phase was determined before (usually ≈1 mg/10 ml) and after partitioning in the water phase by uv spectrophotometry against the appropriate blank. The given partition coefficient, P (Table I), is corrected for the ionized fraction at pH 7.2.

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Homologous *N*-Alkylnorketobemidones. Correlation of Receptor Binding with Analgesic Potency

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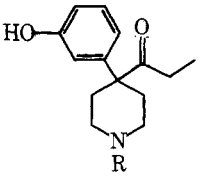
For a homologous series of *N*-alkylnorketobemidones a statistically significant correlation was found between the relative abilities to bind mouse brain homogenate *in vitro* and their *in vivo* mouse hot-plate analgesic potencies. The correlation between *in vitro* binding in the presence of 100 m*M* sodium and analgesic potency was not as good as that found in the absence of sodium. A statistically significant correlation was also found between their analgesic potencies and their abilities to antagonize electrically induced contractions of the guinea pig ileum.

It has been demonstrated previously that the analgesic potencies of a wide range of narcotic analgesics generally parallel their binding affinities to a stereospecific opiate receptor in brain homogenates.[1] Some notable exceptions are etorphine,[1] meperidine,[2] and codeine.[1] The first two compounds probably deviate because their high lipid solubilities[3] allow very rapid penetration into the brain. Codeine may first require metabolic O-demethylation before it is active.[1] A statistical correlation between *in vitro* binding and *in vivo* analgesic or antagonist potency has not been reported heretofore. One difficulty which can be foreseen is that of possible metabolic and/or distribution differences between the various classes of analgesics (*vide supra*). However, if such a correlation were achieved within some series of analgesics in which differences due to metabolism and/or distribution were minimized, it would provide additional evidence that events occurring at the *in vitro* receptor were in fact related to *in vivo* analgesic or antagonist activity. Herein we report the correlation between *in vitro* binding and *in vivo* analgesic potencies of a homologous series of analgesics all of which appear to have similar profiles of metabolism and distribution.

Results and Discussion

The preparation and analgesic potencies of the *N*-alkylnorketobemidones (1–7) have been reported.[4] We have extended this series through the decyl homolog (see Experimental Section) and the analgesic data in mice are presented in Table I. Binding assays were performed using mouse brain homogenate in the presence and absence of 100 m*M* sodium as described previously[2] and the concentration of drug necessary to displace one-half of the stereospecific

Table I. Binding and Analgesic Data



Compd	R	Hot-plate analgesic, m*M* ED_{50}[a]	Inhibition of [^{3}H]naloxone binding (1 n*M*), ED_{50}, n*M*[b]		Ratio of +NaCl/−NaCl
			No sodium	100 m*M* sodium	
1	Methyl	2.1 (1.4–2.8)	7–10	70	7–10
2	Ethyl	67.2 (52.0–87.0)	400	1500–2000	3.8–5
3	Propyl	16.0 (13.2–19.1)	200	800–1000	4–5
4	Butyl	4.6 (3.8–5.9)	50	600–700	12–14
5	Pentyl	0.78 (0.62–1.0)	8	30	3.8
6	Hexyl	7.5 (5.5–10.3)	20	40	2
7	Heptyl	9.0 (7.0–11.6)	20	40–50	2–2.5
8	Octyl	26.5 (20.2–34.9)	200	200	1
9	Nonyl	Inactive	700	700	1
10	Decyl	Inactive	500	600–700	1.2–1.5

[a] 95% confidence limits are shown in parentheses. Eight mice were used at each dose and a minimum of five doses for each compound was used.[12] [b] Refers to n*M* concentration of drug required to inhibit stereospecific [^{3}H]naloxone binding by 50%. Binding was performed using mouse brain homogenates (see ref 1 for methods).

[^{3}H]naloxone binding (ED_{50}) is shown in Table I. Correlation coefficients obtained by the method of least-squares analysis for the correlation between analgesic potency and the two types of binding are shown in Table II. (The correlation is only for the analgesically active compounds 1–8.) Also included in Table II is the coefficient for the correlation of analgesic potency and ability to inhibit electrically induced contractions of the guinea pig ileum.[5]

As shown in Table II, there is a highly significant correlation ($R = 0.9512$, $p \leq 0.001$) between analgesic potency and binding in the absence of sodium. This suggests that the relative *in vivo* potencies within this series are dependent primarily upon the ability to bind the receptor and that there would appear to be little difference between their *in vivo* distribution and metabolism. The excellent correlation ($R = 0.9886$, $p \leq 0.001$) between analgesic potency and inhibition of electrically induced contractions in the guinea pig ileum tends to support this view. Both metabolic and distribution factors are minimized when measuring a biological response in the isolated ileum.[6] If the ability to bind is, indeed, the primary determinant of relative analgesic potencies in this series, then compounds 1–8 would all appear to have very similar intrinsic activities[7] or abilities to induce a response once bound to the receptor.

Interestingly, the correlation between binding in the presence of sodium and analgesic potency is not as good ($R = 0.8382$, $p \leq 0.01$) as the correlation in the absence of sodium. In a previous evaluation of benzomorphan binding it was suggested that the best correspondence between *in vitro* binding and *in vivo* analgesic activity was obtained when binding was measured in the presence of sodium.[2a] However, this suggestion was based on a limited number of compounds and no rigorous statistical correlation was attempted. Inasmuch as the correlation presented in this paper is only for a phenylpiperidine type of analgesic, it may be profitable in the future to examine statistical correlations between a wider variety of analgesics and the two types of binding conditions.

Another critical factor to consider in attempting to obtain a correlation between *in vitro* binding and *in vivo* analgesic potency could occur with the agonist–antagonists.[8] The agonist–antagonists are generally strong *in vitro* binders relative to potent agonists[2] but in some *in vivo* analgesic tests show little or no activity. For example, nalorphine and cyclazocine, two strong agonist–antagonists, are 7.5 and 290 times more active in the Nilsen than in the hot-plate test,[9] whereas purer agonists such as morphine are approximately equipotent in the two tests. Therefore, the method of measuring analgesia is very important in making correlations if some of the compounds are agonist–antagonists. The correlation presented here may have been facilitated by the fact that none of the compounds were potent antagonists. Whereas some of the compounds had weak antagonistic properties (*vide infra*), they all had nearly identical potencies in both the Nilsen and hot-plate tests.[4]

Table II. Correlation Coefficients. Correlation[c] of m*M* Hot-Plate ED_{50}'s with Binding and Inhibition of the Guinea Pig Ileum

	R	R^2	*p*[a]
No sodium binding	0.9512	0.9047	≤0.001
100 m*M* sodium binding	0.8382	0.7026	≤0.01
Inhibition of guinea pig ileum[b]	0.9886	0.9733	≤0.001

[a] From two-tailed Student's t test. [b] Reference 5. [c] See ref 11.

Previously it was demonstrated that the ratio of the ability to displace [H^3]naloxone in the presence of 100 m*M* sodium and the absence of sodium was 1 for nearly pure antagonists, 1.7–7.0 for the agonist–antagonists tested, and 12 or greater for the predominantly agonist compounds tested.[2b] The sodium/no sodium binding ratios of compounds 1–8 predicted on this basis that the pentyl (ratio of 3.8), hexyl (2.0), and heptyl (2.25) compounds should have antagonist as well as agonist properties. This is in agreement with previous studies in monkeys[4] and was further confirmed in the guinea pig ileum.[5] In the ileum pentyl, hexyl, and heptyl compounds had antagonist potencies of 13, 8, and 4% of nalorphine, respectively. An apparent discrepan-

cy was observed in the case of the octyl compound 8. The binding ratio (1.0) indicated that it should be a nearly pure antagonist. However, no antagonist activity was detected in monkeys[10] (at 5.0 mg/kg) and only about 1% of the antagonist potency of nalorphine was observed in the guinea pig ileum.[5] As shown in Table I, 8 has weak but detectable analgesic potency. The reason for this discrepancy is not clear. The nonyl compound 9 and the decyl compound 10 were also predicted to be nearly pure antagonists based on this ratio (1.0 and 1.35, respectively). However, neither compound had sufficient potency in the hot-plate test, monkeys,[10] or the guinea pig ileum[5] for accurate characterization of its properties.

Some additional aspects of these results seem worthy of comment. The good correlation observed between binding and analgesic potency further suggests that this *in vitro* mixture contains a receptor involved in mediating the analgesic response *in vivo*.

Additionally, the observed correlation suggests minimal differences in metabolism and distribution at the time of peak analgesic effect in this series of compounds. Therefore, this series of compounds might be a useful tool in further investigation of the mechanism of opiate analgesia.

In summary, a statistical correlation was found between the *in vivo* analgesic potencies of a series of *N*-alkylnorketobemidones and both their relative binding abilities and their abilities to antagonize electrically induced contractions of the guinea pig ileum. The statistical correlation between *in vitro* binding in the presence of 100 m*M* sodium and *in vivo* determined analgesic potency was not as good as that which was found in the absence of sodium.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. The structures of compounds were in all cases supported by their nmr, ir, and mass spectra. Elemental analyses (indicated by C, H, Br, and N when within ±0.4% of theoretical values) were performed by the Section on Microanalytical Services and Instrumentation of this laboratory.

***N*-Octylnorketobemidone (8) Hydrobromide.** To 100 ml of DMF was added 5.0 g (0.021 mol) of norketobemidone, 4.5 g (0.024 mol) of 1-bromooctane, and 7.5 g of K_2CO_3, and the mixture was stirred overnight at 90–95°. The solvent was evaporated, the residue taken up in 250 ml of $CHCl_3$ and washed with H_2O, and the organic layer was dried (Na_2SO_4) and evaporated. This residue was dissolved in acetone, acidified to Congo Red with 33% HBr in AcOH, and crystallized by addition of ether. Recrystallization from ether–acetone gave white crystals, mp 137–139°. *Anal.* ($C_{22}H_{36}BrNO_2$) C, H, Br, N.

***N*-Nonylnorketobemidone (9) Hydrobromide.** Using the procedure described above for 8 white crystals were obtained, mp 146–148°. *Anal.* ($C_{23}H_{38}BrNO_2$) C, H, Br, N.

***N*-Decylnorketobemidone (10) Hydrobromide.** Using the procedure described above for 8 white crystals were obtained, mp 139–140°. *Anal.* ($C_{24}H_{40}BrNO_2$) C, H, Br, N.

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Hypocholesteremic Derivatives of Styrylacetic Acid. 1. *gem*-Dimethyl Analogs of Benzalbutyric Acid

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The preparation of α,α-dimethyl analogs of the hypocholesteremic and hypolipemic agent 3-methyl-4-phenyl-3-butenoic acid (β-benzalbutyric acid, BBA) is described. These compounds were prepared as part of a continuing program directed toward a study of the structure–activity interrelationships of styryl- and phenoxyacetic acid antilipemic agents and the preparation of metabolically resistent analogs of BBA. Preliminary results on the *in vitro* ability of the compounds to inhibit cholesterol biosynthesis indicate that α,α-dimethyl substitution reduces activity although the potency of the *p*-chloro analog **6b** was comparable to that of BBA.

The etiology of atherosclerosis has continued to remain obscure despite considerable research in this area. Therapeutic approaches to this disease have therefore centered on the minimization of epidemiologically defined risk factors and primarily on the reduction of serum lipoprotein levels with particular emphasis on cholesterol and its es-