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Effect of centrally Acting Muscle Relaxants on the Morphine-Induced Straub Tail Reaction in Mice[1]

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This investigation was carried out in an attempt to ascertain whether all narcotic analgesics produce the Straub tail reaction (STR), and whether the morphine-induced STR could be depressed by centrally acting muscle relaxants. Morphine, oxycodone and levorphanol elicited the STR dose-dependently but codeine, pethidine and pentazocine gave only slight STR. The peak time of the STR was 30 min after the administration of morphine. Intracerebral injection of morphine evoked the STR, but not markedly. Naloxone and pentazocine depressed the morphine-induced STR considerably. Chlordiazepoxide, diazepam, oxazepam, carisoprodol, chlormezanone, chlorzoxazone, meprobamate, orphenadrine, chlorpromazine, perphenazine and haloperidol also reduced the morphine-induced STR markedly. The morphine-induced STR was inhibited noticeably by intracerebral or systemic injection of baclofen and difenamizole. The intracerebral dose of baclofen required for this inhibition was as low as approximately one-thousandth of that by systemic administration. These findings suggest that the morphine-induced STR is positively depressed by centrally acting muscle relaxants.

Keywords——morphine; Straub tail reaction; centrally acting muscle relaxants; intracerebral injection; baclofen; difenamizole

Several investigators have suggested that the Straub tail reaction (STR)[3] is due to excitation of the spinal cord.[4] We have also investigated the morphine-induced STR with respect to neurotransmitters in the central nervous system (CNS).[5] On the other hand, although the STR has been used to evaluate centrally acting muscle relaxants, at present there is no consistent information concerning the effect of such drugs on the STR.[6] The

1) A preliminary communication has appeared in *Chem. Pharm. Bull.* (Tokyo), **25**, 1837 (1977).
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present study was done to determine whether all narcotic analgesics produce the STR, and whether the morphine-induced STR could be depressed by centrally acting muscle relaxants.

Materials and Methods

Albino male mice of ddY strain weighing between 18 and 25 g were allocated into groups of ten to fifteen for each experiment, except for the intracerebral (*i.c.*) injection of baclofen, 0.1 and 0.2 μg/mouse (twenty mice). Food and water were freely supplied. The following drugs were available: baclofen, its hydrochloride (Ciba-Geigy Ltd.), carisoprodol (Nippon Kayaku Co., Ltd.), chlordiazepoxide hydrochloride, diazepam (Hoffmann-LaRoche), chlormezanone, meprobamate (Daiichi Seiyaku Co., Ltd.), chlorpromazine hydrochloride (Yoshitomi Pharmaceutical Industries Ltd.), chlorzoxazone (Daiwa Pharmaceutical Co.), codeine phosphate, morphine hydrochloride (Shionogi and Co., Ltd.), difenamizole, its hydrochloride, levorphanol tartrate, oxycodone hydrochloride (Takeda Chemical Industries Ltd.), haloperidol (Janssen), naloxone hydrochloride, pentazocine (Sankyo Co., Ltd.), orphenadrine hydrochloride (Dainippon Pharmaceutical Co., Ltd.), oxazepam (Banyu Pharmaceutical Co., Ltd.), perphenazine (Yamanouchi Pharmaceutical Co., Ltd.), pethidine hydrochloride (Tanabe Seiyaku Co., Ltd.). Intracerebral injection was carried out according to the method reported previously.[7] Morphine, baclofen hydrochloride and difenamizole hydrochloride solutions were made up in isotonic saline immediately prior to use and were adjusted to pH 7.0 with 0.1 N NaOH. For systemic administration, chlordiazepoxide, chlorpromazine, codeine, levorphanol, morphine, naloxone, orphenadrine, oxycodone, pentazocine and pethidine were dissolved in isotonic saline, whereas diazepam, oxazepam, carisoprodol, chlormezanone, chlorzoxazone, meprobamate, perphenazine, haloperidol, baclofen and difenamizole were suspended in 0.3% carboxymethylcellulose dissolved in isotonic saline. Morphine, 10 mg/kg, *s.c.*, was injected into the back of the neck 15 min after treatment with test drugs. The STR was then scored at intervals of 10, 30 and 60 min after the administration of morphine. The STR was graded according to the modified numerical scores of Juul[8] 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° (the angles were measured above the horizontal table plane). In addition, the inhibition percentage was calculated from the observation at 30 min after the administration of morphine, using the formula $(A-B)/A \times 100$ where A is the mean score of animals treated with morphine alone, and B is the mean score of animals treated with various agents plus morphine. *P*-values were obtained by Student's *t*-test. The experiment was carried out in a semi-soundproof room at $23 \pm 1°$ and $55 \pm 2.5\%$ relative humidity.

Results

Time Course of the Morphine-Induced Straub Tail Reaction

As indicated in Fig. 1, morphine (2.5—40 mg/kg, *s.c.*) evoked the STR dose-dependently. However, the morphine-induced STR was weak (score below 1.0) at doses of 5 mg/kg and below. The time of peak reaction was 30 min after the administration of morphine. Morphine at 40 mg/kg showed the strongest manifestation of the STR, and the STR continued for 150 min after administration. Morphine at 80 mg/kg *s.c.* produced a marked stereotyped behavior, but the STR induced by 80 mg/kg of morphine was not more pronounced than that induced by 40 mg/kg of morphine.

Dose-Response Curve for the Straub Tail Reaction induced by Various Narcotics and Narcotic Antagonists

As shown in Fig. 2, the ability of morphine to produce the STR was compared to those of levorphanol, oxycodone, pethidine, codeine and pentazocine. When tested 30 min after administration, codeine, pethidine and pentazocine showed only slight STR. Pethidine and pentazocine, 160 mg/kg, *s.c.*, proved to be lethal. However, levorphanol and oxycodone produced the STR markedly, and levorphanol was the most effective among these drugs on the basis of STR-$ED_{1.5}$ (the dose producing the STR of score 1.5). However, neither levorphanol nor oxycodone produced a marked STR compared to morphine. Naloxone, 40 mg/kg, *s.c.*, hardly produced the STR.

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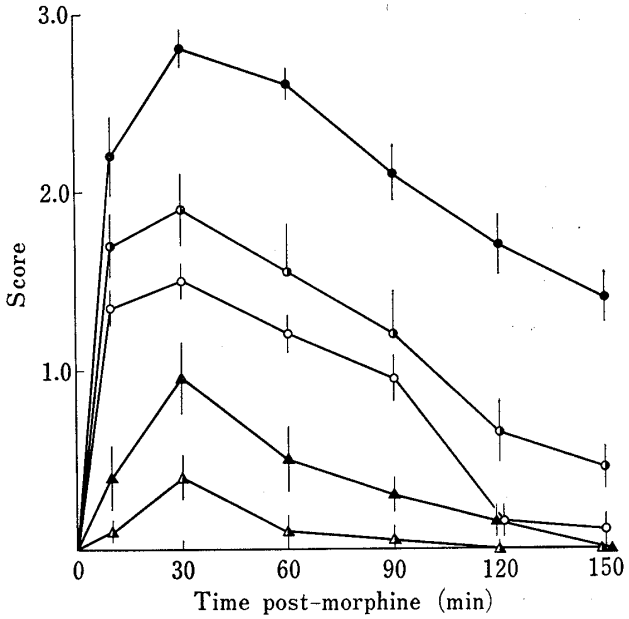

Fig. 1. The Time Course of the Morphine-Induced Straub Tail Reaction in Mice

Morphine, 2.5 mg/kg, *s.c.* (▲——▲); 5 mg/kg, *s.c.* (▲——▲); 10 mg/kg, *s.c.* (○——○); 20 mg/kg, *s.c.* (◑——◑); 40 mg/kg, *s.c.* (●——●). Each point represents the mean of ten to fifteen mice. Vertical bars represent standard errors of the means.

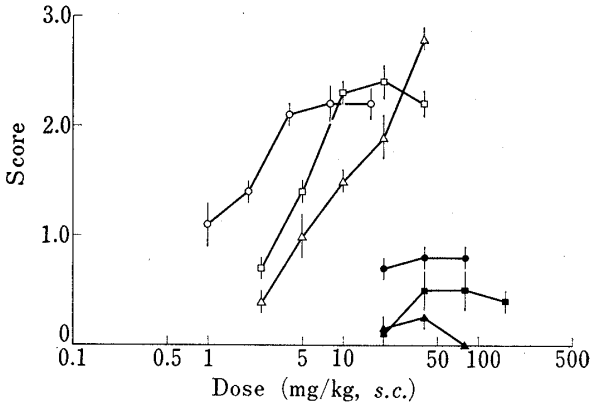

Fig. 2. Dose–Response Curve for the Straub Tail Reaction induced by Various Narcotics and a Narcotic Antagonist

Each value was estimated 30 min after administration. Ordinate, Straub score; abscissa, log dose (mg/kg) given *s.c.* Levorphanol (○——○); oxycodone (□——□); morphine (△——△); pethidine (●——●); codeine (■——■); pentazocine (▲——▲). Each point represents the mean of ten to fifteen mice. Vertical bars indicate the standard errors of the means.

Relationship between Intracerebral Injection of Morphine and the Straub Tail Reaction

As shown in Fig. 3, a peak reaction of the morphine-induced STR was obtained at a dose of 40 μg/mouse, *i.c.* The intensity of that reaction, however, was weak compared to that induced by subcutaneous administration of morphine. The STR induced by a higher dose

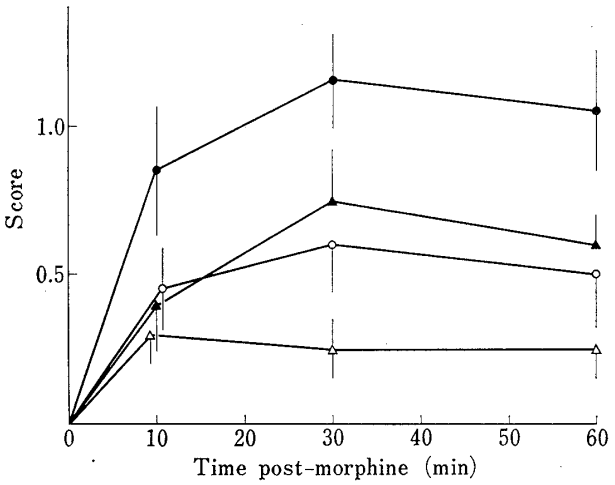

Fig. 3. Relationship between Intracerebral Injection of Morphine and the Straub Tail Reaction

Morphine 2 μg/mouse, *i.c.* (△——△); 20 μg/mouse, *i.c.* (○——○); 40 μg/mouse, *i.c.* (●——●); 50 μg/mouse, *i.c.* (▲——▲). Each point represents the mean score obtained from ten mice. Vertical bars represent standard errors of the means.

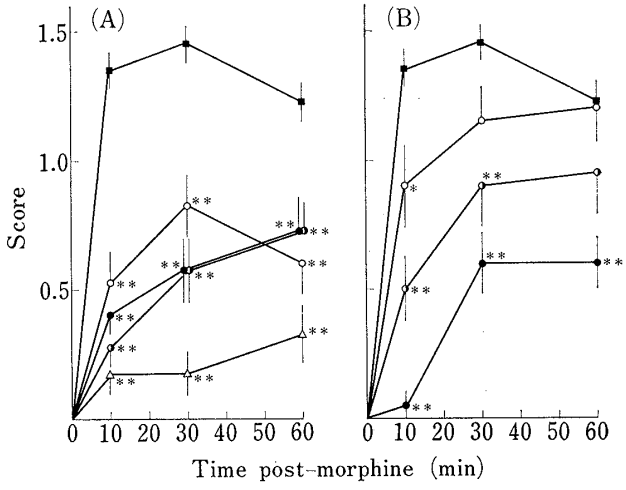

Fig. 4. Effect of Narcotic Antagonists on the Morphine-Induced Straub Tail Reaction

(A) Naloxone (*i.p.*) was administered 15 min before the administration of morphine 10 mg/kg, *s.c.* Control (■——■); naloxone 0.25 mg/kg (○——○); 0.5 mg/kg (◑——◑); 1 mg/kg (●——●); 2 mg/kg (△——△. (B) Pentazocine (*i.p.*) was administered 10 min before the administration of morphine 10 mg/kg, *s.c.* Control (■——■); pentazocine 20 mg/kg (○——○); 40 mg/kg (◑——◑); 80 mg/kg (●——●). Each point represents the mean of ten to fifteen mice. Vertical bars indicate the standard errors of the means. $*p<0.05$, $**p<0.01$ compared to the control group.

of morphine, 50 μg/mouse, was less marked than that induced by 40 μg/mouse. Morphine at 100 μg/mouse proved to be lethal.

Effect of Narcotic Antagonists on the Morphine-Induced Straub Tail Reaction

As shown in Fig. 4, naloxone, 0.25—2.0 mg/kg, *i.p.*, and pentazocine, 20—80 mg/kg, *i.p.*, depressed the morphine-induced STR markedly. Pentazocine decreased the morphine-induced STR as much as naloxone but was much less potent (naloxone depressed the morphine-induced STR at approximately one-hundredth of the dose of pentazocine). Neither of these drugs elicited the STR when given alone.

Effect of Centrally Acting Muscle Relaxants on the Morphine-Induced Straub Tail Reaction

As summarized in Table I, chlordiazepoxide, diazepam and oxazepam decreased the morphine-induced STR markedly. Carisoprodol, chlormezanone, chlorzoxazone and meprobamate inhibited the morphine-induced STR markedly. The antiparkinsonism agent, orphenadrine decreased the morphine-induced STR markedly. The neuroleptics, chlorpromazine,

Table I. Effect of Centrally Acting Muscle Relaxants on the Morphine-Induced Straub Tail Reaction

Drug	Dose (mg/kg)	Route	Score (mean ±S.E.)	Inhibition (%)	*P*
Saline		*i.p.*	1.5±0.1		
Chlordiazepoxide	10	*i.p.*	1.0±0.1	33	<0.05
	20	*i.p.*	0.9±0.2	40	<0.05
Diazepam	2.5	*i.p.*	1.1±0.2	27	N.S.
	5	*i.p.*	0.6±0.1	60	<0.001
Oxazepam	10	*i.p.*	0.8±0.1	47	<0.001
	20	*i.p.*	0.6±0.1	60	<0.001
Carisoprodol	50	*i.p.*	0.9±0.2	40	<0.05
	100	*i.p.*	0.6±0.3	60	<0.01
Chlormezanone	50	*i.p.*	1.1±0.6	27	N.S.
	100	*i.p.*	0.3±0.1	80	<0.001
Chlorzoxazone	50	*i.p.*	1.1±0.2	27	N.S.
	100	*i.p.*	0.7±0.2	50	<0.001
Meprobamate	50	*i.p.*	1.1±0.2	27	N.S.
	100	*i.p.*	0.8±0.2	47	<0.01
Orphenadrine	12.5	*i.p.*	0.8±0.2	47	<0.01
	25	*i.p.*	0.5±0.1	67	<0.001
Chlorpromazine	1	*i.p.*	1.2±0.2	20	N.S.
	2	*i.p.*	0.5±0.3	67	<0.001
Perphenazine	2	*i.p.*	1.2±0.5	20	N.S.
	5	*i.p.*	0	100	—
Haloperidol	5	*i.p.*	0.7±0.2	53	<0.001
	10	*i.p.*	0.2±0.1	80	<0.001
Baclofen	5	*i.p.*	0.3±0.1	80	<0.001
	10	*i.p.*	0.1±0.1	93	<0.001
Difenamizole	10	*i.p.*	0.9±0.1	40	<0.001
	15	*i.p.*	0.6±0.1	60	<0.001
Saline		*i.c.*	1.4±0.1		
Baclofen	0.1[a]	*i.c.*	0.7±0.2	50	<0.001
	0.2[a]	*i.c.*	0.6±0.1	57	<0.001
Difenamizole	100[a]	*i.c.*	0.8±0.1	43	<0.001
	200[a]	*i.c.*	0	100	—

Each drug was administered 15 min before morphine 10 mg/kg, *s.c.* Thirty min after the administration of morphine, the intensity of the STR was assessed according to the scoring system (mean ±S.E. of ten to twenty mice) described in "Materials and Methods." The mean control level in these experiments was 1.5±0.1 (*i.p.* administration of vehicle+morphine 10 mg/kg, *s.c.*) or 1.4±0.1 (*i.c.* administration of vehicle+morphine 10 mg/kg, *s.c.*). The inhibition percentage for each treatment was calculated using the mean values for intensity.
a) μg/mouse. N.S., Not significantly different from the vehicle-treated group.

perphenazine and haloperidol also reduced the morphine-induced STR markedly. Furthermore, the morphine-induced STR was inhibited markedly by intracerebral injection of baclofen and difenamizole. Of particular interest was the fact that the intracerebral dose of baclofen required for this inhibition was as low as approximately one-thousandth of that necessary in the case of intraperitoneal administration. In addition, none of the centrally acting muscle relaxants elicited the STR when given (*i.p.*) without morphine.

Discussion

The time of peak STR was 30 min after the administration of various doses of morphine, corresponding to the peak time of analgesic effect and of brain morphine content.[9] Morphine, oxycodone and levorphanol elicited the STR dose-dependently but codeine, pethidine and pentazocine gave only slight STR. Naloxone (40 mg/kg) scarcely elicited the STR. The dose–response curve for morphine was linear in contrast to those for levorphanol and oxycodone. Therefore, morphine (10 mg/kg, 30 min after administration) was suitable for subsequent drug antagonism studies. In addition, the finding that analgesic doses[10] of morphine, codeine, pethidine and pentazocine gave only slight STR suggests that the STR and analgesia induced by narcotic drugs may be dissociable.

The narcotic antagonists, naloxone and pentazocine depressed the morphine-induced STR. This finding indicates that the STR may be at least partly elicited through the mediation of opioid receptors[11] in the CNS in mice. On the other hand, codeine, pethidine and pentazocine gave only slight STR. It appears that opioid receptors may not be crucial for the manifestation of the morphine-induced STR.

Intracerebral injection of morphine elicited the STR, but not markedly. Heinekamp[4a] concluded, on the other hand, that the STR is partly due to direct excitation of the spinal cord. Leimdorfer[4b] found evidences that the morphine-induced STR is a reflex phenomenon originating in the spinal cord based on electroencephalographic studies. Biochemical data have also supported the view that cerebrospinal pathways may play some role in the manifestation of the morphine-induced STR.[5a,c] In particular, it has been reported that intraspinal injection of morphine elicits the STR.[12] Consequently, the finding that intracerebral injection of morphine gave only slight STR supports the view that one of the sites eliciting the STR may be the spinal cord in addition to the brain. From these results, it appears that the morphine-induced STR may be a suitable animal model for evaluating centrally acting muscle relaxants.

The mechanism by which chlordiazepoxide, diazepam, oxazepam, carisoprodol, chlormezanone, chlorzoxazone, meprobamate, orphenadrine, chlorpromazine, perphenazine, haloperidol, baclofen and difenamizole depress the morphine-induced STR probably involves action on the cerebrospinal pathways without any peripheral action. This assumption is supported by the following evidence: a) diazepam acts at the supraspinal level[13]; b) diazepam, oxazepam and haloperidol inhibit the linguomandibular reflex, a polysynaptic reflex involving interneurons in the reticular formation[14]; c) chlordiazepoxide, diazepam and carisoprodol

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depress the crossed extensor refrex[15]; d) orphenadrine blocks the reticular and spinal facilitation of motor function[16]; e) chlorpromazine, diazepam and orphenadrine depress decerebrate rigidity[17]; f) baclofen affects both the spinal cord and the higher CNS without any peripheral action[18]; g) difenamizole depresses the function of the reticular formation,[19] and the inhibitory action of difenamizole on the morphine-induced STR is facilitated by intracerebral injection of 5-hydroxytryptamine.[20] In addition, neuroleptics such as chlorpromazine and haloperidol block central dopaminergic receptors, thereby interrupting dopaminergic transmission.[21] The action mechanism of benzodiazepines may be mediated by a change in the function of GABA-ergic neurons.[22] The morphine-induced STR was reduced considerably by intracerebral injection of GABA[5b] or one of its derivatives, baclofen.[1] The suppressive effects of neuroleptics and benzodiazepines on the morphine-induced STR are presumably due to blockade of catecholaminergic receptors[5a] and an inhibitory function of GABA in the CNS,[23] respectively. The action of centrally acting muscle relaxants from a neurochemical point of view is being investigated in more detail.

These findings support, in contrast to the results obtained by Srimal *et al.*[6c] or by Ellis and Carpenter,[6e] the view that the STR is positively depressed by centrally acting muscle relaxants. This animal model requires no costly or elaborate equipment and involves neither anesthesia nor surgery. Therefore, the STR appears to be available for comparatively rapid and accurate evaluation of drugs.

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