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Effects of Various Amino Acids and Their Related Agents in Association with Neurotransmitter Substances on the Morphine-induced Straub Tail Reaction in Mice[1]

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Experiments were carried out in an attempt to elucidate the effects of various amino acids and their related agents in association with neurotransmitter substances on the morphine-induced Straub tail reaction (STR) in mice, using intracerebral injection technique. γ-Aminobutyric acid (GABA) and aminooxyacetic acid yielded significant decrement of the STR. Bicuculline provided the significant increment of the STR. DL-γ-Amino-β-hydroxybutyric acid and γ-guanidinobutyric acid had no influence on the STR. Glycine and taurine inhibited the STR significantly. L-Glutamic acid, L-aspartic acid, L-tyrosine and L-tryptophan had no influence on the STR. These results suggested the possibility that GABA, glycine and taurine may prevent the morphine-induced STR by means of an inhibitory action on the central nervous system in mice.

Keywords——morphine; Straub tail reaction; intracerebral injection; GABA; taurine; glycine

Recently, our investigation suggests that the morphine-induced Straub tail reaction [STR] is prevented by a new analogue of γ-aminobutyric acid (GABA), β-(*p*-chlorophenyl)-γ-aminobutyric acid [baclofen].[3] On the other hand, it has been apparent that certain amino acids are in fact synaptic transmitters of major significance in the central nervous system [CNS].[4]

The present study was done to clarify the effects of various amino acids and their related agents on the morphine-induced STR with reference to the effects of baclofen.

Materials and Methods

Mice used in this study were albino males of ddY strain weighing between 18 and 25 g. Food and water were supplied *ad libitum*. Drugs used were as follows: morphine hydrochloride [Shionogi and Co., Ltd.], L-tyrosine, L-tryptophan, L-glutamic acid, L-aspartic acid and GABA [Katayama Kagaku Kogyo Co., Ltd.], DL-γ-amino-β-hydroxybutyric acid (GABOB), γ-guanidinobutyric acid, aminooxyacetic acid (AOAA) and bicuculline [Sigma Chemical Co.]. Since a few amino acids administered to intact adult mammals including rodents do not cross the blood-brain barrier,[5] most of the amino acids employed were given by the intracerebral (*i.c.*) injection technique[6] in a volume of 20 μl/mouse and doses were estimated in terms of μg per mouse where applicable, except the intraperitoneal administration of AOAA and bicuculline. The scoring system used for the determination of the intensity of the STR was as follows: 0=0°, 0.5=1—44°, 1.0=45°, 1.5=46—89°, 2.0=90°, 2.5=91—179°, 3.0=180° above from the horizontal table plane.[7] The STR was estimated 10, 30 and 60 min after the administration of morphine. The amino acids used were given *i.c.* 15

1) Some parts of this work were presented at the 49th General Meeting of the Japanese Pharmacological Society at Osaka, March 1976.
2) Location: *Tenpaku-ku, Nagoya, 468, Japan.*
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min prior to morphine. Morphine, 10 mg/kg, was injected *s.c.* into the back of the neck in mice. The inhibition (%) was calculated from the observation at 30 min after morphine as follows: [(the mean score of control)-(the mean score of animals treated with amino acids or their related agents)/(the mean score of control)] × 100. *P* values were obtained by the Student's *t*-test, comparing the mean score determined for the saline-treated group. The experiments were programmed in a semi-soundproof room at 23±1° and 55±2.5% relative humidity.

Results

As illustrated in Fig. 1, GABA (200 μg/mouse, *i.c.*) showed the tendency to inhibit the STR. GABA (500 μg/mouse, *i.c.*) showed significant inhibition of the STR at 10 and 30 min after the administration of morphine. Inhibitory effects of GABA (*i.c.*) on the STR, however, were attenuated with the passage of time. AOAA (10, 20 and 50 mg/kg, *i.p.*) gave the significant inhibition of the STR. In addition, AOAA (50 mg/kg, *i.p.*) showed remarkable inhibition of the motor coordination. As indicated in Table I, GABOB produced by oxidation of GABA had no influence on the STR in the doses (100 and 200 μg/mouse) given. In the higher dose, GABOB (300 and 400 μg/mouse) proved to be lethal. γ-Guanidinobutyric acid consisted of GABA and arginine did not influence the STR even in the high dose of 600 μg/mouse. Bicuculline (2 mg/kg, *i.p.*), an antagonist of GABA, increased the STR significantly ($p<0.05$). When given L-glutamic acid (100 and 200 μg/mouse, *i.c.*) and L-aspartic acid (50 and 100 μg/mouse, *i.c.*) respectively, the STR was not altered. In the higher dose of both amino acids, the animals proved to be lethal. Glycine (100 and 300 μg/mouse, *i.c.*) inhibited the STR significantly and the inhibitory effect of glycine on the STR continued an hour or more. Taurine (100 μg/mouse, *i.c.*) had no influence on the STR. However, taurine (150 μg/mouse, *i.c.*) showed the significant inhibition of the STR. At 75 min after administration, the inhibitory effect of taurine (150 μg/mouse, *i.c.*) on the STR was attenuated. L-Tyrosine, a precursor of catecholamine, had no influence on the STR in the doses (50 and 100 μg/mouse, *i.c.*) given, but showed the remarkable inhibition of the motor coordination. Likewise, L-tryptophan, a precursor of 5-hydroxytryptamine, did not act on the STR in the doses (100 and 200 μg/mouse, *i.c.*) given. L-Tryptophan (200 μg/mouse, *i.c.*) showed a little inhibition of the motor coordination. In addition, the various amino acids alone did not elicit the STR.

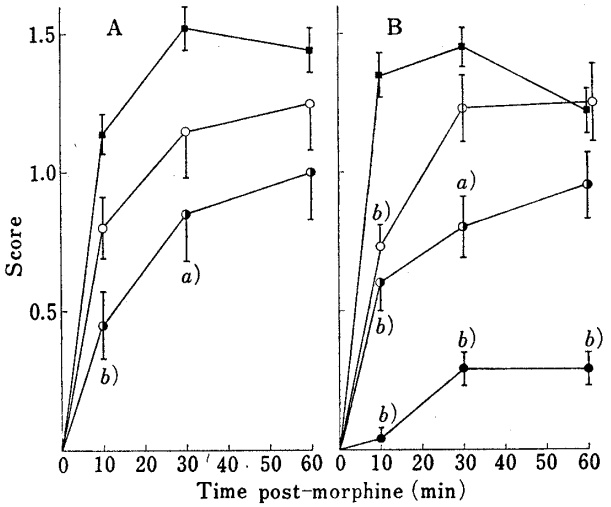

Fig. 1. The Time Course of Effects of GABA and Aminooxyacetic Acid (AOAA) on the Morphine-induced Straub Tail Reaction (STR)

(A) GABA (*i.c.*) was injected 15 min before the administration of morphine 10 mg/kg, *s.c.* Control (■—■); GABA 200 μg/mouse (○—○); GABA 500 μg/mouse (◑—◑).
(B) AOAA (*i.p.*) was injected 30 min before the administration of morphine 10 mg/kg, *s.c.* Control (■—■); AOAA 10 mg/kg (○—○); AOAA 20 mg/kg (◑—◑); AOAA 50 mg/kg (●—●).
Each point represents the mean of ten to fifteen mice. Vertical bars indicate the standard error of the mean. *a*) $p<0.01$; *b*) $p<0.001$ compared to saline-treated group.

Discussion

GABA (500 μg/mouse, *i.c.*) inhibited the STR significantly. AOAA, an inhibitor of GABA-α-ketoglutaric acid-transaminase, yielded significant decrement of the STR dose-dependently. Bicuculline, a GABA antagonist, provided the significant increment of the

Table I. Effects of Amino Acids and Their Related Agents on the Morphine-induced Straub Tail Reaction (STR)

Drugs	Dose (μg/mouse)	Route	Score (mean±S.E.)	Inhibition (%)	p
Saline		*i.c.*	1.5±0.1		
GABA	200	*i.c.*	1.2±0.2	20.0	N.S.
	500	*i.c.*	0.9±0.2	40.0	<0.05
Aminooxyacetic acid	10#	*i.p.*	1.2±0.1	20.0	N.S.
	20#	*i.p.*	0.8±0.1	46.7	<0.001
	50#	*i.p.*	0.2±0.1	86.7	<0.001
Bicuculline	1#	*i.p.*	1.6±0.2	−6.7	N.S.
	2#	*i.p.*	1.9±0.2	−26.7	<0.05
DL-γ-Amino-β-hydroxybutyric acid	100	*i.c.*	1.4±0.1	6.7	N.S.
	200	*i.c.*	1.4±0.3	6.7	N.S.
γ-Guanidinobutyric acid	200	*i.c.*	1.4±0.2	6.7	N.S.
	400	*i.c.*	1.4±0.1	6.7	N.S.
	600	*i.c.*	1.5±0.1	0	N.S.
Taurine	100	*i.c.*	1.5±0.2	0	N.S.
	150	*i.c.*	0.7±0.2	53.3	<0.001
Glycine	100	*i.c.*	1.0±0.2	33.3	<0.02
	300	*i.c.*	0.8±0.2	46.7	<0.01
L-Aspartic acid	50	*i.c.*	1.3±0.2	13.3	N.S.
	100	*i.c.*	1.3±0.2	13.3	N.S.
L-Glutamic acid	100	*i.c.*	1.4±0.2	6.7	N.S.
	200	*i.c.*	1.2±0.1	20.0	N.S.
L-Tyrosine	50	*i.c.*	1.4±0.2	6.7	N.S.
	100	*i.c.*	1.6±0.1	−6.7	N.S.
L-Tryptophan	100	*i.c.*	1.6±0.1	−6.7	N.S.
	200	*i.c.*	1.6±0.1	−6.7	N.S.

Each amino acid and bicuculline was administered 15 min before morphine 10 mg/kg, *s.c.* At 30 min after the administration of morphine, intensity of the STR was assessed according to the scoring system (mean±S.E. of ten to fifteen mice) described in Materials and Methods. The mean control level in this experiment was 1.5±0.1 (intracerebral administration of the vehicle+morphine 10 mg/kg, *s.c.*). Each inhibition (%) was constructed using the mean values for intensity. # mg/kg. N.S.: Not significantly different from saline-treated group.

STR. It has been reported that AOAA evokes the elevation of GABA content in the CNS.[8] Likewise, it has been shown that bicuculline seems to be an adequate antagonist for presynaptic inhibition and for many of the GABA-evoked responses in the spinal cord.[9] In the GABA derivatives, however, GABOB (100 and 200 μg/mouse, *i.c.*) and γ-guanidinobutyric acid (200, 400 and 600 μg/mouse, *i.c.*) had no influence on the STR. In the higher dose, GABOB (300 and 400 μg/mouse, *i.c.*) proved to be lethal. The experiments described above indicate that the STR may be prevented through the mediation of the increase of central GABA-like activity.

On the other hand, it is indicated that glycine shows the inhibitory action of the spinal interneurons[10] and shows the antagonistic effect on the seizure induced by various drugs.[11]

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It is known that taurine inhibits the functional activity of central neurons.[12] Glycine (100 and 300 μg/mouse, *i.c.*) noticeably inhibited the STR until 75 min after administration. Taurine (150 μg/mouse, *i.c.*) depressed the STR significantly. It appears that glycine and taurine may result in the inhibition of the STR due to acting on the neural pathways which required for the development of the morphine-induced STR.

On the contrary, it has been reported that L-glutamic acid and L-aspartic acid are the excitatory amino acids in the CNS.[13] Kuriyama *et al.*[8b] have indicated that AOAA increases GABA content but decreases L-glutamic acid content of mouse brain at 1.5 hr after administration, compared to the vehicle-treated group. L-Glutamic acid and L-aspartic acid, however, did not influence the STR compared to vehicle-injected group, thereby ruling out the function as excitatory amino acids on the STR. It would thus appear that inhibitory effect of AOAA on the STR may be largely due to the increase of GABA content in mouse CNS. We have formerly reported that the morphine-induced STR is relevant to the biogenic amines.[14] L-Tyrosine and L-tryptophan, however, had no influence on the STR despite the precursor of catecholamine or tryptamine. It is also indicated that both these amino acids do not influence the central neurons.[13,15] From these findings, L-tyrosine and L-tryptophan appear to be of no pharmacological siginificance in the CNS except the precursor of monoamines.

GABA, glycine and taurine are potent depressant amino acids and the effect of these amino acids on the STR could well be considered if these amino acids are found to have a role as an inhibitory neurotransmitter in the CNS. It is suggested that GABA may partly participate in the inhibitory effect of baclofen on the STR.[3] In addition, the fact that glycine and taurine inhibit the STR in the relatively low doses suggests that the involvement of the glycine and taurine on the morphine-induced STR cannot be neglected. In discussing this problem, further experiments on the detailed neurochemical aspect (*e.g.* minute distribution of amino acids and their synthetic enzymes in nerve tissues) are being carried out.

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