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Short communication

Cocaine withdrawal enhances pentobarbital-induced sleep in rats: Evidence of GABAergic modulation

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ABSTRACT

We intended to clarify whether pentobarbital-induced sleep in rats is affected during cocaine withdrawal and whether GABAergic systems are involved in this sleep. Cocaine (20 mg/kg) was administered subcutaneously (s.c.) to rats once per day for 6 days. Pentobarbital (42 mg/kg) was administered intraperitoneally (i.p.) to the rats 1 day (acute withdrawal), 8 days (subacute withdrawal), or 14 days (subchronic withdrawal) after withdrawal from cocaine. All rats were fasted for 24 h prior to the pentobarbital injection. Pentobarbital-induced sleeping time was significantly increased during both acute and subacute withdrawal, while sleeping onset latency was not affected. However, sleeping time recovered to normal 14 days after withdrawal. Protein levels of $GABA_A$ receptor γ-subunits and glutamic acid decarboxylase (GAD) were increased in both acute and subacute cocaine withdrawal in the hypothalamus, but were normal after 14 days of withdrawal. These results indicate that pentobarbital-induced sleeping time in cocaine withdrawal is transiently increased. Hypersomnia in cocaine withdrawal might be influenced by functional changes in the GABAergic systems.

Sleep disturbance in cocaine withdrawal is associated with most psychiatric illnesses. Cocaine abusers often experience hypersomnia and propensity for rapid eye movement (REM) sleep during periods of acute and subacute withdrawal from cocaine [1]. However, during sustained withdrawal, chronic cocaine users exhibit decreased sleep, and significant deterioration in sleep is apparent over the first three weeks of abstinence [2,3]. Preclinical and clinical studies have reported conflicting findings as both insomnia and hypersomnia have been observed in cocaine withdrawal [1,2,4–6]. Therefore, we were interested in whether cocaine withdrawal increased pentobarbital-induced sleeping behavior, and whether the expression of $GABA_A$ receptors and glutamic acid decarboxylase (GAD) in the hypothalamus were involved.

We used male Sprague Dawley rats (Samtako, Korea) weighing 220–260 g for all experiments. Animals were housed in acrylic cages (45 cm × 60 cm × 25 cm) with water and food available *ad libitum* under an artificial 12-h light/dark cycle (lights on at 7:00 am) and at a constant temperature (22 ± 2 °C). To ensure adaptation to the new environment, the rats were held in the departmental holding room for one week prior to the start of the experiments. All rats were maintained in accordance with the National Institute of Toxicological Research of the Korea Food and Drug Administration guidelines for the care and use of laboratory animals. Rats were randomly assigned to one of six groups with 12–13 rats in each. Cocaine (20 mg/kg) was administered s.c. to rats once a day for 6 days. The rats were fasted for 24 h prior to the pentobarbital injection but had free access to drinking water. Pentobarbital sodium (42 mg/kg, Han-Lim Pharm. Co., Ltd, Korea) was i.p. injected 24 h (acute withdrawal), 8 days (subacute withdrawal), or 14 days after cocaine withdrawal. Sleep-onset latency and sleep duration were measured. All experiments were performed between 1:00 and 5:00 pm. Those animals that stopped moving in the box within 15 min after pentobarbital injection were immediately transferred to another box, where animals that stayed immobile for more than 3 min were judged to be asleep. The time that elapsed from receiving pentobarbital until each animal lost its righting reflex when positioned delicately on its back represented the latency to onset of sleep. The awakening time, characterized by righting of the animal, was noted. The sleeping time was defined as the time taken for the animal to regain spontaneous movements after having been transferred to the second box. Animals that failed to fall asleep within 15 min after pentobarbital administration were excluded [7,8]. After measuring sleeping behaviors, rats were decapitated and the hypothalamus were removed and treated with lysis buffer. The extracts were centrifuged at 20,000 × *g* for 20 min. Equal amounts

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Table 1
Effects of cocaine withdrawal on the onset and duration of sleep in pentobarbital-treated rats

Group	*n*	1 d withdrawal		8 d withdrawal		14 d withdrawal	
		Sleep latency (min)	Sleeping time (min)	Sleep latency (min)	Sleeping time (min)	Sleep latency (min)	Sleeping time (min)
Control	13	2.9 ± 0.1	82.8 ± 4.9	2.2 ± 0.1	84.6 ± 4.6	2.9 ± 0.2	87.0 ± 7.3
Withdrawal	12	2.6 ± 0.2	100.4 ± 3.3**	2.8 ± 0.5	104.2 ± 3.3***	2.4 ± 0.2	96.3 ± 4.4

Each value represents the mean ± S.E.M.
** $p < 0.01$ vs. control.
*** $p < 0.005$ vs. control.

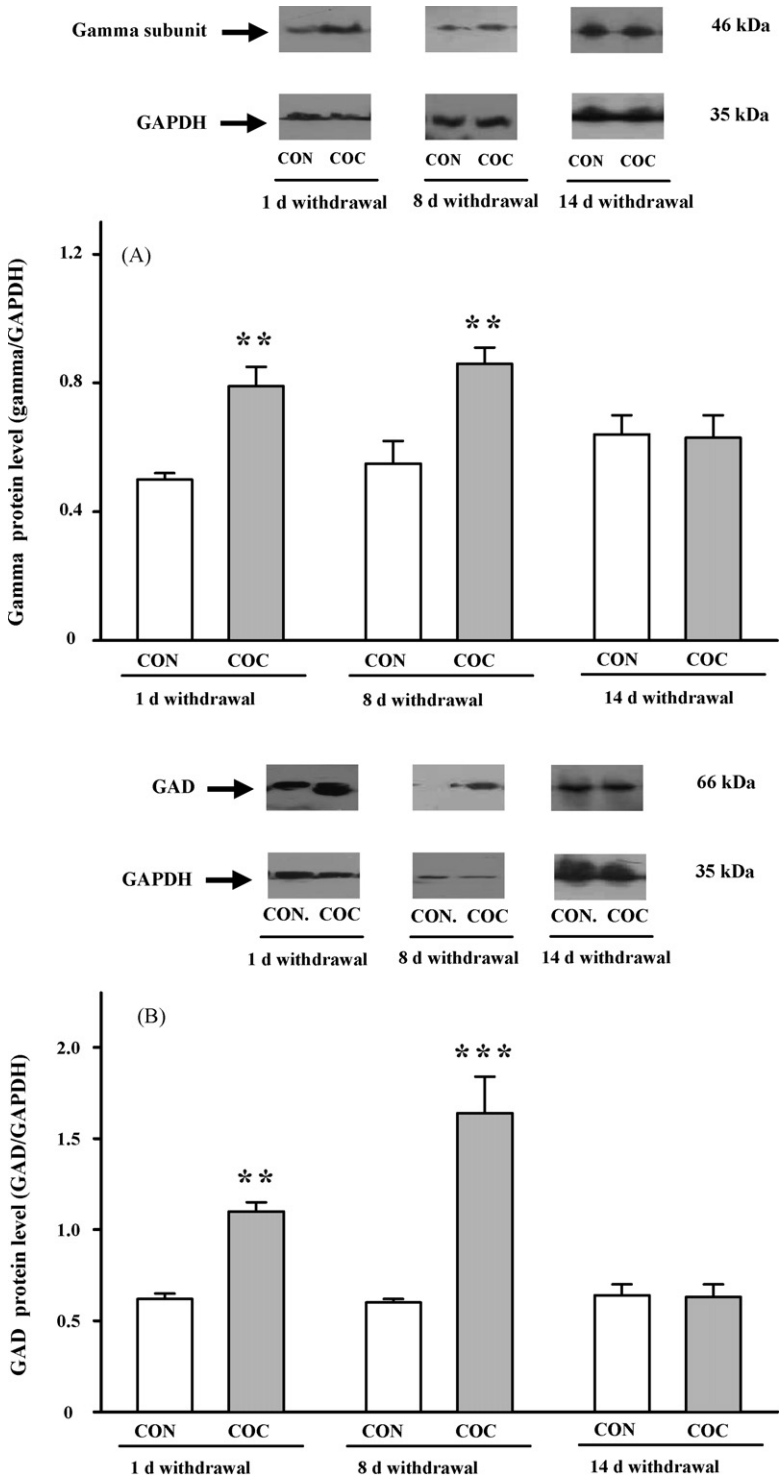

Fig. 1. Effects of cocaine (COC) withdrawal on hypothalamic $GABA_A$ receptor γ-subunit expression in rats. (A) γ-subunits; (B) GAD. Each column represents the mean with S.E.M. ** $p < 0.01$, *** $p < 0.005$, compared with that of the control group.