EFFECTS OF EPOXIDES OF HEROIN, MORPHINE AND CODEINE ON THE ELECTRICALLY STIMULATED GUINEA PIG ILEUM

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Abstract—Modes of action of codeine epoxide, a new metabolite of codeine, and epoxides of morphine and heroin on the electrically stimulated guinea pig ileum were studied. The opiates and their epoxides inhibited the twitch response of the ileum to electrical stimulation. The inhibitory actions of the test drugs were antagonized by naloxone. The activities of epoxides were not strikingly different from those of the parent opiates. Dependence liabilities of the epoxides were also tested on the guinea pig ileum which was treated with a high concentration of the test drugs. Epoxidation of 7,8-double bond in the parent opiates showed a trend toward a decrease in dependence liability.

Codeine-7,8-oxide (codeine epoxide) has, recently, been identified as a new metabolite of codeine in the rat (1). Derivatives of 7,8-oxides of morphine and heroin are assumed to be metabolites of morphine and heroin. As opiate receptors in the cholinergic nerves of the guinea pig ileum are similar to those in the central nervous system (2). The isolated guinea pig ileum is used as a model to investigate the mode of action of narcotic analgesics in the central nervous system. We studied the modes of action of codeine, morphine, heroin and their epoxides on the electrically stimulated guinea pig ileum and our findings are reported herein.

MATERIALS AND METHODS

Male guinea pigs weighing 300 to 350 g were killed, the ileum isolated and a section (3 to 5 cm) taken from the middle ileum was suspended in a 20 ml organ bath filled with Locke Ringer solution kept at 32°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The twitch responses of the ileum to electrical stimulation were recorded isometrically under an initial tension of 1.0 g. The electrodes were made of platinum and the intraluminal electrode was the anode. Rectangular pulses of 0.1 msec duration were used at a frequency of 0.1 Hz and at a voltage sufficient to give a maximal response (3). Locke Ringer solution used had the following composition (mM): NaCl 154, KCl 5.6, CaCl₂ 2.2, MgCl₂ 2.1, NaHCO₃ 5.9 and glucose 2.8. In order to obtain a dose inhibitory response curve of drug, the drug was applied cumulatively.

The guinea pig ileum was also used to assess the dependence liability of the test compounds. Two pieces of ileum were isolated from an adjacent area of the middle
ileum; one was used for the test of a parent compound and the other for evaluating the corresponding epoxide. The piece of ileum was incubated for 24 hr in Locke Ringer solution containing the test compounds in the concentration listed in Table 1, was kept at 20°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide and then washed every 10 min for 60 min with Locke Ringer solution containing one tenth of the concentration required to produce 50% of inhibition (IC50). As listed in Table 1, the concentrations of morphine, heroin and their epoxides in an incubation medium were 100 times higher than their IC50's, however, those of codeine and its epoxide were approximately the same as their IC50's because of the large IC50's. The ileum treated above was adjusted a length of 4 cm under a tension of 1.0 g and electrically stimulated as mentioned in the experiments with the normal untreated ileum. Naloxone (10^{-6} M) was applied to this ileum in the presence of a tenth of IC50 of the test compound and changes in the base line tension and twitch response were observed.

**Drug used:** Morphine hydrochloride (Sankyo, Japan), codeine sulfate (Tanabe, Japan) and naloxone (Sankyo, Japan). Heroin and all the epoxides were synthesized in the Department of Bioorganic and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo (4, 5). The test compounds were dissolved in Locke Ringer solution and the concentrations used referred to their bases.

### RESULTS

All the test compounds dose-dependently inhibited the twitch responses to electrical stimulation as shown in Fig. 1. A parallel line assay was employed for the inhibitory

| Concentration (M) in incubation medium | Concentration (M)**1 in assay medium | S**2 (mm²) | Y'/Y**2 |
|--------------------------------------|-------------------------------------|------------|---------|
| Morphine                             | 1.0×10^{-5}                      | 1.0×10^{-6} | 1530±280 | 2.8±0.3 |
| Morphine epoxide                     | 1.5×10^{-5}                      | 1.5×10^{-6} | 1060±120 | 2.9±0.4 |
| Heroin                               | 1.8×10^{-5}                      | 1.8×10^{-6} | 1980±360 | 2.5±0.4 |
| Heroin epoxide                       | 3.1×10^{-5}                      | 3.1×10^{-6} | 1470±140 | 2.3±0.3 |
| Codeine                              | 1.8×10^{-6}                      | 1.8×10^{-6} | 170±30   | 1.9±0.2 |
| Codeine epoxide                      | 1.0×10^{-6}                      | 1.0×10^{-6} | 120±20   | 1.7±0.2 |

*1: one tenth of IC50 obtained from Fig. 1. *2: mean with S.E. of 6 experiments, see Fig. 3. #: significant difference from the value for the parent compound assessed by paired t-test at P<0.05.
potency ratios using morphine as a standard. The potency ratios of the test compounds relative to morphine are summarized in Table 2. The inhibitory activity of each epoxide was not strikingly different from that of the corresponding parent opiate. The

Table 2. Potency ratios of the test compounds on the electrically stimulated guinea pig ileum.

| Compound           | Potency Ratio (95% confidence limits) |
|--------------------|----------------------------------------|
| Morphine           | 1                                      |
| Morphine epoxide   | 0.67 (0.86–0.48)                       |
| Heroin             | 0.56 (0.76–0.36)                       |
| Heroin epoxide     | 0.32 (0.44–0.20)                       |
| Codeine            | 0.0056 (0.0075–0.0036)                 |
| Codeine epoxide    | 0.010 (0.012–0.008)                    |

*: relative to morphine. No. of experiments=8.

Fig. 2. Effects of naloxone (10^{-6} M) on the inhibitory actions of the test compounds on the electrically stimulated guinea pig ileum. Similar results were observed in 6 strips from 6 guinea pigs.
inhibitory actions of the epoxides were antagonized by a narcotic antagonist, naloxone (10⁻⁶ M), thereby indicating that they were narcotics (Fig. 2).

In the following experiments the ileum treated with a high concentration for 24 hr (see Materials and Methods) was used to compare the dependence liability of a epoxide with that of the corresponding parent opiate. After the ileum had been incubated for 24 hr with the concentration listed in Table 1, the electrically stimulated ileum was challenged by naloxone (10⁻⁶ M) in the presence of one tenth of IC₅₀. Addition of naloxone produced a large contracture of the ileum and increased the twitch response (Fig. 3). An area (S in Fig. 3) surrounded with a base line (dotted line in Fig. 3) and contracture curve was measured and a ratio (y'/y in Fig. 3) of the twitch responses before and after naloxone (10⁻⁶ M) were also calculated. The change(s) in the base line tension of the epoxide-treated ileum after naloxone challenge was smaller than that in the base line tension of the ileum treated by the corresponding parent opiate. The ratios (y'/y) obtained in the ileum treated by the epoxides were not significantly different from those in the ileum treated by the corresponding parent opiates. All the ratios (y'/y) were larger than one and are summarized in Table 1. When the ileum incubated for 24 hr in Locke Ringer solution which did not contain the opiate was challenged by naloxone (10⁻⁶ M) in the presence of morphine (a tenth of IC₅₀: 1.5×10⁻⁸ M), no contracture of ileum was observed and the twitch responses to electrical stimulation were not influenced by morphine (1.5×10⁻⁶ M).

**DISCUSSION**

The potency ratios of codeine and heroin relative to morphine were similar to the values reported by Audigier et al. (6) who estimated the analgesia induced by intracerebroventricular injection of the both drugs to mice and also in binding studies with ³H-etrophine performed on a rat brain homogenate. The inhibitory actions of all the test compounds on the electrically stimulated ileum were antagonized by naloxone. Analgesic of the parent opiates, given s.c. to rats (5), through the inhibitory actions on the twitch response were not strikingly influenced by epoxydation in this study. The analgesic activities in the rats (5) were dependent on affinities and intrinsic activities and also on distribution and metabolism of the test compounds. The potency ratios of the test compounds in this study are considered to reflect their relative affinities to the opiate receptors.

Ehrenpreis et al. (7, 8) found that the small intestine of chronically morphinized guinea pig characteristically contracted upon naloxone exposure. Furthermore, myenteric plexus-longitudinal muscle strips prepared from tolerant/dependent guinea pig and continuously exposed to normorphine, displayed a contracture upon naloxone challenge (9-11). These phenomena are considered to represent a sign of abstinence from narcotics (9-11), since naloxone does not cause any change in the tension of preparations obtained from naive rats. It was demonstrated that the contracture of the preparation after naloxone challenge was more intense in
the presence of opiate than in the absence of it (9, 11). Therefore, Locke Ringer solution containing one tenth of IC50 of one of the test compounds was used as the bath fluid in this study, when naloxone was being administered. The change in the base line tension of epoxide-treated ileum after naloxone challenge was smaller than that in the base line tension of the ileum treated with the corresponding parent opiate. Ehrenpreis et al. (7) also reported that contracture of the ileum by naloxone was not observed during the first few days of administration of morphine but more intense in the ileum from the morphine dependent guinea pig. Therefore, if the changes in the base line tension after naloxone challenge reflect dependence liabilities of narcotic analgesics, these liabilities of the epoxides may be less than those for the corresponding parent opiates.

In the ileum of the guinea pig treated with the test compounds for 24 hr and washed out for 60 min, naloxone increased the twitch response to electrical stimulation in the presence of one tenth of the IC50, this dose having no influence on the untreated ileum. It may be one of the characteristics of the ileum from opiate-dependent guinea pigs that the twitch response is inhibited by lower concentrations of opiates (11). If such is indeed the case, y'/y-value for a drug having a low degree of dependence liability would be expected to be smaller than that for a drug with a high dependence. In the present results, no significant differences between y'/y-values for the epoxides and for the parent opiates were observed. Although we have no explanation for discrepancy in the two parameters for dependence liability, one of reasons may be changes of y'/y-values are smaller than the changes in the base line tension.

The inhibitory activities of epoxides of morphine, codeine and heroin on the electrically stimulated ileum of guinea pig were not strikingly different from those of the parent opiates. Although epoxidation of 7,8-double bond in the opiates tends toward a decrease in dependence liability, epoxides may still have a high degree of dependence liability.

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