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Subsensitivity to the Cough-Depressant Effects of Opioid and Nonopioid Antitussives in Morphine-Dependent Rats: Relationship to Central Serotonin Function

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KAMEI, J., T. MORI, M. OGAWA AND Y. KASUYA. *Subsensitivity to the cough-depressant effects of opioid and nonopioid antitussives in morphine-dependent rats: Relationship to central serotonin function.* PHARMACOL BIOCHEM BEHAV **34**(3) 595–598, 1989.—The present study was designed to determine whether morphine-dependent rats have a decreased sensitivity to the cough-depressant effects of both opioid and nonopioid antitussives. Morphine dependence was induced by treatment with morphine-admixed food (0.5 mg/g of food) for 7 days. The cough reflex was induced by application of electrical stimulation to the tracheal mucosa by the puncture electrode-induced cough method. The cough-depressant effect was evaluated as the antitussive ED_{50} calculated by the method of Litchfield and Wilcoxon. The effects of both opioid (morphine and dihydrocodeine) and nonopioid (dextromethorphan and noscapine) antitussive drugs were diminished in morphine-dependent rats. The values of ED_{50} of these antitussive drugs in morphine-dependent rats were about 3-fold higher than those in control rats. A significantly lower number of serotonin receptors was found in the brainstem of morphine-dependent rats (B_{max}: 2.88 ± 0.32 pmoles/mg protein) than in controls (B_{max}: 4.93 ± 0.50 pmoles/mg protein). It is possible that the decreased sensitivity to both opioid and nonopioid antitussive drugs, in terms of the depression of the cough reflex, in morphine-dependent rats may be due to changes in the number of serotonin receptors.

Morphine dependence Cough reflex Antitussive drugs Serotonin receptors

CHRONIC treatment with morphine is generally accompanied by the development of physical dependence. It has been suggested that chronic treatment with morphine alters the metabolism of serotonin by increasing its rate of turnover (3, 12, 19, 22), and a decrease in the number of serotonin receptors has been found in rats chronically treated with drugs that activate serotonergic mechanisms in the brain (17). Indeed, a reduction in the number of serotonin receptors has been found in the brainstem of morphine-dependent rats (18).

Considerable evidence supports the involvement of brain serotonergic mechanisms in the action of both narcotic and nonnarcotic antitussive drugs (5–7). Furthermore, our previous studies have suggested the possibility that antitussive drugs interact with a mechanism that is associated with central serotonergic receptors (5,8). If central serotonin function plays an important role in the action of antitussive drugs, not only the effects of narcotic antitussive drugs, but also the effects of nonnarcotic antitussive drugs should be diminished in morphine-dependent rats. To test this hypothesis we studied the effect of morphine, dihydrocodeine, dextromethorphan and noscapine on the cough reflex in morphine-dependent rats. In order to ascertain the specificity of the involvement of serotonin receptors, the binding of ^{3}H-serotonin to receptors on brainstem membranes of morphine-dependent rats was also examined.

METHOD

Male Sprague-Dawley rats (Tokyo Animal Laboratory Inc., Tokyo, Japan), weighing about 250 g at the beginning of the experiments, were used. Animals were housed in individual cages under a 12-hr light-dark cycle with food and water continuously available. The room temperature was maintained at 22 ± 1°C, and the relative humidity was maintained at 55 ± 5%. The rats were allowed to adapt to their environment for a period of 1 week.

Physical Dependence

Morphine dependence was induced by treatment with mor-

phine-admixed food. To prepare the drug-admixed food, morphine was mixed with standard powdered food (CA-1, Japan Clea, Tokyo, Japan) at a drug/food ratio of 0.5 mg/g in a mortar. Each rat was allowed to eat the morphine-admixed food and to drink tap water ad lib. The rats were treated with morphine for 7 days, according to the method of Suzuki *et al.* (20). Control animals were similarly treated, but received normal food instead of morphine-admixed food. Physical dependence was assessed by precipitation of a withdrawal syndrome by treatment with naloxone (0.5 mg/kg, SC). After injection of naloxone, body weight was measured at intervals of 15–30 min for 180 min.

Induction of the Cough Reflex

Morphine-dependent and control rats were fixed in a dorsal position under α-chloralose anesthesia (70 mg/kg, IP). The cough reflex was induced by electrical stimulation, by the puncture electrode-induced cough method described previously (9). 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. The stimulus intensity for each animal was set by increasing the voltage. The electrical stimuli used for inducing the cough reflex were given at 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 drug was regarded as effective. When the cough reflex occurred in response to all stimuli, the drug was regarded as ineffective. Only one dose of drug was given to each animal. A minimum of 8 animals was used for each dose of each drug. All antitussive drugs were administered intravenously. The antitussive ED_{50} for antitussive drugs was determined by the method of Litchfield and Wilcoxon (11).

Binding of ^{3}H-Serotonin to Brainstem Membranes

Morphine-dependent and control rats were killed by decapitation, and their brains were rapidly removed. The brainstem (medulla oblongata and pons) was dissected out on ice for the assay of serotonin binding. Crude preparations of membranes were prepared as described by Bennet and Snyder (1) and Nelson *et al.* (13). The brainstem was homogenized in 10 volumes of ice-cold 0.05 M Tris-HCl buffer, pH 7.4, using a Kinematica Polytron (setting 7, 4 × 5 sec). The homogenate was centrifuged at 1000 × g for 10 min and the resulting supernatant was recentrifuged at 50000 × g for 30 min. The pellets were resuspended in cold Tris buffer, incubated at 37°C for 10 min, and centrifuged as above. Pellets were resuspended in 10 volumes of 0.05 M Tris buffer contained 10 μM pargyline and 4 mM $CaCl_2$ (incubation buffer, pH 7.4). Samples of preparations of membranes (60 μg protein/tube) were put in test tubes. ^{3}H-Serotonin was added at concentrations over a range from 1.5 to 50 nM, and 10 μM unlabelled serotonin was used to determine nonspecific binding. Test tubes (containing a final volume of 300 μl) were incubated at 22°C for 30 min and then the reaction mixtures were filtered under reduced pressure through Watman GF/C filters. Filters were washed twice with 5 ml ice-cold Tris buffer for 15 sec. Filters were transferred to counting vials, and counted in a Packard Tri-Carb liquid scintillation spectrometer in 5 ml aqueous scintillant (Echonoflow, New England Nuclear) at a counting efficiency of 50%.

Drugs

5-Hydroxy(G-^{3}H)tryptamine creatinine sulfate (12 Ci/mmol) was obtained from Amersham International (Amersham, Buckinghamshire, UK). Morphine hydrochloride and dihydrocodeine phosphate were purchased from Sankyo Co., Tokyo, Japan and dextromethorphan hydrobromide was purchased from Shionogi Pharmaceutical Co., Osaka, Japan. Noscapine hydrochloride, naloxone hydrochloride and α-chloralose were purchased from Sigma Chemical Co., St Louis, MO. All antitussive drugs and naloxone were dissolved in 0.9% saline immediately before use.

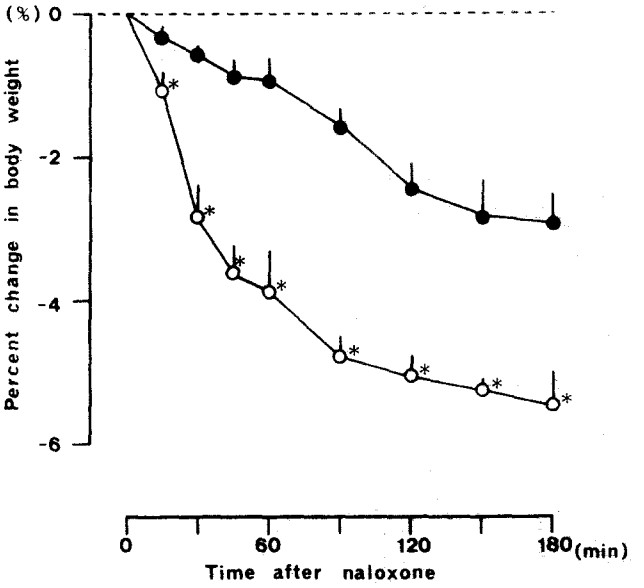

FIG. 1. Time course of changes in body weight during the 3 hr after administration of naloxone (0.5 mg/kg, SC) of control (filled circles) and morphine-dependent (open circles) rats. Each plot represents the mean percent change in body weight of five rats. *$p<0.05$, significantly different from control rats.

Statistics

The results were analysed by a one-way analysis of variance (ANOVA) followed by the Student's *t*-test.

RESULTS

The morphine-dependent rats, when injected with naloxone, showed several signs of withdrawal, in particular, a drop in body weight, diarrhea and body shakes. As shown in Fig. 1, a significantly greater loss of body weight was found in morphine-dependent rats than in control rats. The loss of body weight 60 min after injection of naloxone in control rats and morphine-dependent rats was 0.94 ± 0.29% and 3.86 ± 0.55%, respectively.

Morphine and dihydrocodeine produced a dose-dependent depression in the cough reflex in both morphine-dependent rats and control rats (Table 1). The antitussive ED_{50} (dose required to depress the cough reflex in 50% of rats tested) of morphine and dihydrocodeine were determined to be 0.24 mg/kg and 0.42 mg/kg, respectively, in control rats (Table 3). Chronic treatment with morphine significantly raised the values of ED_{50} for morphine and dihydrocodeine about 2.5-fold, to 0.58 mg/kg and 1.03 mg/kg, respectively (Table 3).

Studies were initiated on the effect of two nonopioid antitussive drugs: dextromethorphan and noscapine. As shown in Table 2, dextromethorphan and noscapine also produced a dose-dependent depression in the cough reflex in both morphine-dependent rats and control rats (Table 2). Moreover, as in the case of the

TABLE 1

ANTITUSSIVE EFFECTS OF MORPHINE AND DIHYDROCODEINE IN MORPHINE-DEPENDENT RATS

Drug	Treatment	Dose (mg/kg, IV)	Response*	%
Morphine	control	0.20	4/9	44.4
		0.30	5/9	55.6
		0.45	7/9	77.8
		0.68	9/9	100.0
	dependent	0.30	1/8	12.5
		0.45	2/8	25.0
		0.68	5/8	62.5
		1.00	7/8	87.5
Dihydrocodeine	control	0.30	2/8	25.0
		0.45	4/8	50.0
		0.68	7/8	87.5
		1.00	8/8	100.0
	dependent	0.68	2/8	25.0
		1.00	4/8	50.0
		1.50	6/8	75.0
		2.30	7/8	87.5

*Animals affected/animals used.

TABLE 2

ANTITUSSIVE EFFECTS OF DEXTROMETHORPHAN AND NOSCAPINE IN MORPHINE-DEPENDENT RATS

Drug	Treatment	Dose (mg/kg, IV)	Response*	%
Dextromethorphan	control	1.30	3/9	33.3
		2.00	5/9	55.6
		3.00	8/9	88.9
		4.50	9/9	100.0
	dependent	3.00	3/8	37.5
		4.50	4/8	50.0
		6.80	6/8	75.0
		10.00	7/8	87.5
Noscapine	control	0.68	3/9	33.3
		1.00	6/9	66.7
		1.50	6/9	66.7
		2.30	8/9	88.9
	dependent	1.00	1/8	12.5
		1.50	3/8	37.5
		2.30	5/8	62.5
		3.40	6/8	75.0

*Animals affected/animals used.

treatment with opioid antitussive drugs, chronic treatment of morphine caused significant reduction of antitussive potency of dextromethorphan and noscapine. The values of ED_{50} for dextromethorphan and noscapine were determined to be 1.67 mg/kg and 0.88 mg/kg in control rats, and 4.08 mg/kg and 1.99 mg/kg in morphine-dependent rats, respectively (Table 3).

Table 4 shows the results of binding of ^{3}H-serotonin to brainstem membranes from morphine-dependent rats. A significantly lower number of binding sites for ^{3}H-serotonin (B_{max}) was found in the brainstem of morphine-dependent rats than in the brainstem of controls. No significant changes were observed in the apparent K_D for the binding of ^{3}H-serotonin to the brainstem membranes of both control and morphine-dependent rats.

DISCUSSION

Some signs of withdrawal, such as body shakes, spontaneous locomotor activity and loss of body weight, have been reported to be useful for the detection and assessment of physical dependence on opioids in rats. Loss of body weight after withdrawal of opioids or injection of naloxone has been considered to be the best index for the detection of opioid dependence in rodents (20,21). Therefore, the loss of body weight after treatment with naloxone was chosen as an indicator of physical dependence on morphine.

The reduction in the number of serotonin (5-HT) receptors in the brainstem of morphine-dependent rats was in complete agreement with the results of Sammanin *et al.* (18). As regards a possible mechanism, these authors suggested that a marked reduction in the number of binding sites for 5-HT in the brainstem of morphine-dependent rats occurs as a consequence of persistent activation of 5-HT functions by chronic treatment with morphine.

We previously suggested that central serotonergic receptors play an important role in the mechanisms of action of antitussive drugs (5,8). Indeed, we demonstrated that neonatal treatment with 5,7-dihydroxytryptamine (5,7-DHT), which causes a supersensitivity to agonists of 5-HT, produced a marked potentiation of the antitussive effect of dihydrocodeine (8). Furthermore, an increased sensitivity to the cough-depressant effect of dihydrocodeine observed in 5,7-DHT-treated rats was abolished by pretreatment with methysergide, a 5-HT receptor blocker. Moreover, the cough-depressant effect of dextromethorphan was abolished by methysergide in pentobarbital-anesthetized cats (5). In the present study, we found that chronic treatment with morphine, sufficient to reduce the number of receptors for 5-HT in the brainstem, resulted in subsensitivity to both opioid and nonopioid antitussives in terms of their cough depressant actions. We were, furthermore, surprised to find that not only the cough-depressant effect of opioid antitussives, but also the cough-depressant effect of nonopioid antitussives, such as dextromethorphan and noscapine, also diminished in morphine-dependent rats. These findings strongly support the hypothesis that central 5-HT receptors play an impor-

TABLE 3

EFFECTS OF CHRONIC TREATMENT OF MORPHINE ON THE ANTITUSSIVE EFFECTS OF MORPHINE, DIHYDROCODEINE, DEXTROMETHORPHAN AND NOSCAPINE

	Morphine	Dihydrocodeine	Dextromethorphan	Noscapine
Control	0.24*	0.42	1.67	0.88
	(0.15–0.38)†	(0.31–0.57)	(1.22–2.30)	(0.57–1.36)
Dependent	0.58	1.03	4.08	1.99
	(0.41–0.82)	(0.71–1.49)	(2.61–6.39)	(1.37–2.89)
	2.42‡	2.48	2.45	2.26

*Antitussive ED_{50} (mg/kg). ED_{50} values were determined by the method of Litchfield and Wilcoxon.

†Ninety-five percent confidence limits of ED_{50}.

‡Antitussive ED_{50} ratio (dependent/control).

tant role in the action of antitussives. It is impossible, however, to conclude that opioids interact directly with the serotonergic receptor mechanisms involved in the regulation of coughing. Moreover, it has been suggested that dextromethorphan (2) and noscapine (10) do not interact directly with receptors for 5-HT. Acute treatment with opioids induces an increase in the rate of turnover of 5-HT (3, 23, 24). 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-DHT (8). It is reasonable to speculate, therefore, that the antitussive action may be related to the enhancement of the function of receptors for 5-HT, and that antitussives interact with receptors indirectly by causing the release (and/or blocking the uptake) of 5-HT.

On the other hand, a number of different 5-HT receptor subtypes have now been identified and it is known that [^{3}H]5-HT labels several types of these receptors (4, 14–16). Furthermore, the interaction between 5-HT receptor subtypes and the cough-depressant effects of antitussives remains to be clarified. However, at present, we cannot identify which subtype(s) of 5-HT receptors function is affected by chronic morphine treatment, and is interacting with the action of antitussives. Further studies are needed to resolve these questions.

In conclusion, cough-depressant effects of both opioid and nonopioid antitussives were markedly reduced in rats chronically treated with morphine, which caused the subsensitivity of the 5-HT receptors. The possibility remains that the activation of 5-HT neurons is needed if antitussives are to be fully active.

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