acknowledge preliminary adrenergic evaluation of compounds 4a and 8a by Professors Milos Hava and Edward Walaszek.

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Relationship between Analgetic ED_{50} Dose and Time-Course Brain Levels of *N*-Alkylnormeperidine Homologues[1]

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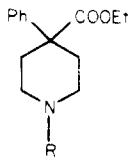
The brain levels of meperidine and three *N*-alkyl homologues were determined at equal analgetic iv doses in mice. The relative levels of the four compounds in brain were found to be closely proportional to their ED_{50} doses even though the compounds exhibit a wide range in partition coefficient and metabolic N-dealkylation. While lipid solubility and metabolism are undoubtedly important factors in the overall time-course brain levels, it appears that during the period of analgetic measurement (5–60 min after injection) these factors do not profoundly affect the relative brain levels because peak uptake occurs within the first 5 min after administration of the homologues. As a consequence, the observed ED_{50} potencies appear to provide a fair approximation of the relative receptor affinities of the four homologues. N-Dealkylation was observed as a major metabolic transformation by mouse liver in vivo for all four compounds, and the extent of this N-dealkylation was found to directly correspond to the rates of N-dealkylation by mouse liver homogenate seen in an earlier study.

Homologous series of analgetics that differ only in the N-substituent often show variation in the ED_{50} potency.[2] Although this simple structural modification usually does not lead to dramatic potency changes in the 4-phenylpiperidine class, the question concerning the origin of this potency variation is as yet unresolved. Thus, variation in ED_{50} potency may be due to receptor-related events, differential access to the biophase, or some combination of these.

For example, a homologous series of alkylnorketobemidones shows a good correlation between relative ability to bind mouse brain homogenate in vitro and their in vivo hot-plate analgetic potencies.[3] This observation suggests that within this series factors such as metabolism and distribution do not appear to have a profound effect on relative potency. A similar result was found in the same type of study on a series of *N*-alkyl derivatives of benzazocine.[4] By contrast, we have previously reported[5] a correlation between the analgetic ED_{50} doses of *N*-alkyl derivatives of normeperidine (1, R = H) in mice and their in vitro rates of N-dealkylation. The homologues which exhibited a greater tendency toward N-dealkylation also had higher ED_{50} values. The high correlation suggested that the relative rates of N-dealkylation in vitro are similar to those in vivo and that the variation in ED_{50} potency with chain length is related primarily to differences in availability to the biophase as a result of metabolism. Other studies[6,7] involving intraventricular administration of analgetics tend to implicate liposolubility as an important parameter contributing to relative potency based on standard modes of administration.



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In an effort to determine if the iv ED_{50} potencies of homologous *N*-alkylnormeperidines in mice parallel their brain levels, we have examined the time-course brain concentrations of four members (1, R = Me, *n*-Pr, *n*-Bu, *n*-hexyl) of this series. The results of the present study indicate that the observed potencies represent an approximation of their relative affinities for analgetic receptors.

Table I. Comparison of Analgetic Potencies of *N*-Alkylnormeperidines Based on Intravenous ED_{50} and Brain Concentration

N-Alkylnormeperidine	Partition coeff[a]	ED_{50} iv,[b] μmol/kg	Rel potency,[c] iv	Brain concn (integral 5-60 min),[d] nmol min/g	Rel potency,[c] integral brain concn
Methyl	19	32.8 (23.9-48.5)	1.0	854 (795-918)	1.0
n-Propyl	70	45.9 (36.2-103.1)	0.7 (0.5-1.1)	828 (760-902)	1.0 (0.9-1.1)
n-Butyl	136	19.1 (16.2-27.0)	1.7 (1.2-2.7)	274 (247-305)	3.1 (3.0-3.3)
n-Hexyl	608	3.7 (2.7-4.4)	8.8 (5.8-14.7)	126 (120-134)	6.8 (6.6-7.0)

[a] From octanol-phosphate buffer, pH 7.4 (see Experimental Section). [b] Reference 8. [c] Relative to meperidine = 1. [d] Extrapolated value.

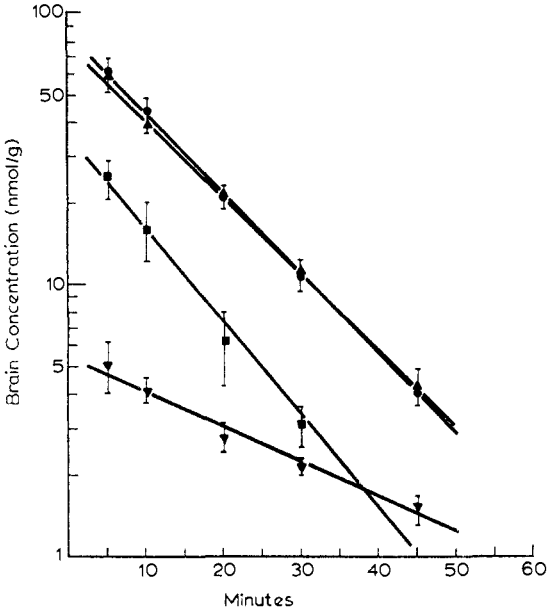

Figure 1. Decline of brain levels of *N*-alkylnormeperidines with time after iv administration of an ED_{50} dose in mice (●, meperidine; ▲, *n*-propyl; ■, *n*-butyl; ▼, *n*-hexyl). Each point is the mean observation on six animals ± the standard error.

Results and Discussion

Mouse whole brain and whole liver concentrations of the four *N*-alkyl derivatives were determined at 5, 10, 20, 30, and 45 min following a bolus ED_{50} dose in the lateral tail vein. The graphic representations of the results obtained for each compound are shown in Figures 1 and 2. As can be seen in Figure 1, the brain levels of all four compounds decrease throughout the time frame examined. The pattern of decline for all four compounds is statistically indistinguishable from a linear regression between log brain concentration and time over the 5–45-min interval, and this is consistent with a monoexponential kinetic pattern of brain concentration during this time period. Thus all four compounds appear to attain peak levels in the brain within 5 min.

The primary objective of the present study was to compare the relative potencies of the four homologues based on ED_{50} dose with those based on brain concentration. Since the analgetic potencies of the four homologues were obtained[8] by the method of Eddy[9] and consequently represent the cumulative biological response from 5 to 60 min after injection, the corresponding measure for the amount of each homologue present in brain during this period is represented by the area under the brain concentration–time curve. These areas are related to the amount of compound in whole brain at equiactive doses that is available to the biophase over the time interval and, consequently, should reflect the rank order affinities of the compounds for the receptors.

It is noteworthy that the rank orders of ED_{50} potencies and cumulative brain levels among the four homologues remain the same (Table I). Moreover, even though the rank order ratios afford slightly different values, they are statistically indistinguishable from one another. Since the linear correlation between the log ED_{50} dose and the log normalized[10] brain levels is significant ($r^2 = 0.90$), it appears that the ED_{50} doses do provide at least a fair approximation of the relative receptor affinities of the four compounds.

The four homologues vary considerably in lipid solubility

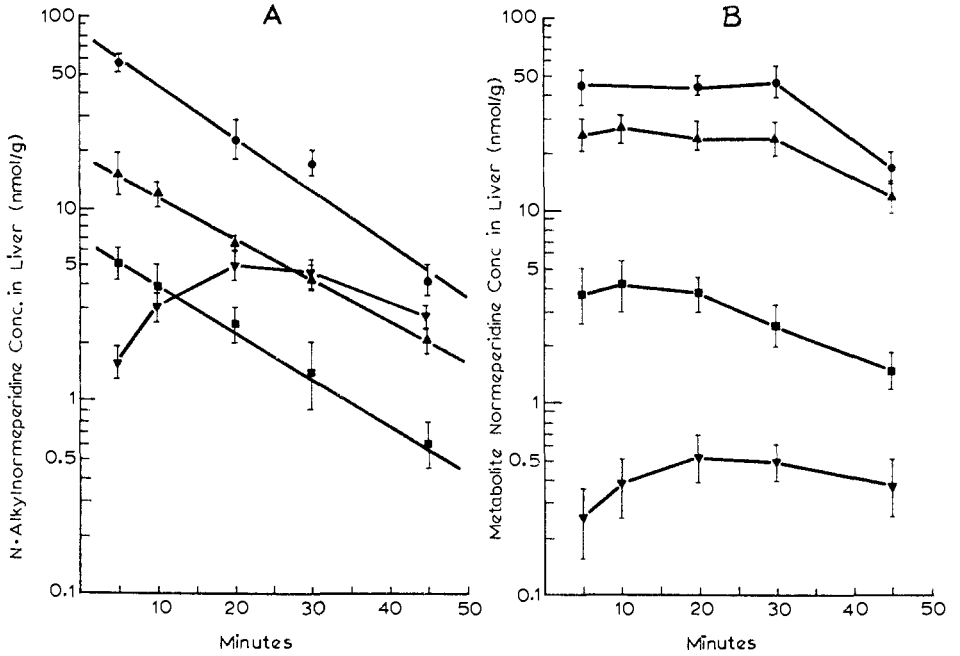

Figure 2. Time-course profiles of *N*-alkylnormeperidines (A) and their corresponding metabolite normeperidine (B) concentrations in the liver of mice following iv administration of an ED_{50} dose (●, meperidine; ▲, *n*-propyl; ■, *n*-butyl; ▼, *n*-hexyl). Each point is the mean observation on six animals ± the standard error.

Table II. Comparison of the Time-Course Integral Concentrations of Metabolite Normeperidine and N-Alkylnormeperidine Parent in Liver over 5-45 min

N-Alkylnormeperidine	N-Alkyl parent concn-time integral, nmol min/g	Normeperidine concn-time integral, nmol min/g	Normeperidine-N-alkyl parent integral ratio (N/P)	Percent N-dealkylation in vitro[a]
Methyl	926.7	1733.1	1.87	38.4
n-Propyl	263.3	917.7	3.49	62.8
n-Butyl	83.8	121.7	1.45	24.8
n-Hexyl	153.3	17.9	0.12	8.6

[a] Reference 5.

(Table I) and in their rates of metabolism (Table II), but these factors do not appear to play a significantly crucial role in regulating the brain levels during the period of analgetic measurement. Thus, linear correlation between log P vs. log normalized brain levels is poor ($r^2 = 0.066$) and no meaningful correlation defining a parabolic[11] relationship was found. Similarly, log normalized brain levels vs. log N-dealkylation in vivo afforded low correlation ($r^2 = 0.518$). While liposolubility and metabolism are undoubtedly important factors, the low correlations might reflect the extremely rapid access (peak levels within 5 min) of the four homologues into the brain after iv administration. Although the range in lipid solubility between the lowest and highest homolog is large, the partition coefficient of the lowest member is still high, and it is possible that the differential access of the compounds to the brain is minimal because of the rate-limiting effect of blood flow. This finding is in harmony with the finding[7] that with compounds of high lipophilicity there is much less difference between iv ED_{50} and intraventricular ED_{50} values when compared to compounds having much lower partition coefficients.

A second area of concern was to investigate a possible relationship between N-dealkylation of the four meperidine homologues in vitro and in vivo. Although all four compounds are known[5] to be N-dealkylated by mouse liver homogenate leading to a common metabolite (normeperidine), only meperidine has been reported[12] to undergo this transformation in the intact animal.

In our studies, normeperidine was the only basic metabolite detected in the liver samples. This finding confirms N-dealkylation as a major metabolic transformation in vivo for the four homologues. Figure 2 shows the time-course levels of the four homologues in liver, along with those of metabolite normeperidine derived from each compound. As can be seen, the levels of meperidine and N-propyl- and N-butylnormeperidine decrease throughout the time-course, while those of N-hexylnormeperidine show a peak during the same period. In addition, the three lower chain homologues undergo rapid N-dealkylation as evidenced by the high levels of metabolite by 5 min that in some cases equals the amount of parent present, while the amount of normeperidine from N-hexylnormeperidine is proportionally much lower.

The actual amount of each meperidine homologue and its metabolite accumulating in liver over the 5–45-min period, of course, cannot be directly measured because of their continuous exit from the tissue. However, the areas under the liver concentration–time curves do provide an estimate of the relative amounts of parent compound and metabolite present over the time-course if, as a first approximation, each member of the series has a similar exit profile. Values for the areas under the liver concentration–time curves for each N-alkyl parent and its metabolite normeperidine are given in Table II. The ratio (N/P) of metabolite to N-alkyl parent areas directly relates to the percent N-dealkylation as used in the in vitro data of an earlier study,[5] and the two measures serve as a basis of comparison for the two sources of data. The two parameters exhibit an excellent linear correlation ($r^2 = 0.98$) which in the present case establishes the liver homogenate data to be a good measure of predicting the rate of metabolism by the same tissue in the intact animal. In addition, the strong correlation indicates that N-dealkylation alone can account for the disappearance of each meperidine homologue from liver with time.

The rank order of time-course levels of the four N-alkylnormeperidine derivatives in liver parallels dose except for the N-propyl compound. In proportion to dose, the level of N-propylnormeperidine should be greater than that of meperidine. The levels actually observed could be accounted for in part by its (N-propyl) more rapid rate of metabolism. The reason for the slow accumulation of N-hexylnormeperidine in liver is not clear. One might speculate on noting the proportionally lower concentrations of each homologue in the liver relative to brain at early times (Figures 1 and 2), along with the partition coefficients (Table I), that the high lipid solubility of the N-hexyl compound may limit the amount initially taken up by the liver in relation to other tissues. With time the slower rate of metabolism of the N-hexyl compound may allow it to accumulate via redistribution processes and thereby lead to a peaking of concentration during the time frame observed.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. Radioactivity was measured with a Beckman LS-150 or Packard 3375 liquid scintillation spectrometer. The identity and radiochemical purity of radioactive materials were determined by TLC on alumina or silica gel sheets. The corresponding authentic unlabeled materials were used to check the physical properties of all radioactive materials.

[^{3}H]Normeperidine Hydrochloride. N-Nitrosonormeperidinic acid sodium salt[13] (0.5 g) was randomly labeled with tritium by catalytic exchange in tritiated solvent (Amersham/Searle) and, after purification of the regenerated acid by fractional crystallization from EtOH, it was esterified by treatment with diazoethane and denitrosated with HCl–urea. In a representative preparation, 100 mg (0.43 mmol) of [^{3}H]-N-nitrosonormeperidinic acid in EtOH (20 ml) was treated with diazoethane in Et_2O in portions until the yellow color of the reagent persisted. The lower boiling components were evaporated on a steam bath and the residual EtOH solution was concentrated in vacuo to about 30 ml. The concentrate was cooled in an ice bath and 3 g of HCl gas, 250 mg of urea, and a few lumps of Drierite were added. The mixture was refluxed for 1.5 hr and filtered. The filtrate was made neutral and the solvent was replaced with H_2O. After regeneration of the amine base with excess NH_3 and partitioning into Et_2O, the base was acidified (EtOH–HCl) and diluted with EtOH–Et_2O and the solution seeded with authentic unlabeled hydrochloride. There was isolated 90.3 mg (78%) of [^{3}H]normeperidine hydrochloride crystals which was recrystallized once from Et_2O–EtOH: mp 129–130°; sp act. 2.5 mCi/mmol; radiochemically pure by TLC [silica gel; EtAc–NH_4OH (95:5)].

[^{3}H]Meperidine Hydrochloride. [^{3}H]Normeperidine hydrochloride (0.56 mmol, 2.5 mCi/mmol) was dissolved in EtOH (15 ml). To the solution cooled in ice was added 60 mg of 5% Pd/C, 6 drops of glacial AcOH, and 0.35 ml of formalin. The reaction mixture was stirred under 1 atm of H_2 for 1 hr and then filtered. The filtrate was concentrated under vacuum and H_2O (10 ml) was added. A few drops of dilute HCl were added and the aqueous solution was washed once with Et_2O, made basic with excess NH_3, and extracted with Et_2O. The Et_2O extracts were dried ($MgSO_4$), acidified (ethanolic HCl), diluted with EtOH–Et_2O, and seeded with authentic unlabeled hydrochloride. After 1 day, white crystals (81 mg) were isolated and recrystallized once

from $(CH_3)_2CO$–Et_2O: mp 162–163°; sp act. 2.4 mCi/mmol; radiochemically pure by TLC [silica gel; EtAc–NH_4OH (95:5)].

N-Propyl[^{3}H]normeperidine Hydrochloride. [^{3}H]Normeperidine hydrochloride (104 mg, 0.39 mmol, sp act. 0.9 mCi/mmol) was mixed with 70 mg of 5% Pd/C, 3 drops of glacial AcOH, and 1.9 mmol of propionaldehyde in EtOH (15 ml) and the mixture was stirred for 1 hr under 1 atm of H_2. The usual work-up afforded 111 mg (93%) of crude hydrochloride which was recrystallized once from $(CH_3)_2CO$–Et_2O: mp 190–192°; sp act. 0.52 mCi/mmol; radiochemically pure by TLC [silica gel; Et_2O–NH_4OH (98:2)].

N-Butyl[^{3}H]normeperidine Hydrochloride. To 100 mg (0.37 mmol, sp act. 2.8 mCi/mmol) of [^{3}H]normeperidine hydrochloride in EtOH (15 ml) were added 50 mg of 5% Pd/C, 4 drops of glacial AcOH, and 1.9 mmol of butyraldehyde. After stirring the mixture for 1 hr under 1 atm of H_2, the usual work-up afforded 103 mg (84%) of white crystals which was recrystallized from $(CH_3)_2CO$–Et_2O: mp 182–183°; sp act. 2.4 mCi/mmol; radiochemically pure by TLC [silica gel; Et_2O–NH_4OH (98:2)].

N-Hexyl[^{3}H]normeperidine Hydrochloride. A mixture of [^{3}H]normeperidine hydrochloride (0.22 mmol, sp act. 2.3 mCi/mmol), 40 mg of 5% Pd/C, 3 drops of glacial AcOH, and 1.1 mmol of 1-hexanal in EtOH (10 ml) was stirred under 1 atm of H_2 for 1 hr. The reaction mixture on usual work-up afforded 49.9 mg of product which was recrystallized twice from EtAc–Et_2O: mp 159–160°; sp act. 2.1 mCi/mmol; radiochemically pure by TLC [silica gel; Et_2O–NH_4OH (92:8)].

Time Course Studies. ED_{50} doses of N-alkyl[^{3}H]normeperidines in saline were administered as a bolus to adult male Swiss–Webster mice (27–32 g) in the lateral vein of the tail. The solutions were prepared so that each animal received 0.033 ml/10 g of body weight. Mice were sacrificed by decapitation at various time intervals (5, 10, 20, 30, or 45 min) following administration and the brain and liver were removed from the carcass, rinsed with saline, blotted dry, weighed, and homogenized with 4.5 (brain) or 4.0 ml (liver) of 0.01 N HCl in glass tissue grinders.

Each brain or liver homogenate was mixed with 5 μg each of unlabeled N-alkylnormeperidine hydrochloride and unlabeled normperidine hydrochloride, and 0.5 nCi of [^{14}C]-N-butylnormeperidine hydrochloride which was employed as an internal standard. The mixture was basified (pH 12) with 1 N NaOH, shaken, and centrifuged with 10 ml of C_6H_6. The C_6H_6 phase (7 ml) was shaken with 3.0 ml of 0.1 N HCl and 2.5 ml of this phase was made basic with 1 N NaOH and shaken with 5.0 ml of C_6H_6. An aliquot (4.5 ml) of the C_6H_6 phase was made acidic with 5% HCl in EtOH and evaporated in vacuo to about 100 μl. EtOH (1 ml) was added and the mixture was again evaporated to about 40 μl. This volume was applied as a spot to a 1.5 × 8 cm scribed section of a 10 × 20 cm silica gel sheet (Eastman). After all brain or liver extracts were applied to various sections of a sheet, it was developed in Et_2O–NH_4OH (98:2). Samples of unlabeled N-alkylnormeperidine were included on each sheet to serve as positional markers. The developed chromatogram was allowed to dry and the scribed sections were cut out and the section length was cut into two portions according to the relative position of the unlabeled compound standards visualized with I_2. Each portion was placed in a vial and mixed with 1 ml of MeOH and 15 ml of liquid scintillation cocktail (Permablend I, Packard). The radioactivity of both nuclides (^{3}H and ^{14}C) was measured and all samples were counted for sufficient time to yield ≤2.5% error. Ratios of ^{3}H to ^{14}C cpm of the upper portion of each scribed section of chromatogram were compared with a preestablished linear standard curve of ratios (^{3}H/^{14}C) vs. amounts of N-alkyl[^{3}H]normeperidine hydrochloride added to blank brain or liver homogenate and processed in the same way to convert radioactivity to weight measure. Similarly, ratios of the ^{3}H cpm of the lower portion of the chromatogram section to the ^{14}C cpm of the upper portion were compared with a similar standard curve for [^{3}H]normeperidine to obtain nanomoles of [^{3}H]normeperidine present in each brain or liver sample.

Percentage recoveries of labeled compounds from brain and liver were 82–92%. The identity of radioactive materials in the C_6H_6 extracts was confirmed also by TLC on basic alumina (Eastman) using EtAc as the development solvent. The amounts of radiolabeled materials in liver were corrected for the amounts present in the residual blood volume of liver homogenate. The latter was estimated by the method of Meijer[14] and is represented by a mean value of 5.8% (v/w).

Partition Coefficients. Partition coefficients for the four N-alkylnormeperidines were determined from a 1-octanol–phosphate buffer (pH 7.4) system. Each phase was presaturated with the other before use. 1-Octanol (1 ml), 0.9 ml of phosphate buffer, and 0.1 ml of N-alkyl[^{3}H]normeperidine hydrochloride in phosphate buffer were shaken for 10 min and centrifuged for 10 min. Analyses were carried out on 100-μl aliquots of each phase by liquid scintillation spectrometry. The concentrations employed for each N-alkyl[^{3}H]normeperidine hydrochloride were below 10^{-4} M but sufficient to provide adequate cpm above background for each aliquot. Partitioning experiments were performed in triplicate and each phase was assayed in duplicate. Partition coefficients were calculated from the ratio of cpm of octanol aliquot to cpm of buffer aliquot and corrected for the relative quenching between the two phases.

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