Effects of L-Tryptophan on the Effects of Antitussives

Junzo KAMEI,* Tomoko MORI, and Yutaka KASUYA

Department of Pharmacology, School of Pharmacy, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142, Japan

(Received September 20, 1989)

The effects of L-tryptophan and the major constituents of cough medicines on the antitussive effect of dihydrocodeine were examined in a comparative study with anesthetized rats. The antitussive effect of dihydrocodeine was enhanced by simultaneous administration of noscapine or methylephedrine. In rats treated with noscapine and methylephedrine together, the effect of dihydrocodeine was more markedly enhanced. Furthermore, L-tryptophan reduced by half the antitussive ED₅₀ (AtD₅₀) of dihydrocodeine. There was no difference between the AtD₅₀ of dihydrocodeine when administered in combination with noscapine and methylephedrine and that of dihydrocodeine when combined with noscapine and L-tryptophan. The AtD₅₀ of noscapine and dextromethorphan was also reduced by about half when what was administered with L-tryptophan. By contrast, the liability with respect to physical dependence on dihydrocodeine was not enhanced by the simultaneous administration of L-tryptophan. These results suggest that L-tryptophan can be considered to be a useful constituent of antitussive preparations.

Keywords — antitussive drug; L-tryptophan; serotonin; antitussive effect; cough reflex

Introduction

Dihydrocodeine is generally used as a major constituent of antitussive and expectorant drugs (antitussive preparations). Abuse of antitussive preparations that contain dihydrocodeine and other major constituents, such as noscapine, methylephedrine, chlorpheniramine and caffeine, has been recently reported.^{1–4)} It is, therefore, necessary to seek a preferable formulation for antitussive preparations that is free from harmful side effects. However, little information is available about the interaction between dihydrocodeine and other constituents, with particular reference to the cough-depressant effect of dihydrocodeine.

It has been reported that an increase in levels of serotonin in the brain has a depressant influence on the cough reflex.⁵⁾ Moreover, since a decrease in levels of brain serotonin reduces the cough-depressant effect of both opioid and non-opioid antitussive drugs,⁶⁾ we have proposed that the activation of serotonin neurons is needed if antitussive drugs are to be fully active. Serotonin is formed from tryptophan, for which the major source is dietary protein. Administra-

tion of L-tryptophan increases plasma tryptophan concentrations and results in increased synthesis of serotonin in the brain.⁷⁾

Therefore, the present study was designed with two basic aims: 1) to investigate the pharmacological interaction between dihydrocodeine and other major constituents of antitussive preparations with reference to cough-depressant effects; and 2) to investigate the usefulness of L-tryptophan as a constituent of antitussive preparations.

Materials and Methods

Animals — Male Sprague-Dawley rats (Tokyo Animal Laboratory Inc., Tokyo, Japan), initially 8 weeks of age, weighing about 250 g were housed, in groups of six per cage, under a 12 h light-dark cycle with food and water continuously available. The rats were allowed to adapt to their environment for a period of one week.

Induction of the Cough Reflex — The cough reflex was induced by electrical stimulation using the puncture electrode method described in previous reports.^{6,8,9)} Briefly, animals

* To whom correspondence should be addressed.

were fixed in a dorsal position under α -chloralose (70 mg/kg) anesthesia. The puncture electrode, which was made of stainless-steel wire (0.2 mm in diameter, 10 cm in length) and coated with epoxy for insulation, was inserted into the trachea through a guiding cannula (injection needle, 23G). The guiding cannula was pulled out as soon as the electrode had been inserted into the trachea. 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 ms; the voltage was 2–4 V; and the duration of application was 10 s. The stimulus intensity for each animal was set by gradually increasing the voltage. The thoracic movements of the rat were measured with a force-displacement transducer (Nihon-Kohden, 611T), used as an indicator of respiration and the cough reflex. Recordings were made on a polygraph (Nihon-Kohden, RM-6100).

Calculation of the Antitussive ED_{50} (AtD_{50}) — The electrical stimuli used for inducing the cough reflex were applied 5, 10, 15, 30, 45 and 60 min after administration of antitussive drugs. When no cough reflex occurred in response to even one stimulus, the antitussive drug was regarded as effective. When the cough reflex occurred in response to all stimuli, the antitussive drug was regarded as ineffective. Only one dose of a drug was given to each animal. A minimum of 6 animals was used for each dose of each drug. All antitussive drugs were administered intravenously. The AtD_{50} for antitussive drugs was determined by the method of Litchfield and Wilcoxon.¹⁰⁾

Physical Dependence — The effect of L-tryptophan on the liability of dihydrocodeine to cause physical dependence was studied. For the development of physical dependence, rats were treated with dihydrocodeine and L-tryptophan, separately or together, admixed with food. To prepare the drug-admixed food, dihydrocodeine and L-tryptophan were mixed with standard powdered food (CA-1, Japan Clea, Tokyo, Japan) at a ratio of drug to food 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*.

The rats were treated with drugs for 7 d, according to the method of Suzuki *et al.*¹¹⁾ Control animals were similarly treated but received normal food instead of drug-admixed food. Physical dependence was assessed by precipitation of withdrawal syndrome by treatment with naloxone (0.5 mg/kg, s.c.). After injection of naloxone, body weight was measured at intervals of 15–30 min for 180 min.

Measurement of Brain Serotonin Levels — The levels of serotonin (5-HT) in brain were estimated by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). After each rat was decapitated, its brain was rapidly removed and the brainstem (medulla oblongata, pons and midbrain) dissected out on ice. The brainstem was homogenized in 0.1 M perchloric acid which contained 2.5 ng/10 μ l 3,4-dihydroxybenzylamine (DHBA) as an internal standard, and the homogenate was centrifuged at $10000 \times g$ for 20 min at 4 °C. After centrifugation, 10 μ l of the supernatant was injected into the HPLC-ECD system. The chromatographic mobile phase was 0.1 M citrate buffer (pH 2.6) that contained 5% acetonitrile, 0.05 mM ethylenediaminetetra acetic acid (EDTA) and 60 mg/l sodium octyl sulfate. The flow rate was 1.0 ml/min. The potential of the electro-chemical detector was set at 0.8 V against an Ag/AgCl reference electrode. The HPLC-ECD system consisted of a model PM-30A pump (Bioanalytical System Inc. (BAS), U.S.A.) with an electrochemical detector (LC-4B, BAS) and 5 μ m ODS biophase column (BAS; 4.6 mm $\times$ 250 mm).

Drugs — Dihydrocodeine phosphate and *dl*-methylephedrine hydrochloride were purchased from Sankyo Co., Tokyo, Japan, and Maruishi Co., Osaka, Japan, respectively. L-Tryptophan, dextromethorphan hydrobromide, chlorpheniramine maleate, noscapine hydrochloride, caffeine and naloxone hydrochloride were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. These drugs were combined in accordance with the generally consistent ratio of constituents of over-the-counter (OTC) antitussive preparations (dihydrocodeine : noscapine : methylephedrine : caffeine : chlorpheniramine = 1 : 2 : 2 : 2 : 0.4). The following combi-

nations were used in this experiment: dihydrocodeine; noscapine; dextromethorphan; dihydrocodeine : noscapine = 1 : 2; dihydrocodeine : noscapine : methylephedrine = 1 : 2 : 2; dihydrocodeine : noscapine : methylephedrine : caffeine : chlorpheniramine = 1 : 2 : 2 : 2 : 0.4; dihydrocodeine : L-tryptophan = 1:5; dihydrocodeine : noscapine : L-tryptophan = 1 : 2 : 5; dihydrocodeine : noscapine : caffeine : chlorpheniramine : L-tryptophan = 1 : 2 : 2 : 0.4 : 5; noscapine : L-tryptophan = 1 : 5; and dextromethorphan : L-tryptophan = 1 : 5. All antitussive drugs were dissolved in saline. The doses given refer to each compound measured as the base. The effects of constituents with reference to cough-depressant effects were assessed by the changes in the AtD_{50} of dihydrocodeine, noscapine and dextromethorphan.

Results

1. Effects of L-Tryptophan and Major Constituents of Antitussive Preparations on the Antitussive Effect of Dihydrocodeine

Dihydrocodeine produced a dose-dependent depression of the cough reflex. The AtD_{50} of dihydrocodeine was determined to be 0.45 mg/kg. As shown in Table I, the antitussive effect of dihydrocodeine was enhanced by simultaneous administration of noscapine or methylephedrine, as evidenced by a decrease in the

TABLE I. Effects of Major Constituents of Antitussive Preparations on the Antitussive Activity of Dihydrocodeine in Rats

Drugs	AtD_{50} ^{a)} (mg/kg, i.v.)
Dihydrocodeine (DC)	0.45 (0.37–0.58) ^{b)}
DC + noscapine (NC)	0.30 (0.21–0.42)
DC + methylephedrine (ME)	0.33 (0.22–0.49)
DC + NC + ME	0.16 (0.10–0.27)
DC + NC + ME + caffeine + chlorpheniramine	0.30 (0.19–0.47)

^{a)} Antitussive ED_{50} . AtD_{50} values were determined by the Litchfield-Wilcoxon method. ^{b)} 95% confidence limits of AtD_{50} .

TABLE II. Effects of L-Tryptophan and Major Constituents of Antitussive Preparations on the Antitussive Activity of Dihydrocodeine in Rats

Drugs	AtD_{50} ^{a)} (mg/kg, i.v.)
Dihydrocodeine (DC)	0.45 (0.37–0.58) ^{b)}
DC + L-tryptophan	0.20 (0.15–0.26)
DC + noscapine (NC) + L-tryptophan	0.14 (0.10–0.20)
DC + NC + L-tryptophan + caffeine + chlorpheniramine	0.16 (0.10–0.25)

^{a)} Antitussive ED_{50} . AtD_{50} values were determined by the Litchfield-Wilcoxon method. ^{b)} 95% confidence limits of AtD_{50} .

AtD_{50} of dihydrocodeine. In combination with dihydrocodeine, both noscapine and methylephedrine together markedly enhanced the antitussive effect of dihydrocodeine (Table I). The antitussive effect of dihydrocodeine was also enhanced by simultaneous administration of noscapine, methylephedrine, caffeine and chlorpheniramine (AtD_{50} of dihydrocodeine without additional constituents, 0.45 mg/kg; with additional constituents: 0.30 mg/kg; Table I). However, the AtD_{50} of dihydrocodeine in this combination was about 2-fold higher than the AtD_{50} of dihydrocodeine in the combination of dihydrocodeine, noscapine and methylephedrine (Table I). By contrast, in the presence of L-tryptophan, the dihydrocodeine-induced antitussive effect was also markedly enhanced. In fact, L-tryptophan reduced the AtD_{50} of dihydrocodeine by 50% (Table II). Moreover, there was no

TABLE III. Effects of L-Tryptophan on the Antitussive Activity of Dextromethorphan and Noscapine in the Rats

Drugs	AtD_{50} ^{a)} (mg/kg, i.v.)
Dextromethorphan (DM)	1.60 (1.15–2.22) ^{b)}
DM + L-tryptophan	0.79 (0.50–1.12)
Noscapine (NC)	1.06 (0.80–1.51)
NC + L-tryptophan	0.61 (0.42–0.88)

^{a)} Antitussive ED_{50} . AtD_{50} values were determined by the Litchfield-Wilcoxon method. ^{b)} 95% confidence limits of AtD_{50} .

TABLE IV. Effects of L-Tryptophan, Dihydrocodeine and Dextromethorphan on Levels of Serotonin (5-HT) in Rat Brainstem

	5-HT (ng/g brain tissue)		
	15 min	30 min	60 min
Saline	329.6 ± 17.2	267.4 ± 12.4	304.6 ± 19.4
L-Tryptophan	403.6 ± 23.8 ^{a)}	394.5 ± 31.5 ^{a)}	466.8 ± 11.5 ^{a)}
Dihydrocodeine	268.2 ± 38.7	327.6 ± 47.7	406.8 ± 15.7 ^{a)}
Dihydrocodeine + L-tryptophan	412.8 ± 28.7 ^{a,b)}	379.6 ± 18.6 ^{a)}	454.5 ± 63.6 ^{a)}
Dextromethorphan	300.0 ± 25.2	234.3 ± 37.9	342.8 ± 99.3
Dextromethorphan + L-tryptophan	547.9 ± 33.6 ^{a,b)}	376.7 ± 14.9 ^{a)}	428.8 ± 28.3 ^{a)}

Values are expressed as the mean ± S.E. of results from at least four animals. Statistical analysis was performed by Student's *t*-test. *p*-values of less than 0.05 were considered significant. Significant differences from saline values are indicated as *a)* *p* < 0.05. Significant differences from values of dihydrocodeine or dextromethorphan treated group are indicated as *b)* *p* < 0.05.

difference between the AtD₅₀ of dihydrocodeine in combination with noscapine and methylephedrine and that in the combination of dihydrocodeine with noscapine and L-tryptophan (Tables I and II). L-Tryptophan was tested in place of methylephedrine as a constituent in the combination of dihydrocodeine, noscapine, caffeine and chlorpheniramine. The AtD₅₀ of dihydrocodeine in combination with noscapine, L-tryptophan, caffeine and chlorpheniramine was determined to be 0.16 mg/kg (Tables I and II).

2. Effect of L-Tryptophan on the Antitussive Effects of Dextromethorphan and Noscapine

As shown in Table III, the AtD₅₀ values of dextromethorphan and noscapine were 1.60 mg/kg and 1.06 mg/kg, respectively. The antitussive effects of dextromethorphan and noscapine were enhanced by the simultaneous administration of L-tryptophan. Indeed, the AtD₅₀ of dextromethorphan and noscapine were reduced by about 50% when each was combined with L-tryptophan (dextromethorphan + L-tryptophan, 0.79 mg/kg; noscapine + L-tryptophan, 0.61 mg/kg).

3. Effect of L-Tryptophan on Brain Levels of Serotonin

The level of 5-HT in the brainstem was significantly increased at 15, 30 and 60 min after administration of L-tryptophan (2.5 mg/kg, i.v.). Furthermore, when L-tryptophan was administered with dihydrocodeine or dextromethorphan, the level of 5-HT was significantly elevated

at 15, 30 and 60 min later as compared with saline control values (Table IV). Moreover, 15 min after administration of drugs, the level of 5-HT in rats which received dihydrocodeine or dextromethorphan in combination with L-tryptophan was significantly higher than which received dihydrocodeine or dextromethorphan

Fig. 1. Time Course of Changes in Body Weight, during the 3 h after Administration of Naloxone (0.5 mg/kg, s.c.), of Control (○), Dihydrocodeine-Treated (●), L-Tryptophan-Treated (△) and Dihydrocodeine + L-Tryptophan-Treated (▲) Rats

Each plot represents the mean changes in body weight (as a percentage) ± S.E. of five rats. Statistical analysis was performed by Student's *t*-test. *a)* *p* < 0.05, significantly different from control rats.

alone (Table IV).

4. Effect of L-Tryptophan on the Potential for Physical Dependence on Dihydrocodeine

Dihydrocodeine-dependent rats, when injected with naloxone, showed a decrease in body weight. As shown in Fig. 1, a significantly greater loss of body weight was found in rats given dihydrocodeine and dihydrocodeine plus L-tryptophan than in control rats. However, the naloxone-precipitated decrease in body weight in dihydrocodeine-dependent rats tended to be suppressed in rats given dihydrocodeine and L-tryptophan simultaneously. Furthermore, rats given normal food also exhibit a slight loss of body weight ($> 2\%$) after injection of naloxone (Fig. 1). This loss of body weight was slightly suppressed by feeding with L-tryptophan, but this effect was not significant.

Discussion

The antitussive effect of dihydrocodeine was enhanced by simultaneous administration of the major constituents of standard antitussive preparations, such as noscapine, methylephedrine, caffeine and chlorpheniramine. Noscapine is claimed to be an antitussive drug; and apart from its antitussive effect, it has no significant actions on the CNS in doses within the therapeutic range.¹²⁾ It is possible, therefore, that the enhancement of the antitussive effect of dihydrocodeine by noscapine may simply be due to the synergetic action of the two antitussive drugs. However, the mechanisms of the increases in the dihydrocodeine-induced antitussive effect caused by other constituents are obscure. In this connection, Numata *et al.*¹³⁾ found that serum levels of dihydrocodeine were increased by simultaneous administration of methylephedrine, caffeine and chlorpheniramine. In their paper,¹³⁾ they speculate that pharmacokinetic interactions occur with antitussive preparations, thereby enhancing the availability of the constituents. This speculation appears to be supported by our observations that methylephedrine, caffeine and chlorpheniramine do not exhibit antitussive effects by themselves, but they do enhance the cough-depressant effect of dihydrocodeine. However, this speculation alone does

not explain the mechanisms by which these three compounds enhance the cough-depressant effect of dihydrocodeine. Much further work is needed to elucidate these mechanisms.

We noted that the enhancement of the antitussive effect of dihydrocodeine by noscapine and methylephedrine was diminished when caffeine and chlorpheniramine were administered simultaneously. In this connection, Schlosberg, *et al.*¹⁴⁾ have shown that administration of caffeine increases levels of 5-HT and 5-hydroxyindoleacetic acid in the brain, subsequent to the increased uptake of tryptophan by the brain. As mentioned above, the 5-HT in the brain plays an important role in the action of antitussive drugs. However, Suzuki *et al.*¹⁵⁾ reported that the antitussive effect of dihydrocodeine was not changed by the simultaneous administration of noscapine and chlorpheniramine. We suggest, therefore, that chlorpheniramine might antagonize the enhancement of the antitussive effect of dihydrocodeine when chlorpheniramine is combined with other constituents. However, we do not have a more precise explanation at present.

L-Tryptophan, a precursor of serotonin (5-HT), increased the antitussive effect of dihydrocodeine, noscapine and dextromethorphan. A considerable body of evidence supports the involvement of brain serotonergic mechanisms in the action of antitussive drugs.^{5,6,8,9)} In fact, an increase in levels of 5-HT in the brain causes a marked inhibition of the cough reflex.⁵⁾ Moreover, decreased depressant effects of opioid and non-opioid antitussive drugs on the cough reflex were seen when levels of 5-HT in the brain were reduced.⁶⁾ It seems likely, therefore, that the increased antitussive effect of dihydrocodeine, noscapine or dextromethorphan by L-tryptophan may be attributed to the increase in levels of 5-HT in the brain. Indeed, the level of 5-HT in the brain was significantly increased after administration of L-tryptophan alone, or it combined with dihydrocodeine or dextromethorphan.

In the present study, no significant change was observed in levels of 5-HT in the brain after administration of dihydrocodeine, except for 60 min after administration. Moreover, the same

result was observed after administration of dextromethorphan. Our previous studies suggested that central 5-HT receptors play an important role in the action of antitussives.^{8,9)} However, there are no evidences that both opioid and non-opioid antitussives interact directly with serotonergic receptor mechanisms involved in the regulation of coughing. Acute treatment with opioids induces an increase in the rate of turnover of 5-HT.^{16,17)} The increase in the rate of turnover of 5-HT may lead to an increase in the rate of release of 5-HT. Previously, we found that naloxone completely reversed an increased sensitivity to the cough-depressant effect of dihydrocodeine in rats neonatally treated with 5,7-dihydroxytryptamine.⁸⁾ Thus, it is reasonable to speculate that the antitussive action of dihydrocodeine and dextromethorphan may be related to the enhancement of rate of turnover of 5-HT, but not related to increase of levels of 5-HT in the brain. It is possible, therefore, that the administration of L-tryptophan alone, or combined with antitussives may induce an increase of levels of 5-HT in the brain which become more easily available to the brain. On the other hand, the levels of 5-HT in the brain show a change with the passage of time after treatment with saline. Similar fluctuations were also observed after administration of L-tryptophan and/or antitussives. Thus, it seems likely that these fluctuations in the levels of 5-HT in the brain might be the spontaneous one.

There have been several reports of abuse of antitussive preparations as a result of the inclusion of ephedrine or its derivatives.¹⁸⁻²²⁾ Thus, a preferable formulation for antitussive preparations might contain L-tryptophan in place of methylephedrine and cause a similar or more marked increase in the cough-depressant effect of dihydrocodeine than that observed with preparations that contain methylephedrine.

Loss of body weight after either withdrawal of the drug or injection of naloxone provides an excellent method for the detection of opioid dependence.^{11,23)} Using the loss of body weight after treatment with naloxone as an indicator of the magnitude of physical dependence on dihydrocodeine, we examined the effects of L-tryptophan on naloxone-precipitated loss of body

weight. In the present study, simultaneous feeding with L-tryptophan did not enhance the naloxone-precipitated loss of body weight that followed feeding with dihydrocodeine. This result suggests that L-tryptophan did not enhance the liability of dihydrocodeine to cause physical dependence. Furthermore, the naloxone-precipitated loss of body weight in both dihydrocodeine-dependent and control rats tended to be suppressed by L-tryptophan. L-Tryptophan is an essential constituent of the diet and is used in dietary supplements. Therefore, it is possible that L-tryptophan is a useful and safe constituent of antitussive preparations, not only because of a positive interaction in antitussive effects, but also for nutritional support.

In conclusion, we have demonstrated a positive interaction between dihydrocodeine and other major constituents of antitussive preparations, with reference to the cough reflex, and we have shown that L-tryptophan enhances those positive interactions. Therefore, L-tryptophan can be considered as a useful constituent that does not enhance the potential for the abuse of opioid-containing antitussive preparations.

Acknowledgments We thank Miss S. Muranaka for her expert technical assistance.

References

- 1) N. Tani, S. Kaneko, S. Minamikawa, H. Miki and H. Haga: A clinical study of SS-Bron solution-W dependency, *Jpn. J. Alcohol Drug Dependence*, **19**, 205-210 (1984).
- 2) J. Watanabe, M. Takahashi and H. Kaneto: Studies on tolerance to and dependence on SS Bron solution-W: Effects of major constituents on the action of dihydrocodeine in mice, *Jpn. J. Alcohol Drug Dependence*, **21**, 205-215 (1986).
- 3) T. M. Jelley: Phensedyl abuse, *Br. J. Psychiatry*, **151**, 418-419 (1987).
- 4) M. Borde and S. H. Nizamie: Dependence on a common cough syrup, *Lancet*, **1988**, 760-761.
- 5) J. Kamei, T. Hosokawa, S. Yanaura and T. Hukuhara: Involvement of central serotonergic mechanisms in the cough reflex, *Jpn. J. Pharmacol.*, **42**, 531-538 (1986).
- 6) J. Kamei, M. Ogawa and Y. Kasuya: Monoamines and the mechanisms of action of antitussive drugs in rats, *Arch. Int. Pharmacodyn.*, **290**, 117-127 (1987).
- 7) R. W. Fuller: Drugs altering serotonin synthesis and metabolism, "Neuropharmacology of serotonin," ed.

- by A. R. Green, Oxford University Press, Oxford, 1985, pp.1—20.
- 8) J. Kamei M. Ogawa and Y. Kasuya: Supersensitivity of 5,7-dihydroxytryptamine-treated rats to the respiratory depressant and antitussive effects of dihydrocodeine, *Eur. J. Pharmacol.*, **153**, 305—308 (1988).
 - 9) J. Kamei, T. Mori, M. Ogawa and Y. Kasuya: Subsensitivity to the cough-depressant effects of opioid and non-opioid antitussives in morphine-dependent rats: relationship to central serotonin function, *Pharmacol. Biochem. Behav.*, **34**, 595—598 (1989).
 - 10) J. T. Litchfield and F. Wilcoxon: A simplified method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Ther.*, **96**, 99—113 (1949).
 - 11) T. Suzuki, M. Shimada, T. Yoshii, J. Uesugi and S. Yanaura: Development of physical dependence on and tolerance to morphine in rats treated with morphine-admixed food, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **7**, 63—71 (1983).
 - 12) J. H. Jaffe and W. R. Martine: Opioid analgesics and antagonists, "The Pharmacological Basis of Therapeutics," 7th ed., ed. by A. Goodman Gilman, L. S. Goodman, T. W. Rall and F. Murad, Macmillan, New York, 1985, pp.491—531.
 - 13) H. Numata, T. Oka, T. Tsuda and T. Yanagita: Pharmacokinetic interactions among ingredients of antitussive preparations, *Jpn. J. Pharmacol.*, **43**, 196P (1987).
 - 14) A. J. Schlosberg, J. D. Fernstrom, M. C. Kopczynski, B. M. Cusack and M. A. Gillis: Acute effects of caffeine injection on neutral amino acids and brain monoamine levels in rats, *Life Sci.*, **29**, 173—183 (1981).
 - 15) T. Suzuki, E. Yoshida, Y. Yoshii, Y. Koike, M. Misawa and S. Yanaura: The effects of noscapine and chlorpheniramine on physical dependence and antitussive activity of dihydrocodeine, *Jpn. J. Psychopharmacol.*, **8**, 337—343 (1988).
 - 16) G. G. Yarbrough, D. M. Buxbaum and E. Sanders Bush: Increased serotonin turnover in the acutely morphine treated rat, *Life Sci.*, **10**, 977—983 (1971).
 - 17) G. G. Yarbrough, D. M. Buxbaum and E. Sanders-Bush: Biogenic amines and narcotic effects. II. Serotonin turnover in the rat after acute and chronic morphine administration, *J. Pharmacol. Exp. Ther.*, **185**, 328—355 (1973).
 - 18) M. A. Diaz, T. N. Wise and G. N. Semchyshyn: Self medication with pseudoephedrine in a chronically depressed patient, *Am. J. Psychiatry*, **136**, 1217—1218 (1979).
 - 19) K. M. Leighton: Paranoid psychosis after abuse of Acetified, *Br. Med. J.*, **284**, 789—790 (1982).
 - 20) C. R. Pugh and S. M. Howie: Dependence on pseudoephedrine, *Br. J. Psychiatry*, **149**, 798 (1986).
 - 21) A. M. Whitehouse and J. N. Duncan: Ephedrine psychosis rediscovered, *Br. J. Psychiatry*, **150**, 258—261 (1987).
 - 22) M. T. Lambert: Paranoid psychosis after abuse of proprietary cold remedies, *Br. J. Psychiatry*, **151**, 548—550 (1987).
 - 23) T. Suzuki, Y. Yoshii and S. Yanaura: Induction of physical dependence on morphine in mice by the drug-admixed food method, *Jpn. J. Pharmacol.*, **34**, 319—325 (1984).