Effects of L-Tryptophan on the Development of Tolerance to the Antitussive Effects of Dihydrocodeine

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ABSTRACT—The effects of L-tryptophan on the development of tolerance to the antitussive effects of dihydrocodeine were examined in rats. Chronic co-administration of L-tryptophan with dihydrocodeine prevented the development of tolerance to the antitussive effects of dihydrocodeine. Furthermore, the antitussive effects of dihydrocodeine in dihydrocodeine-tolerant rats were fully restored by acute co-administration of L-tryptophan, as evidenced by a decrease of about 50% in the ED50 value of dihydrocodeine. Thus, it is concluded that L-tryptophan may antagonize the development of tolerance to the antitussive effects of dihydrocodeine.

Our previous studies indicated that L-tryptophan, a precursor of serotonin (5-HT), increased the antitussive effect of dihydrocodeine by increasing the levels of 5-HT in the brainstem (1). Moreover, L-tryptophan did not enhance the ability of dihydrocodeine to cause physical dependence (1). From these results, we suggested that L-tryptophan might be considered to be a useful constituent of antitussive preparations. However, chronic administration of opiates results in the development of tolerance to their pharmacological effects. Tolerance to the antitussive effects of opiates is thought to arise as a result of the development of subsensitivity of serotonin receptors (2). Thus, L-tryptophan might also enhance the development of tolerance to the antitussive effects of dihydrocodeine. In the present study, therefore, we investigated the effect of L-tryptophan on the development of tolerance to the antitussive effects of dihydrocodeine.

Male Sprague-Dawley rats, initially 8 weeks of age and weighing about 250 g, were housed in groups of six per cage, under a 12-hr light-dark cycle. For the development of tolerance to dihydrocodeine, rats were treated with dihydrocodeine and L-tryptophan, separately or together, admixed with food for 7 days, according to the method of Suzuki et al. (3).

To prepare the drug-admixed food, dihydrocodeine (Sankyo) and L-tryptophan (Sigma) were mixed with standard powdered food (CA-1, Japan Clea) at a ratio of drug to food (Weight/Weight) as follows: dihydrocodeine, 0.5 mg/g; L-tryptophan, 2.5 mg/g. Each rat was allowed to eat the drug-admixed food and to drink tap water ad libitum. Control animals were similarly treated but received normal food instead of drug-admixed food. None of the drug treatments, however, had significant effects on either the food intake or the body weight of rats.

The cough reflex was induced by electrical stimulation to the tracheal mucosa according to the puncture electrode method under chloralose anesthesia (70 mg/kg, i.p.) as described in a previous report (4). The electrical stimulation used for inducing the cough reflex
consisted of a square-wave pulse with a frequency of 40 Hz; the duration of the pulse was 1 msec; the voltage was 2–4 V; and the duration of application was 10 sec. Prior to administration of the antitussive drug, the stimulation was carried out for the 5 min intervals in order to obtain the constant cough reflex for the control. Then the electrical stimulation was repeated at 5, 10, 15, 30, 45 and 60 min after administration of antitussive drugs. The antitussive effect was judged as positive when no cough reflex occurred at all and was judged as negative when the cough reflex occurred in response to all 6 stimuli. The ED$_{50}$ for dihydrocodeine was determined using the method of Litchfield and Wilcoxon. In some animals, the acute effect of L-tryptophan on the antitussive tolerance to dihydrocodeine was also examined. In these experiments, the combination ratio of L-tryptophan to dihydrocodeine was 5 to 1.

Dihydrocodeine produced a dose-dependent depression in the cough reflex in control rats (Table 1). As shown in Table 2, the ED$_{50}$ value of dihydrocodeine in control animals was determined to be 0.42 mg/kg. A certain degree of tolerance to the antitussive effects of dihydrocodeine appeared in rats treated chronically with dihydrocodeine (Table 1). Thus, the ED$_{50}$ value increased about 2.5-fold when assayed 7 days after starting the administration of dihydrocodeine in the diet. However, chronic co-administration of L-tryptophan with dihydrocodeine blocked the development of tolerance to dihydrocodeine (Table 1). Indeed, there was no difference between the ED$_{50}$ value for dihydrocodeine in control rats and that in rats chronically treated with dihy-

| Treatment                        | Dose (mg/kg, i.v.) | Response * | %   |
|----------------------------------|--------------------|------------|-----|
| None                             | 0.30               | 4/10       | 40.0|
|                                  | 0.45               | 5/10       | 50.0|
|                                  | 0.68               | 6/10       | 60.0|
|                                  | 1.00               | 9/10       | 90.0|
| Dihydrocodeine (chronic)         | 0.68               | 3/10       | 30.0|
|                                  | 1.00               | 5/10       | 50.0|
|                                  | 1.50               | 6/10       | 60.0|
|                                  | 2.25               | 9/10       | 90.0|
| Dihydrocodeine (chronic) + L-Tryptophan (chronic) | 0.30               | 4/10       | 40.0|
|                                  | 0.45               | 5/10       | 50.0|
|                                  | 0.68               | 6/10       | 60.0|
|                                  | 1.00               | 8/10       | 80.0|
| Dihydrocodeine (chronic) + L-Tryptophan (acute) | 0.20               | 3/8        | 37.5|
|                                  | 0.30               | 4/8        | 50.0|
|                                  | 0.45               | 5/8        | 62.5|
|                                  | 0.68               | 6/8        | 75.0|
| L-Tryptophan (chronic) alone     | 0.13               | 3/10       | 30.0|
|                                  | 0.20               | 5/10       | 50.0|
|                                  | 0.30               | 7/10       | 70.0|
|                                  | 0.45               | 8/10       | 80.0|

* Animals affected/animals used.
Table 2. Effects of acute or chronic treatment with L-tryptophan on the antitussive ED₅₀ values for dihydrocodeine in rats chronically treated with dihydrocodeine

| Treatment                          | AtD₅₀ᵃ) of dihydrocodeine (mg/kg, i.v.) |
|-----------------------------------|----------------------------------------|
| None                              | 0.42 (0.28 - 0.64)ᵇ)                   |
| Dihydrocodeine (chronic)          | 1.03 (0.72 - 1.48)                     |
| Dihydrocodeine (chronic) + L-Tryptophan (chronic) | 0.43 (0.26 - 0.71) |
| Dihydrocodeine (chronic) + L-Tryptophan (acute) | 0.30 (0.16 - 0.55) |
| L-Tryptophan (chronic) alone      | 0.20 (0.14 - 0.29)                     |

ᵃ)Antitussive ED₅₀. AtD₅₀ values were determined by the Litchfield-Wilcoxon method.ᵇ)95% confidence limits of AtD₅₀.

Dihydrocodeine and L-tryptophan (Table 2). In addition, the ED₅₀ value of dihydrocodeine was reduced by about 50% in rats that were chronically fed with L-tryptophan alone (Table 2). Furthermore, the acute treatment with L-tryptophan restored the antitussive effect of dihydrocodeine in dihydrocodeine-tolerant rats, as evidenced by a decrease in the ED₅₀ value for dihydrocodeine by about 50% (Table 2).

The development of tolerance to dihydrocodeine was demonstrated in the present study. The mechanism of development of tolerance to the antitussive effects of dihydrocodeine is not well known. A considerable body of evidence supports the involvement of brain serotonin receptors in the antitussive action of opioid drugs (2, 5, 6). Previously, we demonstrated that rats treated chronically with morphine develop tolerance to the antitussive effects of morphine and dihydrocodeine (2). Furthermore, a significantly lower number of 5-HT receptors was found in the brainstem of such tolerant rats than in controls (2). These observations suggest that the decreased sensitivity to opioid antitussive drugs, in terms of the depression of the cough reflex, in rats treated chronically with opioid antitussive drugs may be due to the development of sub-sensitivity of serotonin receptors (2). The data presented here demonstrate clearly that an acute treatment with L-tryptophan restores the antitussive effect of dihydrocodeine in dihydrocodeine-tolerant rats. Our previous study has demonstrated that L-tryptophan increases the antitussive effect of dihydrocodeine in normal rats (1). Furthermore, we have shown that the level of 5-HT in the brainstem was significantly elevated after administration of L-tryptophan alone or when it was given in combination with dihydrocodeine (1). It seems likely, therefore, that the increased antitussive effect of dihydrocodeine as a result of acute treatment with L-tryptophan in dihydrocodeine-tolerant rats may be attributed to an increase in the level of 5-HT in the brain as 5-HT becomes more easily available to the brain, and that this increase counterbalances the deficiency in 5-HT receptor functions following chronic treatment with dihydrocodeine.

It has been suggested that chronic treatment with opiates alters the metabolism of serotonin by increasing its rate of turnover (7–10). Samanin et al. (11) suggested that a marked reduction in the number of binding sites for 5-HT in the brainstem of opiate-dependent rats occurs as a consequence of persistent activation of 5-HT function by chronic treatment.
with opiates. If L-tryptophan activates 5-HT functions by increasing not only the synthesis but also the release of 5-HT in the brain, chronic treatment with L-tryptophan ought to enhance the development of tolerance to dihydrocodeine by causing more severe down-regulation of receptors for 5-HT. However, in the present study, simultaneous feeding with L-tryptophan blocked but did not enhance the ability of dihydrocodeine to cause tolerance to its own antitussive effects. Moreover, the antitussive effects of dihydrocodeine were significantly increased in rats that were chronically treated with L-tryptophan alone. Therefore, these results seem to support the idea that L-tryptophan may enhance the rate of turnover of 5-HT, and that a corresponding increase in 5-HT synthesis and release can counteract down-regulation of receptors for 5-HT and/or produce the sensitization.

In conclusion, we have found that chronic administration of L-tryptophan with dihydrocodeine blocked the development of tolerance to the antitussive effects of dihydrocodeine. In this regard, we have reported previously that simultaneous feeding of L-tryptophan with dihydrocodeine does not enhance the ability of dihydrocodeine to cause physical dependence (1). Therefore, L-tryptophan can be considered as a useful constituent of antitussive preparations that does not enhance the development of either physical dependence on or development of tolerance to dihydrocodeine.

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