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THE EFFECTS OF GABA-ERGIC DRUGS ON NALOXONE-PRECIPITATED WITHDRAWAL SIGNS IN CHRONICALLY MORPHINE-TREATED MICE

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SUMMARY

1) Chronic administration of morphine hydrochloride in the drinking water of mice induced physical dependence. Jumping and diarrhea, two signs of withdrawal, were produced by intraperitoneal (IP) injection of naloxone.
2) Baclofen, bicuculline and picrotoxin decreased the number of mice's jumping episodes and frequency of diarrhea.
3) Muscimol decreased jumping but not diarrhea.
4) The data indicate that GABA-Ergic system is possibly involved in the morphine physical dependence, but further studies are required to elucidate the roles of GABA-A or GABA-B receptor sites in physical dependence.

INTRODUCTION

The withdrawal syndrome can be precipitated following the termination of morphine injection,

removal of morphine pellets (1, 2), or the administration of opiate antagonists (3, 4) in chronic morphine-treated animals. Abstinence signs were also induced following naloxone injection in the rats (5). It is well documented that GABA mediates a variety of pharmacological events including sedative, analgesic, anticonvulsant responses and thermoregulation (6, 7), feeding (6-11), and analgesia (8, 10, 11). The involvement of GABA in the state of morphine-dependence has been also proposed in several investigations (12-14). Two different receptor sites named GABA-A and GABA-B receptors have been suggested (15).

The present study was accomplished to induce physical dependence in mice by the administrating of morphine Hcl in their drinking water, and evaluate the effects of GABA-A and GABA-B agonists or antagonists on the morphine abstinence syndrome.

MATERIALS AND METHODS

Male albino mice, 25-30 grams, were housed 10 per cage in a room maintained at 20±1c with 12-hour-light schedule. Food and water were available at all times. Morphine Hcl was chronically administered in their drinking water ad libitum to induce the state of dependence. The drug was provided in increasing concentrations (48 h apart) of 0.1, 0.15, 0.2, 0.3, and finally 0.5 mg/ml (5). On the eleventh day, withdrawal syndrome was assessed after IP administration of 2 mg/kg of naloxone hydrochloride to chronically morphine-treated mice. Observation of the withdrawal signs (jumping and diarrhea) was started immediately after naloxone injection and continued for 60 minutes. To evaluate the effects of GABA-Ergic drugs on withdrawal syndrome, saline or drugs were injected intraperitoneally 30 minutes before the administration of naloxone.

The different treated groups were compared statistically using the student's t-test for the number of jumping episodes or Fisher's test for the frequency of jumping and diarrhea.

DRUGS

The following drugs were used: muscimol (Fluka Ag, Chem.), baclofen (Ciba-Geigy), (+)-bicuculline (Sigma), picrotoxin (Sigma), morphine, Hcl (MacFarlan & Smith) and naloxone, Hcl (Dupont). All drugs were dissolved in water except (+)-bicuculline that was first dissolved in one drop of acetic acid and then diluted with water. The drugs were prepared immediately before usage.

RESULTS AND DISCUSSION

As shown in Table 1, chronic oral administration of morphine, Hcl in drinking water in increasing concentrations produced a marked and significant physical dependence in

mice. The administration of naloxone (2 mg/kg IP) to morphine-treated mice induced jumping (70.3%) and diarrhea (90.1%). This method has been used to develop dependence in rats (5). The involvement of GABA in the morphine physical dependence (12-14) and the existence of the two distinct GABA receptor sites, named GABA-A and GABA-B (15), has been shown. Therefore, the evaluation of the roles of GABA agonists and antagonists on dependence is an interesting task. Baclofen (5mg/kg IP), bicuculline (2 mg/kg IP), and picrotoxin (1.5 mg/kg IP) decreased the jumping frequency, jumping episodes, and frequency of diarrhea precipitated by naloxone. Muscimol (1 mg/kg IP) also decreased jumping but not diarrhea. Considering the release of GABA by baclofen (16, 17) and displacing [3H] GABA from the conventional binding sites in the brain membranes (18, 19), the effects of baclofen, a GABA-B agonist and muscimol, a GABA-A agonist (20) upon jumping may be mediated through GABA-A receptor sites.

The presence of GABA neurons within the entric nervous system and the possible role of GABA-B system in the intestine to modulate the peristaltic activity by attenuating the release of acetylcholine (20, 21) may represent the effect of baclofen in decreasing the frequency of diarrhea. Bicuculline also releases GABA by blocking GABA presynaptic receptor sites which may describe the effect of the drug on jumping and diarrhea. We have no explanation for the decreasing effect of picrotoxin on withdrawal signs at the moment. The precise mechanism(s) of these GABA-Ergic drugs on physical dependence require(s) more elucidation.

The mice received morphine Hcl in their drinking water for ten days as indicated in the section of METHODS. On the eleventh day, they were pretreated intraperitoneally with saline, baclofen, muscimol, bicuculline, or picrotoxin 30 minutes prior to naloxone (2 mg/kg IP) injection; all drugs were injected intraperitoneally.

The statistical significant difference was calculated by student's t-test for the number of jumping episodes and Fisher's test for the frequency of diarrhea and jumping.

Treatment % (mg/kg IP)	Jumping Frequency		Jumping episodes mice Mean±SEM	Diarrhea Frequency	
	Mice Jumping mice tested			Mice with diarrhea mice tested	%
Saline 0.1 ml	90/128	70.3	25.3±2.6	123/128	96.1
Baclofen 5	0/12	0.0**	0.0±0.0**	9/12	75.5*
Muscimol 1	3/22	13.6**	0.5±0.4**	21/22	95.4
Bicuculline 2	2/22	9.1**	6.3±4.4**	15/22	68.3**
Picrotoxin 1.5	6/30	20.0**	3.2±1.7**	24/30	80.3*

* P<0.005

** P<0.001 different from saline-treated mice

Table 1. Abstinence signs precipitated by naloxone in morphine-dependent mice, in the presence or absence of GABA-Ergic drugs

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