be due to some weak antagonist property that the C-3 hexyl group confers

Table I. Opiate Receptor Affinities and Analgetic Activities of Prodine-Type Compounds

Compd[a]	Receptor affinity,[b] K (nM)	ED_{50}, mg/kg sc, in mice,[c] hot-plate (95% confidence limits)
α-1	80	0.92 (0.73-1.17)
β-1	20	0.18 (0.13-0.23)
α-2	40	0.40 (0.30-0.60)
β-2	200	3.5 (2.5-4.8)
(+)-α-3	3	0.03 (0.02-0.04)[d]
α-3	6	0.09 (0.07-0.10)
β-3	400	11.7 (9.4-14.6)
α-4	*e*	Inactive at 80
β-4	100	54.4 (14.9-198.8)[f]
Morphine	3	1.2 (0.9-1.3)

[a] All compounds were administered as HCl salts. [b] Expressed as the concentration required for 50% inhibition of specific binding of [^{3}H]dihydromorphine. [c] Reference 1. [d] This specimen was obtained from Professor P. S. Portoghese (ref 5) through Dr. May's collection. [e] This compound, at high concentration (10^{-5} M), displaces both specifically and nonspecifically bound [^{3}H]dihydromorphine. [f] Poorly dose-related response.

to the molecule in the same way as C_5–C_7 *N*-alkyl chains do in the ketobemidones, an analogous class of piperidine compounds.[7] The hexyl derivative α-**4**, which was inactive as an analgetic in the hot-plate test (Table I), competed with [^{3}H]dihydromorphine for receptor binding at a high concentration (10^{-5} M), but in contrast to all the piperidines tested, it displaced both specifically and nonspecifically bound dihydromorphine, as Klee and Streaty found for some tranquilizers or pseudocholinesterase inhibitors.[4a]

From the results here reported it seems reasonable to assume that changes in analgetic activity seen in the α/β pairs may be clearly related to receptor interaction. The β configuration appears to be superior to the α configuration only when the C-3 axial substituent is a methyl group (1). However, with lengthening of the side chain (**2**, **3**) it is the α configuration, with an equatorial C-3 substituent, which better fulfills the steric requirement of the receptor.

Experimental Section

Syntheses and configurations of the piperidines tested were reported previously.[1] The [7,8-^{3}H]dihydromorphine (specific activity 51.2 Ci mmol^{-1}) was obtained from New England Nuclear Corp. The material was stored after dilution with 0.32 M sucrose to a concentration of 10^{-8} M at -20 °C. All operations involving [^{3}H]dihydromorphine were carried out in darkness or in very dim, indirect daylight because of the photosensitivity of this compound.[8]

Binding experiments were carried out as described by Klee and Streaty.[4] Rat brain homogenates were prepared in 0.32 M sucrose

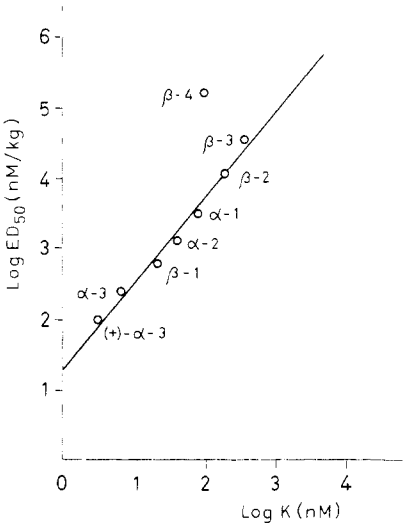


Figure 1. Correlation between analgetic activities and receptor affinities of α- and β-prodine-type compounds. The line was drawn from log y = 1.16 log x + 1.38; correlation coefficient r = 0.99. The value for β-4 was omitted in the calculation of the regression line because this compound showed a very poorly dose-related analgetic response.

(10 ml/g of brain) by the general procedure of Whittaker.[9] Osborne Mendel rats (150–200 g) from the NIH colony were used. The homogenates were centrifuged for 10 min at 1000g and the supernatant fraction was then centrifuged at 10000g for 20 min. This pellet (P_2) was resuspended in the original volume of 0.32 M sucrose and used within 24 h of storage at 0 °C or stored in 2-ml aliquots at −60 °C (at this temperature the pellet was stable for many months).

Binding experiments were routinely carried out as follows. P_2 fractions were diluted with 8 vol of 0.32 M sucrose–0.01 M Tris–HCl, pH 8.0. This suspension (900 μl), which contained approximately 0.5 mg of protein, was used in each polycarbonate assay tube which also contained the substance to be tested as competitor of [^{3}H]dihydromorphine binding. As the final concentration, 100 μl of 10^{-8} M solution of [^{3}H]dihydromorphine in 0.32 M sucrose was added to each tube. Two blank tests without inhibitor and 14 increasing concentrations of the inhibitor were used in each run. Samples were kept at 0 °C prior to incubation. After incubation at 37 °C for 10 min, the samples were centrifuged at 19000 rpm (45000g) for 10 min in a refrigerated Sorvall centrifuge. The supernatant fluid was carefully withdrawn with a syringe equipped with a blunt tipped No. 18 needle and the pellet was rinsed with 1 ml of 0.32 M sucrose and again carefully withdrawn with the syringe. The pellet was suspended in 500 μl of 1% Triton X-100 and transferred to a scintillation vial. The assay tube was rinsed with a second 500 μl of 1% Triton which was added to the same vial after which 7.5 ml of Triton–toluene scintillation fluid[10] was added and the radioactivity determined.

Specific binding is defined to include only that portion of [^{3}H]dihydromorphine binding which can be displaced by narcotic analgesics or antagonists and not by their inactive stereoisomers.[11] The proportion of the total binding, which is specific by this criterion, decreases with increasing concentration of [^{3}H]dihydromorphine. At 10^{-9} M [^{3}H]dihydromorphine, the concentration used in our experiments, specific binding is approximately one-half of the total binding.

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9-Hydroxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine Derivatives as Analgesics

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Three 1,3-dimethyl-9-hydroxy-1,2,3,4,5,6-hexahydro-1,5-methano-3-benzazocine derivatives (**7**–**9**) have been synthesized and tested as analgesics. The synthesis of these compounds involved conversion of 1-methyl-7-methoxy-β-tetralone (**1**) by Mannich reaction with $MeNH_2$ and HCHO to give the 11-ketone **2**, from which **7**, **8**, and **9**, respectively, were obtained. These compounds have analgesic activity, and **7** was found to be comparable to codeine.

The compounds described in Table I were prepared and tested as analgesics because the closely related 2,3,4,5,-6,7-hexahydro-1,6-methano-1*H*-3-benzazonine[1] and 2,3,-4,5,6,7-hexahydro-1,6-methano-1*H*-4-benzazonine derivatives,[2] ring C enlarged benzomorphans, have been found to possess analgesic activity. The substituents chosen were those which often enhance this activity of 6,7-benzomorphans.[3]

Chemistry. Conversion of 1-methyl-7-methoxy-3,4-dihydro-2(1*H*)-naphthalenone (**1**)[4] to the desired framework **2** was achieved by Mannich reaction with $MeNH_2$ and HCHO in one step.[5] The carbonyl group in **2** was reduced to methylene by Wolff–Kishner reaction, giving compound **3**. On the other hand, ketone **2** was treated with $Ph_3P{=}CH_2$ to give the 11-methylene compound **4**. Hydrogenation of **4** over Pt catalyst in EtOH gave 11α-methyl