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SHORT COMMUNICATION

Possible involvement of GABA in morphine analgesia

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Many attempts have been made to correlate the analgesic action of morphine with alterations of content and/or metabolism of various putative neurotransmitters, and possible involvements of the changes of cerebral acetylcholine [1, 2], catecholamine [3–5] and 5-hydroxytryptamine [6, 7] contents in the occurrence of morphine analgesia were reported. Concerning γ-aminobutyric acid (GABA), a major candidate for inhibitory neurotransmitter in the central nervous system (CNS), little information is available whether or not the change of this compound in the CNS may actually related to the analgesic action of morphine. Ho *et al.* [8] demonstrated that administration of GABA enhances the formation of morphine tolerance and dependence in mice. In this report, we describe that GABA may also be involved in the analgesic action of morphine.

Mice of STD-ddy strain weighing 22–26 g were used. Analgesic responses were measured by a caudal compression method [9]. Mice were divided in four groups and pretreated with various drugs prior to the administration of morphine–HCl (15 mg/kg, i.p.) or aminopyrine (150 mg/kg, i.p.). The schedules used for pretreatment were as follows: A, saline (1 hr before); B, aminooxyacetic acid–HCl (AOAA: 25 mg/kg, i.p., 1 hr before); and C, semicarbazide–HCl (SCZ: 125 mg/kg, i.p., 5 hr before), administered respectively prior to the morphine or aminopyrine administration. In the experiments using D, bicuculline–HCl (BCL: 2 mg/kg, i.p.), BCL was administered simultaneously with morphine or aminopyrine. After administering morphine or aminopyrine, the threshold for pain was determined every 30 min. for 3 hr. GABA levels in the brain were determined fluorometrically [10] after extracting with 75% EtOH [11].

Maximal analgesic effects of morphine and aminopyrine appeared at 30 min after the administration in male mice (Fig. 1). In morphine-treated male mice, the pretreatment with AOAA significantly prolonged morphine-induced analgesic responses, whereas subconvulsive doses of SCZ and BCL strongly attenuated the morphine analgesia. These effects were most obvious 1 hr after the morphine administration. The potentiating effect of AOAA on morphine induced analgesia was maintained more than 2 hr. The pretreatments with AOAA, SCZ and BCL, however, did not modify the analgesic action of aminopyrine. Similarly, pretreatment with AOAA in female mice also induced a significant prolongation of morphine analgesia without affecting the analgesic effect induced by aminopyrine (Fig. 2). To ascertain the effect of these drugs on GABA contents, GABA levels in the brain were determined when most significant potentiation by AOAA and/or attenuation by SCZ and BCL on the morphine analgesia was observed (1 hr after morphine administration). As shown in Table 1, the pretreatment with AOAA increased the cerebral GABA content by 180 per cent, whereas that with SCZ decreased it by 32 per cent. On the other hand, the pretreatment with BCL did not show any noticeable change of GABA content in the brain. These results suggest that AOAA may induce a potentiating effect on morphine analgesia by increasing the cerebral GABA, whereas SCZ attenuates analgesic effect of morphine by decreasing GABA content in the brain. The data also indicate that antagonistic effect of BCL on morphine analgesia is not related to the alteration of cerebral content of GABA. To examine a possibility that the attenuating effects of both SCZ and BCL on morphine induced analgesia may represent a simple reflection of general and unspecified effects as convulsant drugs, experiments having simultaneous administrations of a subconvulsive dose of strychinine and morphine were also carried out. No significant change in morphine-induced analgesia, however, was observed in animals having simultaneous administration of strychinine. These results suggest that the attenuating effects of SCZ on morphine analgesia is rather specific and may be related to the decrease of brain GABA, while that of BCL may be due to known effects of this compound as a GABA antagonist [12].

It has been considered that GABA may not be involved in the occurrence of analgesic action of morphine, since

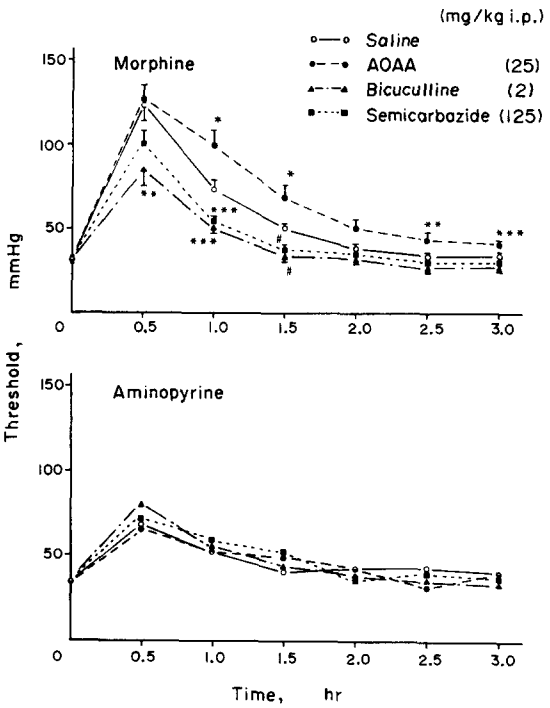

Fig. 1. Effect of various drugs on morphine and aminopyrine induced analgesic responses in male mice. Abscissa: time after morphine (Mor, 15 mg/kg, i.p.) or aminopyrine (Apy, 150 mg/kg, i.p.) administration. Ordinate: threshold for pain stimuli. Schedules for the administration of drugs and number of animals used (in parenthesis) are as follows: saline, 1 hr before Mor [16] or Apy [10]. AOAA, 1 hr before Mor [14] or Apy [5]. Semicarbazide, 5 hrs before Mor [17] or Apy [5]. Bicuculline, simultaneously administered with Mor [12] or Apy [5]. * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, # $P < 0.001$, compared with each control (saline) value, respectively.

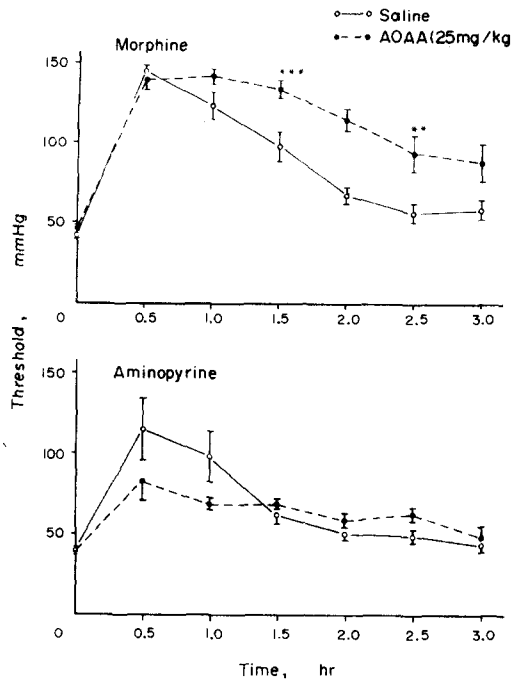

Fig. 2. Effect of AOAA on morphine and aminopyrine induced analgesic responses in female mice. Abscissa: time after morphine (15 mg/kg, i.p.) or aminopyrine (150 mg/kg, i.p.) administration. Ordinate: threshold for pain stimuli. Schedules for the administration of drugs and number of animals used (in parenthesis) are as follows: saline, 1 hr before morphine [11] or aminopyrine [4]. AOAA, 1 hr before morphine [12] or aminopyrine [5]. ** $P < 0.02$, *** $P < 0.01$ compared with each control (saline) value, respectively.

no change of GABA content in the brain mass was detected following a single administration of morphine [13, 14]. Our findings described here, however, indicate that the increase of brain GABA potentiates and the decrease of this compound attenuates the morphine analgesia. By considering additional facts the antagonistic action of bicuculline on morphine analgesia (Fig. 1), enhancement of morphine tolerance and dependence by GABA administration [8] and our recent findings that a significant increase of GABA in the specific regions of spinal cord and thalamus involved in the pathway for the perception of pain (in preparation), we are attempting to suggest that functional alterations of the GABA system in the CNS may also be an important factor for the occurrence of analgesic action of morphine.

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Table 1. Effect of various drugs on GABA content in mouse brain

Treatment	GABA Content ± S.E.M. (μmoles/g)	% of control
Control	2.46 ± 0.05	100
Morphine		
+ Saline	2.33 ± 0.12	94.8 ± 4.6
+ AOAA (25 mg/kg, i.p.)	6.81 ± 0.63*	276.9 ± 29.2
+ Semicarbazide (125 mg/kg, i.p.)	1.67 ± 0.14*	67.8 ± 5.7
+ Bicuculline (2 mg/kg, i.p.)	2.23 ± 0.06	90.8 ± 2.7

Each value in this table represents the mean ± S.E.M. obtained from 5–6 animals. Each determination was made in duplicate. Animals were decapitated 1 hr after morphine (15 mg/kg, i.p.) administration. For other experimental conditions, see the footnotes to Fig. 1.

* $P < 0.01$, compared with the control value.

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