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Narcotic agonist and antagonist potencies of a homologous series of *N*-alkyl-norketobemidones measured by the guinea-pig ileum and mouse vas deferens methods

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The narcotic agonist and antagonist potencies of the series of *N*-alkyl-norketobemidones from norketobemidone to decylnorketobemidone have been determined. The values obtained in the electrically stimulated preparations of the guinea-pig ileum and the mouse vas deferens are closely correlated. The agonist potencies observed in the guinea-pig ileum agree well with those found in the mouse hot-plate test (Oh-ishi & May) and those obtained by determining the inhibition of naloxone binding in brain homogenates (Wilson, Rogers, Pert & Snyder). The antagonist potencies in the guinea-pig ileum and, to a lesser extent, those in the mouse vas deferens agree with the values obtained in the morphine-dependent monkey.

It has been shown that the depressant effects of narcotic analgesic drugs on the electrically evoked contractions of the longitudinal muscle of the guinea-pig ileum or of the mouse vas deferens accurately predict the analgesic potencies of these drugs in man (Kosterlitz, Waterfield & Berthoud, 1974; Hughes, Kosterlitz & Leslie, 1975; Kosterlitz & Waterfield, 1975). Recently, the complete homologous series of *N*-alkyl-norketobemidones (up to *N*-decyl) has been synthesized (Oh-ishi & May, 1973; May, personal communication) and the agonist potencies of the compounds have been assessed by the mouse hot-plate test and the inhibition of the binding of [^{3}H]naloxone in homogenates of rat brain (Wilson, Rogers & others, 1975). In this paper, the results obtained in the guinea-pig ileum and the mouse vas deferens are presented and compared to the data of Wilson & others (1975).

METHODS

The methods used are those already described for the assay in the guinea-pig ileum (Kosterlitz & Watt, 1968; Kosterlitz & others, 1974) and the mouse vas deferens (Hughes & others, 1975). The principle involved is the depression of the longitudinal contraction elicited by coaxial or field electrical stimulation (0·1 Hz, 0·5–1 ms, supramaximal voltage). The inhibition of the contraction is correlated to the same degree of depression read off a dose-response curve for normorphine. Morphine and normorphine are equiactive in these preparations, the onset and offset of action being more rapid with normorphine. Antagonist activity, if present, is measured by determining the dissociation constant K_e which is obtained from $K_e = a/(DR-1)$ where 'a' is the concentration of the drug with antagonist activity and DR is the dose-

ratio, i.e. the ratio of concentrations of agonist required to give the same depression of the twitch in the presence and absence of the antagonist.

RESULTS

Correlation between guinea-pig ileum and mouse vas deferens

Agonist activity. The results obtained with the homologous series of ketobemidones ranging from >NH to $>N[CH_2]_9CH_3$ are shown in Fig. 1. The relative agonist potencies (normorphine = 1) show two clear peaks, one at $>NCH_3$ and the other at $>N[CH_2]_4CH_3$. The values found in the guinea-pig ileum and the mouse vas deferens run parallel, with the exception that the pentyl homologue is about twice as potent in the mouse vas deferens than in the guinea-pig ileum. When the logarithms of the relative potencies in the mouse vas deferens are plotted against those in the guinea-pig ileum, a very close correlation is obtained (r = 0·990).

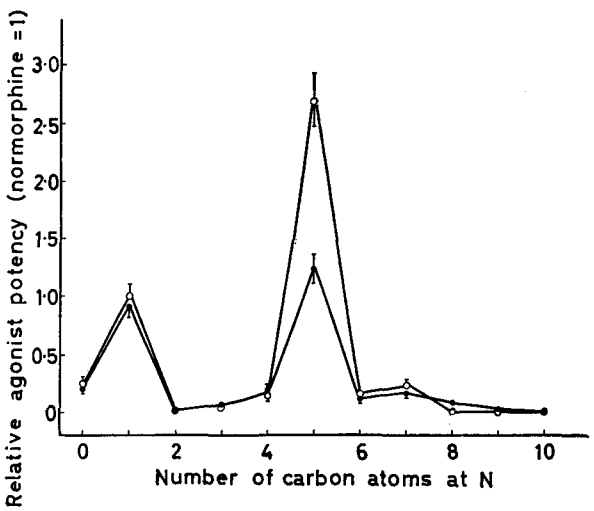

FIG. 1. The effect of changing the length of the side chain at N in *N*-alkyl-norketobemidones on the relative agonist potencies, measured by the depression of the electrically evoked longitudinal contractions of the guinea-pig ileum and mouse vas deferens. Abscissae, number of carbon atoms in the side chain (norketobemidone to decyl-norketobemidone). Ordinate, relative agonist potency (normorphine = 1). The filled circles (●) represent the means of 3 to 7 observations in the guinea-pig ileum and the open circles (○) the means of 3 to 6 observations in the mouse vas deferens, and of 2 observations for the C_{10} homologue. The vertical bars are the standard errors when they are larger than the size of the symbols.

Antagonist activity. In compounds without agonist activity the dissociation constant K_e measures antagonist potency. In compounds with dual agonist and antagonist activity, K_e can be measured only when the dose-ratio is greater than 2; since the ratio ID50/K_e (P_a) corresponds to the value of (DR-1) at a drug concentration equal to its ID50, the P_a value should be 1·5 or larger. When these criteria are applied to the results given in Table 1, the compounds which show definite antagonist activity in the guinea-pig ileum are the hexyl and heptyl homologues followed by the nor- and octyl derivatives. The relative antagonist potencies for hexyl and heptyl compounds are 8 and 4% of that of nalorphine. The pentyl derivative has a low P_a value so that its activity as an antagonist is doubtful. In the mouse vas deferens, the hexyl homologue is the most potent antagonist, followed by the octyl, nonyl and

Table 1. *Antagonist potencies of* N-*alkyl-norketobemidones in the guinea-pig ileum and mouse vas deferens.*

Substitution at N	Guinea-pig ileum K_e (nM)	P_a ($ID50/K_e$)	Antagonist potency (nalorphine = 1)	Mouse vas deferens K_e (nM)	Antagonist potency (nalorphine = 1)
H	230 ± 44 (4)	2·1	0·02	—	0
CH_3	—	—	0	—	0
C_2H_5	—	—	0	5350 ± 780 (3)	0·005
C_3H_7	—	—	0	3010 ± 715 (4)	0·01
C_4H_9	—	—	0	2220 ± 141 (3)	0·01
C_5H_{11}	40·8 ± 7·7 (7)	1·4	?	—	0
C_6H_{13}	54·9 ± 6·6 (5)	9·4	0·08	98·7 ± 19·2 (4)	0·29
C_7H_{15}	109 ± 22 (5)	4·7	0·04	1476 ± 53 (4)	0·02
C_8H_{17}	349 ± 59 (4)	2·7	0·01	438 ± 21 (4)	0·07
C_9H_{19}	—	—	0	678 ± 40 (3)	0·04
$C_{10}H_{21}$	—	—	0	—	0

The values are the means ± s.e.; the numbers of observations are given in brackets. P_a takes into account the agonist and antagonist potencies and is a measure of the effective antagonist potency. The K_e values for nalorphine are 4·47 in the guinea-pig ileum (Kosterlitz & Watt, 1968) and 28·7 in the mouse vas deferens (Hughes & others, 1975). K_e was not calculated when the dose ratio was <2.

heptyl derivatives. None of the other homologues shows antagonist activity of more than 1% of that of nalorphine. Therefore, in both models, the hexyl homologue stands out for its antagonist activity. In the morphine-dependent monkey (Swain, Villarreal & Seevers, 1973; E. L. May, personal communication), the hexyl compound has 5% of the activity of nalorphine, the heptyl homologue has weak antagonist action, the pentyl derivative has an atypical antagonist-like effect, and the octyl, nonyl and decyl homologues have no effect.

Correlation between depression in guinea-pig ileum and mouse hot-plate test

The correlation between the results in the mouse hot-plate test (Oh-ishi & May, 1973; E. L. May, personal communication) and those in the guinea-pig ileum was obtained by plotting the ED50 values (μmol kg^{-1}) of the hot-plate test against the ED50 values (μM) in the guinea-pig ileum (Fig. 2). In view of the wide potency range, logarithms were used. The value for norketobemidone has been omitted in the calculation of the regression line because the determination of the antinociceptive potency was complicated by a low ceiling effect (E. L. May, personal communication). The correlation coefficient was $r = 0{\cdot}972$ and the slope of the regression line $b = 1{\cdot}00$, and, therefore, the agreement between the two methods is considered to be good.

Correlation between depression in guinea-pig ileum and inhibition of naloxone binding in rat brain homogenate

For this comparison two sets of values for the inhibition of [^{3}H]naloxone were available, one obtained in the absence of Na^+ and the other in the presence of 100 mM Na^+ (Wilson & others, 1975). When the concentrations causing 50% inhibition of naloxone binding were plotted against the concentrations causing 50% inhibition of the evoked contraction of the longitudinal muscle of the guinea-pig ileum, different results were obtained for no Na^+ and 100 mM Na^+ (Fig. 3). In the absence of Na^+, the correlation coefficient was $r = 0{\cdot}921$ and the slope of the regression line $b = 1{\cdot}08$.

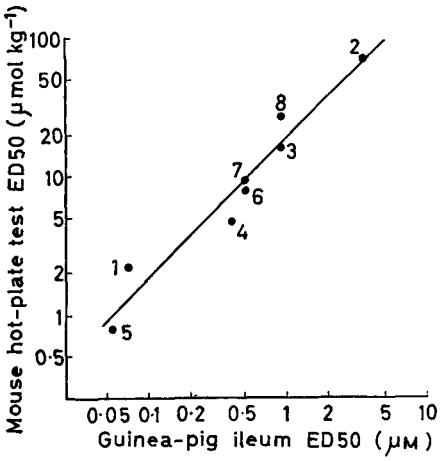

FIG. 2. Comparison of the agonist effects in the guinea-pig ileum and the hot-plate test in the mouse (Oh-ishi & May, 1973; E. L. May, personal communication). Abscissa, concentrations inhibiting the electrically evoked contractions of the longitudinal muscle of the ileum by 50% (ED50; μM); ordinate ED50 values of the hot-plate test (μmol kg^{-1}). The values are plotted logarithmically. The numbers beside the points indicate the number of carbon atoms in the N-side chain. Correlation coefficient r = 0·972. The line was drawn from $\log y = 1{\cdot}00 \log x + 1{\cdot}24$.

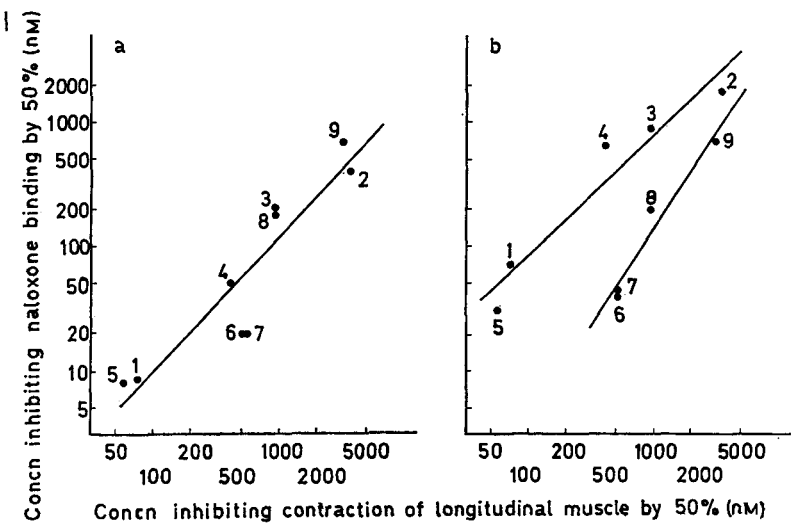

FIG. 3. Comparison of the agonist effects in the guinea-pig ileum and the inhibitory effects on naloxone binding in rat brain homogenates (cf. Wilson & others, 1975). Abscissae, concentrations inhibiting the electrically evoked contractions of the longitudinal muscle of the ileum by 50%, nM); ordinate, concentrations inhibiting naloxone binding in rat brain homogenates by 50% (nM). The values are plotted logarithmically. The numbers beside the points indicate the number of C atoms in the *N*-side chain. (a) no Na^+ in incubation medium of brain homogenate. Overall correlation coefficient, r = 0·921. The line was drawn from $\log y = 1{\cdot}08 \log x - 1{\cdot}16$. Correlation coefficients for C_1–C_5, r = 0·989, and for C_6–C_9, r = 0·948. (b) 100 mM Na^+ in incubation medium of brain homogenate. Overall correlation coefficient, r = 0·747. The upper regression line was drawn for C_1–C_5 (r = 0·962; $\log y = 0{\cdot}96 \log x - 0{\cdot}01$) and the lower line for C_6–C_9 (r = 0·973; $\log y = 1{\cdot}53 \log x - 2{\cdot}45$).

The only compounds which seemed to deviate were the hexyl and heptyl homologues. Pert, Pasternak & Snyder (1973) have shown that the presence of 100 mM Na^+ depresses binding of agonists but not of antagonists. This phenomenon also applies to the N-alkyl-norketobemidones. In the presence of 100 mM Na^+, the regression

line for the compounds without significant antagonist activity was shifted in a parallel manner to the left but the points plotted for the hexyl, heptyl and octyl homologues were changed only a little. The correlation coefficient calculated for all compounds was low ($r = 0{\cdot}747$); it was much higher when it was calculated separately for the compounds without antagonist action (C_1–C_5; $r = 0{\cdot}962$) and with antagonist action (C_6–C_9; $r = 0{\cdot}973$).

DISCUSSION

This investigation has confirmed that there is good agreement between the agonist potencies of narcotic analgesics estimated by the depression of the responses of the two models, the electrically stimulated guinea-pig ileum and mouse vas deferens (Hughes & others, 1975). Further, the correlation between the values obtained by the guinea-pig ileum assay and those found in the mouse hot-plate test is also very close. This finding is the more remarkable since, in the *in vivo* test, variations in absorption, distribution, metabolism and excretion may have modifying influences. The doses required in the *in vivo* test are about one order of magnitude larger than those needed in the *in vitro* assay. This observation confirms the well-known fact that a considerable proportion of the injected drug never interacts with morphine receptors in the central nervous system.

It is of interest that in the *N*-alkyl-norketobemidone series, two peaks of maximum agonist activity have been found in the methyl and pentyl homologues. A similar observation has been made by Winter, Orahovats & Lehman (1957) in the morphine series where the ethyl, propyl and butyl homologues showed much reduced activity and the pentyl and hexyl homologues were about as active as morphine itself. In the series of α-5,9-dimethyl-2-R_1-2′-hydroxy-6,7-benzomorphans peaks of antinoceptive potency occur when R_1 is methyl or pentyl (May & Sargent, 1965); in the guinea-pig ileum, the agonist potencies of the methyl and pentyl homologues are 40 and 48% of the potency of morphine (M. Hutchinson & A. A. Waterfield, personal communication).

Weak antagonist activities have been found in assays on the guinea-pig ileum, mouse vas deferens and morphine-dependent monkey only for the hexyl and heptyl homologues, the hexyl being more potent than the heptyl derivative. The pentyl homologue has possibly weak antagonist activity, but in the guinea-pig ileum this is low compared with its agonist activity and it cannot be discerned in the mouse vas deferens; in the morphine-dependent monkey, its action is atypical for an antagonist because its effects are not dose-dependent, the onset of action is very slow, and morphine does not fully reverse the withdrawal syndrome (Swain & others, 1973). The octyl homologue shows a very weak antagonist activity in the guinea-pig ileum and the mouse vas deferens but not in the monkey and for the nonyl homologue an antagonist activity is discernible only in the mouse vas deferens.

Finally, it is of interest that there is a very close correlation between agonist activity in the guinea-pig ileum and inhibition of naloxone binding in brain homogenates for the homologues having side chains with 1 to 5 carbon atoms. In this comparison, the values for 50% inhibition of binding have been correlated with 50% inhibition of agonist activity because it is impossible to obtain true values of the dissociation constants (K_e) for agonists without significant antagonist activity. There is little doubt that the correlation between the ED50 values obtained for agonist activity in the guinea-pig ileum and those calculated from the inhibition of naloxone binding in

brain homogenates is close, as has already been shown for a wider range of agonists (Kosterlitz & Waterfield, 1975).

On the other hand, the correlation coefficient for the complete series of homologues from *N*-methyl to *N*-nonyl is lower than the coefficient for *N*-methyl to *N*-pentyl. The difference is particularly marked when binding values are used which have been obtained in the presence of 100 mM Na^+. This finding can probably be explained by the observation (Pert & others, 1973) that the presence of 100 mM Na^+ decreases the ability of agonists but not of antagonists to inhibit naloxone binding. The fact that those homologues, *N*-hexyl to *N*-octyl, which have antagonist activity in the guinea-pig ileum, the mouse vas deferens or the morphine-dependent monkey, are responsible for the poor overall correlation agrees well with this concept.

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