INTERACTION OF MORPHINE- AND CODEINE-EPOXIDES WITH OPIATE RECEPTOR

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Since codeine-7,8-oxide (codeine-epoxide) was identified as a new metabolite of codeine in the rat (1), morphine-7,8-oxide (morphine-epoxide) was assumed to be a new metabolite of morphine. The findings of Takayanagi et al. (2) suggested that these compounds inhibited the twitch response of the guinea-pig ileum to electrical stimulation by a naloxone reversible process. Furthermore, epoxides of morphine and codeine had potent antinociceptive effects in rats and tolerance developed more slowly than their parent compounds when the equipotent doses for antinociceptive action were used (3, 4). However, direct studies to test the interactions of these compounds with opiate receptors remain to be elucidated. In the present study, we examined the binding of the epoxides of codeine and morphine to a membrane preparation from rat brain.

The crude mitochondrial fraction from whole rat brain without the cerebellum, weighing 250 to 350 g, was suspended in ice-cold 50 mM Tris/HCl buffer, pH 7.4, and centrifuged at 17,000×g for 10 min. The pellet was resuspended in ice-cold 50 mM Tris/HCl buffer containing 100 mM NaCl and incubated at 0°C for 60 min to facilitate dissociation of endogenous inhibitors of ligand binding (5). NaCl was removed from the membrane fraction by three further centrifugations, resuspended in ice-cold 50 mM Tris/HCl buffer, and stored at −20°C until use.

The measurement of labeled ligand binding in the rat brain membrane preparation was performed using a filtration method. Potencies of competing ligands were determined by co-incubating of unlabeled ligands with [3H]-dihydromorphine (DHM) at 1 nM. The binding reaction was performed at 25°C for 60 min. Non-specific binding was determined in the presence of naloxone at 1 μM. The binding reaction was stopped by rapid filtration through a Whatman glass fiber filter (GF/C). The filters were rinsed twice with 5 ml of ice-cold 50 mM Tris/HCl buffer. They were then placed in liquid scintillation counting vials containing 10 ml of a toluene scintillator and counted in an ALOKA LSC-800 liquid scintillation counter. The protein concentration in the incubation medium was 1 mg protein/ml which was determined by the method of Lowry et al. (6) using bovine serum albumin (F-V) as the standard.

Drugs used: morphine hydrochloride, naloxone hydrochloride and codeine phosphate (Sankyo). [3H]-dihydromorphine (specific activity: 70.7 Ci/mmol, New England Nuclear). Morphine- and codeine-epoxide were synthesized according to the method of Uba et al. (1). Other chemicals used were of analytical grade.

The binding of [3H]-DHM to the membrane preparation from rat brain was displaced by the simultaneous addition of non-radioactive
ligands (Figs. 1 and 2). The abilities of morphine- and codeine-epoxide to displace [3H]-DHM were slightly less potent than their parent compounds. The IC50 calculated from their displacement curves were 4.0 nM (morphine), 8.9 nM (morphine-epoxide), 383 nM (codeine) and 1359 nM (codeine-epoxide).

These results suggest that epoxidation of the 7,8-double bond in the parent opiates tends to decrease their affinities to the opiate receptor(s) on rat brain membranes. The difference between the IC50 values of morphine and morphine-epoxide was in agreement with that of the twitch response of guinea-pig ileum to electrical stimulation and that of the antinociceptive effect in rats which reported by Takayanagi et al. (2, 4). However, the IC50 value of codeine-epoxide calculated from the displacement curve was about 3 times less than that of codeine, notwithstanding that the inhibition of the twitch response of guinea-pig ileum to electrical stimulation and the antinociceptive action induced by codeine-epoxide were about twice as potent as those of codeine (2, 3). These discrepancies remain to be explained. However, it has been suggested that the concept of multiple opiate receptors has been demonstrated on the basis of pharmacological and biochemical results (7-10), and in addition to the mu receptor, other receptor subtypes are also related to the inhibition of the twitch response of guinea-pig ileum to electrical stimulation and the antinociceptive action induced by opiates (11, 12). Further studies on the interactions of codeine-epoxide with multiple opiate receptors seem to be required.

References


