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Effects of Epoxidation on the Actions of Normorphine, Norcodeine, *N*-Allylnormorphine(Nalorphine) and *N*-Allylnorcodeine on the Electrically Stimulated Guinea Pig Ileum

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The effects of epoxidation of normorphine, norcodeine, *N*-allylnormorphine (nalorphine) and *N*-allylnorcodeine on the twitch response of guinea pig ileum to electrical stimulation were studied. Normorphine, norcodeine and their epoxides are narcotic agonists, while nalorphine, *N*-allylnorcodeine and their epoxides are agonist–antagonists. The agonistic effects (*via* mu-receptor) of normorphine and norcodeine were not influenced by epoxidation of the 7,8-double bond. Epoxidation also little influenced the competitive antagonistic action (*via* mu-receptor) of nalorphine and *N*-allylnorcodeine against morphine. On the other hand, the agonistic action (*via* kappa-receptor) of nalorphine and *N*-allylnorcodeine was considerably decreased by epoxidation.

Keywords——normorphine epoxide; norcodeine epoxide; *N*-allylnormorphine epoxide; nalorphine epoxide; *N*-allylnorcodeine epoxide; mu-receptor; kappa-receptor; opioid-receptor; guinea-pig ileum

Codeine-7,8-oxide (codeine epoxide) has, recently, been identified as a new metabolite of codeine in the rat.[1] Thus, 7,8-oxides might be formed as metabolites of other related compounds. The previous findings of Takayanagi *et al.*[2] indicated that codeine epoxide and morphine epoxide inhibited the twitch response of the isolated guinea pig ileum to electrical stimulation by a naloxone reversible process. It was, moreover, reported[3,4] that codeine epoxide and morphine epoxide have a potent antinociceptive action and that tolerance developed more slowly to them than to their parent drugs when equipotent doses for antinociceptive action were used. The existence of multiple opiate-receptors in the brain and in the peripheral tissues has been well-documented on the basis of biochemical and pharmacological studies,[5,6] and it is also well known that morphine, normorphine, codeine and norcodeine predominantly interact with mu-receptor, while nalorphine (*N*-allylnormorphine) interacts with kappa-receptor.[5,6] The guinea pig ileum contains both mu- and kappa-receptors and can be used as a model to investigate the modes of action of a narcotic agonist, such as morphine, and a narcotic agonist–antagonist, such as nalorphine.[7] Therefore, the effects of epoxidation of the 7,8-double bond in normorphine, norcodeine, nalorphine and *N*-allylnorcodeine on the twitch response of the guinea pig ileum to electrical stimulation were studied in this work.

Materials and Methods

Chemicals——Normorphine, normorphine-7,8-oxide (normorphine epoxide), norcodeine, norcodeine-7,8-oxide (norcodeine epoxide), *N*-allylnormorphine (nalorphine), nalorphine-7,8-oxide (nalorphine epoxide), *N*-allylnorcodeine and *N*-allylnorcodeine -7,8-oxide (*N*-allylnorcodeine epoxide) were synthesized by us. Naloxone was kindly supplied by Sankyo Pharmaceutical Company.

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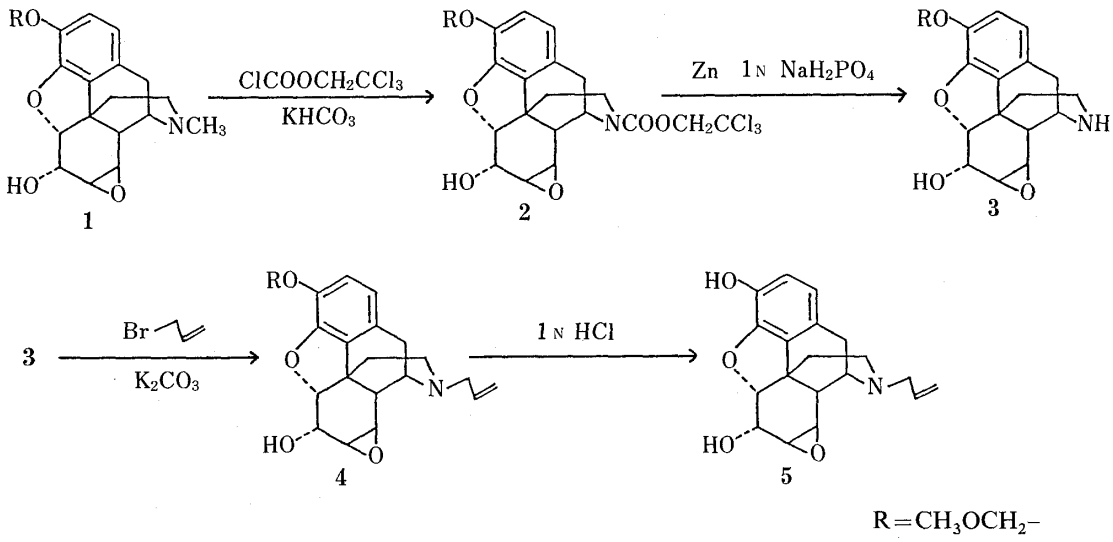

Chart 1

Normorphine-7,8-oxide, norcodeine-7,8-oxide, *N*-allylnormorphine-7,8-oxide (nalorphine-7,8-oxide) and *N*-allylnorcodeine-7,8-oxide were synthesized according to Chart 1. N-Demethylation of 3-methoxymethylmorphine-7,8-oxide (**1**)[8] to compound **3** was accomplished *via* the N-trichloroethoxycarbonyl derivative (**2**) by modifying DeGraw's method.[9] Synthesis of *N*-allylnormorphine-7,8-oxide (**5**) was completed by N-allylation of **3** according to the procedure of Rice *et al.*[10] and subsequent deprotection of the methoxymethyl group of compound **4**. In a similar manner, *N*-allylnorcodeine-7,8-oxide was obtained from codeine-7,8-oxide.[11] The overall yield of *N*-allylnormorphine-7,8-oxide from **1** was 19.3% (4 steps) and that of *N*-allylnorcodeine-7,8-oxide from codeine-7,8-oxide was 50.9% (3 steps). The physical properties of *N*-allylnormorphine-7,8-oxide (**5**) are summarized below. *N*-Allylnormorphine-7,8-oxide (nalorphine-7,8-oxide) colorless needles (from CH_3CN) mp 236—238 °C (dec.). ^{1}H-NMR (CD_3OD) δ: 3.54 (m, 1H, C_9-H), 3.72 (m, 1H, C_6-H), 4.66 (d, 1H, J=5 Hz, C_5-H), 5.26 (m, 2H, C_{19}-H), 5.91 (m, 1H, C_{18}-H), 6.59 (AB system 6.53, 6.65, 2H, J=8 Hz, C_1-H, C_2-H). ^{13}C-NMR (CD_3OD): 53.2, 54.9, 57.0 (3d, C_7, C_8, C_9), 59.0 (t, C_{17}), 118.6 (t, C_{19}), 135.9 (d, C_{18}). MS: 327 (M^+), 298 ($M^+ - CHO$). *Anal.* Calcd for $C_{19}H_{21}NO_4$: C, 69.70; H, 6.47; N, 4.28. Found C, 69.52; H, 6.57; N, 4.25.

Pharmacological Materials and Methods——Male guinea pigs weighing 250 to 300 g were killed, the ileum was isolated, and a section (3 to 5 cm) taken from the middle ileum was suspended in a 20 ml organ bath filled with Krebs solution kept at 37 °C and gassed with carbogen. Two platinum electrodes (2 × 35 mm) were placed at an interval of 5 mm and field stimulation of the ileum was carried out by passing a rectangular pulse of 0.1 ms duration, supramaximal voltage, and a frequency of 0.1 Hz between the two platinum electrodes.[2,12] The ileal preparation responded to a single pulse. The twitch responses to electrical stimulation were recorded isometrically with an initial tension of 1.0 g. Krebs solution used had the following composition (mM); NaCl 118, KCl 4.75, $CaCl_2 \cdot 2H_2O$ 2.54, $NaHCO_3$ 25, KH_2PO_4 1.19, $MgSO_4$ 1.20 and glucose 11.0.

Concentration–action curves of agonists and of agonist–antagonists were obtained cumulatively. Agonistic activity was expressed as a pD_2 values, which is the negative logarithm of the concentration (M) required to produce 50% of the maximum response to the drug (EC_{50}). The EC_{50} was estimated graphically. Antagonistic activity was expressed as pA_2 value, which is the negative logarithm of the concentration (M) of an antagonist that requires doubling the concentration of the agonist in order to keep the effect constant. To test antagonism between agonists and antagonists, the ileal preparations were incubated with an antagonist for 5 min, after the control concentration–action curve of the agonist had been obtained in the absence of the antagonist. Then the curve of the agonist was again obtained in the presence of the antagonist. The shift of the concentration–action curve of the agonist by the antagonist was estimated graphically. The pA_2 value of the antagonist was calculated from the shift of the curve of the agonist and the concentration of antagonist, using the table of Van Rossum.[13] When an agonist–antagonist was used instead of an antagonist, the technique used to estimate its pA_2 value was as described in Results.

Results

All the test drugs concentration-dependently inhibited the twitch responses to electrical stimulation, as shown in Fig. 1. A parallel line assay was employed for estimation of potency ratios, using morphine as a standard. The potency ratios of the test drugs relative to morphine are summarized in Table I.

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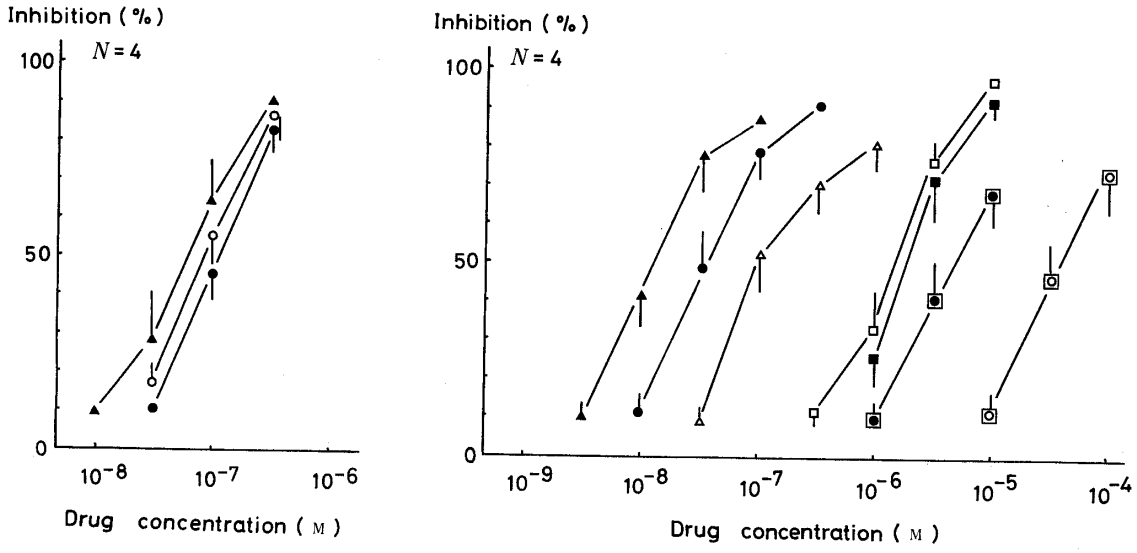

Fig. 1. Concentration–Action Curves of Test Drugs

Each value is the mean with S.E. of 4 experiments.
[Left] ●, morphine; ▲, normorphine; ○, normorphine epoxide. [Right] ●, morphine; ▲, nalorphine; △, nalorphine epoxide; ■, norcodeine; □, norcodeine epoxide; ◉, *N*-allylnorcodeine; ◎, *N*-allylnorcodeine epoxide.

TABLE I. Potency Ratios of the Test Drugs on the Electrically Stimulated Guinea Pig Ileum

	Potency ratio (95% confidence limits)[a]
Morphine	1
Normorphine	1.86 (2.18—1.54)
Its epoxide	1.22 (1.55—0.89)
Norcodeine	0.21 (0.23—0.19)
Its epoxide	0.24 (0.29—0.19)
Nalorphine	2.96 (3.36—2.56)
Its epoxide	0.42 (0.56—0.28)
N-Allylnorcodeine	0.017 (0.020—0.014)
Its epoxide	0.002 (0.0024—0.0016)

a) Relative to morphine (No. of experiments=4).

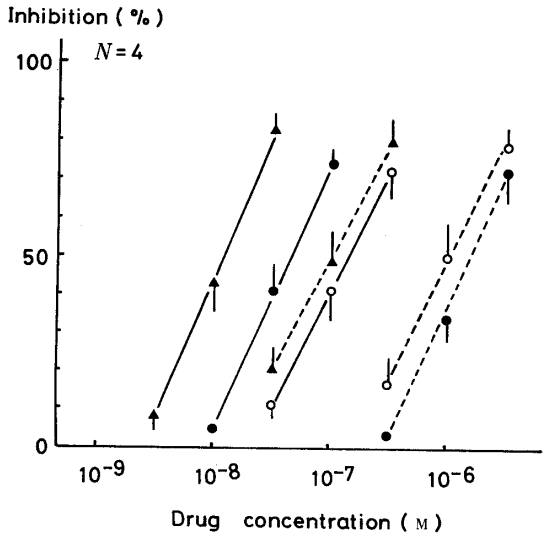

Fig. 2. Effects of Naloxone (10^{-7} M) on the Concentration–Action Curves of Morphine, Nalorphine and Nalorphine Epoxide

Each value is the mean with S. E. of 4 experiments. ●, morphine; ▲, nalorphine; ○, nalorphine epoxide. Solid line, agonist alone; dotted line, with naloxone 10^{-7} M.

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TABLE II. The pA_2 Values of Naloxone, a Competitive Antagonist against Nalorphine, Its Epoxide, *N*-Allylnorcodeine, Its Epoxide, Morphine and Codeine

Agonist	pA_2 value of naloxone[a]
Nalorphine	7.88 ± 0.13[b]
Its epoxide	7.78 ± 0.20[b]
N-Allylnorcodeine	7.91 ± 0.19[b]
Its epoxide	7.83 ± 0.15[b]
Morphine	8.51 ± 0.11
Codeine	8.55 ± 0.21

a) Mean ± S.E. of 4 experiments. *b*) Significant difference from the pA_2 values against morphine and codeine at $p < 0.05$.

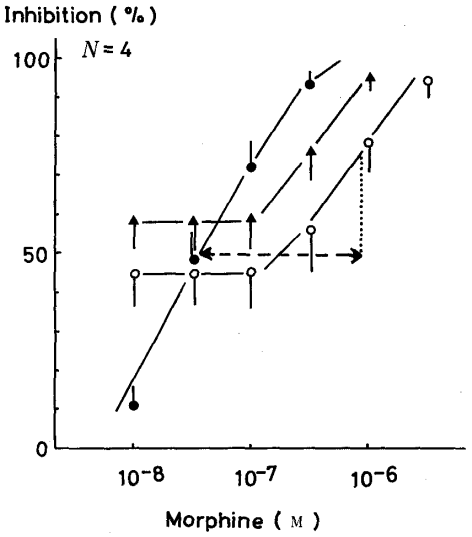

Fig. 3. Effects of Nalorphine and Its Epoxide on the Concentration–Action Curve of Morphine

Each value is the mean with S. E. of 4 experiments. ●, morphine alone; ▲, with nalorphine 3×10^{-8} M; ○, with nalorphine epoxide 10^{-7} M. ←→: shift of the curve of morphine.

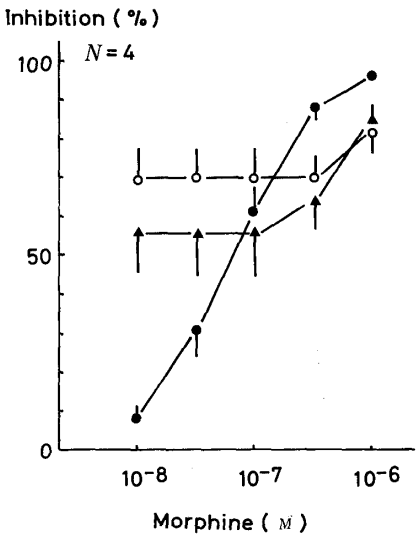

Fig. 4. Effects of *N*-Allylnorcodeine and Its Epoxide on the Concentration–Action Curve of Morphine

Each value is the mean with S. E. of 4 experiments. ●, morphine alone; ▲, with *N*-allylnorcodeine 10^{-5} M; ○, with *N*-allylnorcodeine epoxide 10^{-4} M.

In order to characterize the sites of action of N-allyl derivatives and their epoxides, antagonism between naloxone and some test drugs was studied. The test drugs used in the experiments were nalorphine, nalorphine epoxide, *N*-allylnorcodeine, *N*-allylnorcodeine epoxide, morphine and codeine. The concentration–action curves of the test drugs were shifted in parallel towards higher concentrations (Fig. 2) and the pA_2 values of naloxone were estimated from the shifts, as shown in Table II. The pA_2 values of naloxone against nalorphine, nalorphine epoxide, *N*-allylnorcodeine and *N*-allylnorcodeine epoxide were almost the same, but were significantly different from those against morphine and codeine.

Nalorphine epoxide, *N*-allylnorcodeine and *N*-allylnorcodeine epoxide behaved as agonist–antagonists on the electrically stimulated ileum of guinea pig. In the following experiments we tested whether or not the newly synthesized drugs also have an antagonistic action to morphine. It is established that with low concentrations of a pure agonist, an agonist–antagonist acts as a synergist but that with higher concentrations a competitive antagonism is observed.[13] Nalorphine (3×10^{-8} M), nalorphine epoxide (10^{-7} M), *N*-

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TABLE III. The pA_2 Values of Nalorphine, *N*-Allylnorcodeine and Their Epoxides against Morphine

	pA_2 value[a]
Nalorphine	8.34±0.13
Its epoxide	8.37±0.18
N-Allyl norcodeine	5.80±0.23
Its epoxide	5.25±0.22

a) Mean±S.E. of 4 experiments.

allylnorcodeine (10^{-5} M) and *N*-allylnorcodeine epoxide (10^{-4} M) increased the inhibitions of twitch response by morphine (less than 10^{-7} M) but decreased those by morphine (more than 10^{-7} M), suggesting that they are agonist–antagonists (Figs. 3 and 4). Concentrations which produced 50% response were obtained from the concentration–action curves of morphine in the absence and presence of one of the test drugs. The pA_2 value of the test drug was calculated from the estimated difference (see the dotted line between the curves for morphine alone and for morphine with nalorphine epoxide, in Fig. 3). As summarized in Table III, the pA_2 values of nalorphine and *N*-allylnorcodeine were practically equal to those of their epoxides.

Discussion

The 7,8-oxides of nalorphine, *N*-allylnorcodeine, normorphine and norcodeine may be formed as metabolites, since codeine-7,8-oxide (codeine epoxide) has been identified as a new metabolite of codeine.[1] Therefore, the effects of epoxidation on the actions of derivatives of normorphine were studied. As it is known that guinea pig ileum contains mu- and kappa-receptors,[7] the electrically stimulated ileum of guinea pig was used in this study. Morphine and normorphine have been used predominantly as prototype mu-receptor agonists,[5,6] and the epoxides of morphine and codeine are known to have a selectively high affinity to mu-receptor.[3,4,14] Inhibition of the twitch response of the electrically stimulated ileum by normorphine epoxide and norcodeine epoxide is considered to occur through the mu-receptor. The potency ratios of normorphine and norcodeine were practically uninfluenced by epoxidation of the 7,8-double bond.

The pA_2 value of naloxone against ethylketocyclazocine, a kappa-receptor agonist, was equal to that against nalorphine in the electrically stimulated ileum of guinea pig, suggesting that nalorphine is a kappa-receptr agonist.[15] In the present study, pA_2 values of naloxone against nalorphine, *N*-allylnorcodeine and their epoxides were practically equal to these against ethylketocyclazocine and nalorphine estimated by Takemori *et al.*,[15] and were significantly different from those against morphine and codeine (Table II). These results suggest that all the N-allyl derivatives used interact with the kappa-receptor. Moreover, there is a large difference between the pA_2 values of nalorphine and of *N*-allylnorcodeine. This fact suggests that conversion of 3-OH to OCH_3 in N-allyl derivatives reduces the affinity to the kappa-receptor, though further studies are required to confirm this. Epoxidation of the 7,8-double bond in N-allyl derivatives considerably reduced the inhibitory activity towards the twitch response. The above-mentioned results indicate that epoxides of N-allyl derivatives have a purer narcotic antagonistic action than the parent drugs.

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