Pharmacological Studies of Lappaconitine. Analgesia Produced by Intracerebroventricular, Intracisternal and Intrathecal Injections

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The antinociceptive effects of lappaconitine (LA) and morphine (MOR) when injected intracerebroventricularly (i.c.v.), intracisternally (i.cist.) or intrathecally (i.t.) were investigated in mice by use of the tail pinch, hot plate and acetic acid-induced writhing tests. In addition, the effects of naloxone (NLX) administered subcutaneously on the antinociceptive action of LA and MOR were studied. LA and MOR after i.c.v., i.cist. and i.t. injection produced dose-dependent antinociception. MOR was more potent in the tail pinch and acetic acid-induced writhing tests by i.t. injection than by i.c.v. or i.cist. injection, but the potency in the hot plate test was independent of the injection site. On the other hand, like MOR, LA was more potent after i.t. injection than after i.c.v. or i.cist. injection in the tail pinch and acetic acid-induced writhing test. Its antinociceptive action was less potent than that of MOR. The antinociceptive actions of MOR were antagonized by NLX (1 mg/kg, s.c.) in every test and injection site, whereas the antinociceptive actions of LA were not inhibited, except for the antinociception on the tail pinch test, which was partially antagonized by NLX. It is suggested that LA mainly produced an NLX-resistant antinociceptive effect at the spinal site.

Keywords — lappaconitine; antinociception; intracerebroventricular injection; intracisternal injection; intrathecal injection; morphine; naloxone; mouse

In previous papers, we demonstrated that lappaconitine (LA) has a centrally acting antinociceptive action,¹⁾ and its action is not mediated *via* the opioid receptors.²⁾ In order to clarify the active sites in the central nervous system, LA was injected intracerebroventricularly (i.c.v.), intracisternally (i.cist.) and intrathecally (i.t.) in mice. The antinociceptive effects of LA after these injections were examined by use of the tail pinch, hot plate and acetic acid-induced writhing tests.

Materials and Methods

Drugs — Lappaconitine hydrobromide (LA) and morphine hydrochloride (MOR, Takeda, Japan) were dissolved in artificial cerebrospinal fluid (ACSF), and naloxone hydrochloride (NLX, Sigma, U.S.A.) was dissolved in saline. NLX at 1mg/kg was administered s.c., 5 min before LA or MOR injection. The doses were given in terms of the salts.

Animals — Male mice of Std:ddY strain weighing 20 to 30 g were used. Animals were maintained in a temperature- and humidity-

controlled room (22—24 °C, 50—60%) and allowed free access to food and water.

Procedures for the i.c.v., i.cist. and i.t. Injection — The procedure for i.c.v. injection in mouse was essentially that described by Haley and McCormick.³⁾ Hamilton microsyringes bearing a 27-gauge needle with a stop at 3 mm from the needle tip were utilized for administration. The animals were gently restrained, and 10 μ l of ACSF or drug solution was administered into the lateral ventricle at a rate of 1.0 μ l/s. The procedure for i.cist. injection was adapted from the method described by Ueda *et al.*⁴⁾ Hamilton microsyringes equipped with 27-gauge needles which were curved (40 °) at 3.5 mm from the tips were used for injection. The tip of the needle was inserted into the cleft and the bent part of the needle was kept in close contact with the internal surface of the occiput for the entire length (3.5 mm). A volume of 10 μ l of ACSF and drug solution was injected slowly into the cisterna magna and the needle was withdrawn. The i.t. injection procedure essentially followed the method described by Hylden and Wilcox.⁵⁾ The lumbar puncture was performed using a

28-gauge needle on a Hamilton microsyringe. The needle was inserted between the L5 and L6 vertebrae, and drugs were delivered in a volume of 5 μ l. The success rates of i.c.v., i.cist. and i.t. injection were approximately 90%, as determined by dye injections in a separate group of animals.

Measurement of Antinociceptive Activity

— The procedures of the tail pinch, hot plate and acetic acid-induced writhing tests in mice were as described in a previous paper.¹⁾ In the tail pinch test, the latency of the biting response and in the hot plate test, the latency of the licking a hind paw or jumping response was measured at 5, 15, 30, 45, 60 and 90 min after the injection of LA or MOR, respectively. These latencies were converted to percentage effect by means of the formula:

$$\text{percentage effect} = \frac{(\text{drug latency} - \text{control latency})}{(\text{cut-off value} - \text{control latency})} \times 100$$

where the cut-off time was set at 15 s (tail pinch) or 45 s (hot plate). In the acetic acid-induced writhing test, 0.7% acetic acid solution at a volume of 0.1 ml/10 g b.w. was administered i.p., 5 min after LA or MOR injection.

Statistical Analysis — The maximum percentage effect or percent inhibition of writhing was plotted against the log dose and the 50% effective dose (ED_{50}) was calculated from the log dose-effect curve. In the NLX experiment, antinociceptive activity was expressed as a percent-

Fig. 1. Dose-Response Curves for the Antinociceptive Effects of LA (Open Symbols) and MOR (Closed Symbols) Injected i.c.v. (○, ●), i.cist. (△, ▲) and i.t. (□, ■) in Mice

age relative to the mean percentage effect of a respective NLX-nontreated group. Data were statistically evaluated using Student's *t* test and differences were considered significant at $p < 0.05$.

TABLE I. Mean Effective Dose of i.c.v., i.cist. and i.t. Injected LA and MOR in the Mice

Drug		ED_{50} (95% confidence limit)					
		i.c.v.		i.cist.		i.t.	
Tail pinch	LA	2333	(1674–4538)	2413	(1967–3593)	1127	(1031–1304)
	MOR	457	(415–500)	518	(462–582)	64.9	(58.9–71.7)
Hot plate	LA	607	(475–721)	532	(418–636)	644	(541–808)
	MOR	20.0	(17.4–22.5)	14.2	(11.2–18.1)	18.6	(15.7–21.8)
Acetic acid-induced writhing	LA	308	(260–354)	233	(206–266)	62.5	(49.3–74.0)
	MOR	47.9	(43.2–53.1)	14.4	(13.1–16.0)	0.488	(0.373–0.531)

Doses are shown as ng/mouse. Responses were determined at the time of peak effect after drug administration. $n = 15-20$

Results

Antinociceptive Effect

Dose-response curves and ED₅₀ values are shown in Fig. 1 and Table I.

Tail Pinch Test — The ED₅₀ value of LA after i.t. injection was 1127 ng/mouse, and the intensity of the effect was twice that after i.c.v. injection (ED₅₀: 2333 ng/mouse) and i.cist. injection (ED₅₀: 2413 ng/mouse). As in the case of LA, i.t. injection of MOR showed more potent antinociceptive activity than that of i.c.v. and i.cist. injection. The ED₅₀ values of MOR after i.c.v., i.cist. and i.t. injection were 457, 518 and 64.9 ng/mouse, respectively.

Hot Plate Test — LA and MOR showed no difference between the antinociceptive potencies at the three injection sites. The ED₅₀ values of LA and MOR were 607 and 20.0 ng/mouse, respectively, in the i.c.v. injection, 532 and 14.9 ng/mouse, respectively, in the i.cist. injection, and 644 and 18.6 ng/mouse, respectively, in the i.t. injection.

Acetic Acid-Induced Writhing Test — The ED₅₀ value of LA after i.t. injection was 62.5 ng/mouse, and the intensity of the effect was 4 to 5 times greater than that after i.c.v. (ED₅₀: 308 ng/mouse) and i.cist. injection (ED₅₀: 233 ng/mouse). The ED₅₀ values of MOR after i.c.v., i.cist. and i.t. injection were 47.9, 14.4 and 0.488 ng/mouse, respectively, and i.t. injection

was 100 times and 30 times more potent than i.c.v. and i.cist. injection, respectively.

Inhibition of LA or MOR Induced Antinociception by Pretreatment with NLX

Results expressed as percentages are shown in Table II.

The antinociceptive actions of MOR after i.c.v., i.cist. and i.t. injection were antagonized by NLX pretreatment. The degrees of inhibitions were 72 to 83%, 63 to 85% and 71 to 85%, respectively. Only in the tail pinch test was LA-induced antinociception inhibited by NLX; the percentage of inhibition for i.c.v., i.cist. and i.t. injection were 32, 33 and 36%, respectively. However, the antinociceptions of LA in the hot plate and acetic acid-induced writhing tests were not antagonized by NLX. The percentages of inhibition for i.c.v., i.cist. and i.t. injection were 22, 6 and 24%, respectively, in the hot plate test, and 7, 19 and 17%, respectively, in the acetic acid-induced writhing test.

Discussion

Antinociceptive effects of LA after i.c.v., i.cist. and i.t. injection were compared with those of MOR, and influence of NLX treatment upon these antinociceptive effects was studied.

When injected i.c.v., i.cist. and i.t., LA and MOR showed dose-dependent antinociceptive activities against mechanical, heat and chemical

TABLE II. Effect of s.c. Administered NLX on i.c.v., i.cist. and i.t. Injected LA or MOR

	Drug	i.c.v.	i.cist.	i.t.
Tail pinch	LA	100±11	100±11	100±12
	LA + NLX	68±12	67±10 ^{a)}	64±10 ^{a)}
	MOR	100±6	100±6	100±6
	MOR + NLX	21±3 ^{b)}	15±2 ^{b)}	18±3 ^{b)}
Hot plate	LA	100±10	100±10	100±9
	LA + NLX	78±9	94±9	76±10
	MOR	100±10	100±7	100±6
	MOR + NLX	17±3 ^{b)}	32±4 ^{b)}	15±3 ^{b)}
Acetic acid-induced writhing	LA	100±5	100±6	100±6
	LA + NLX	93±6	81±7	83±6
	MOR	100±6	100±3	100±4
	MOR + NLX	28±4 ^{b)}	37±7 ^{b)}	29±4 ^{b)}

The data are expressed as percentages relative to the mean percentage effect of the respective NLX nontreated group, and values are mean ±S.E. of 15–20 mice. Significant differences from nontreated groups, ^{a)} $p < 0.05$, ^{b)} $p < 0.001$.

nociception. However, in the tail pinch test, i.c.v. and i.cist. injected LA did not show definite antinociceptive activity and i.c.v. injection produced flatter dose-response curve. At these antinociceptive doses, no gross signs of behavioural depression were detected. In the hot plate test, the antinociceptive activities of LA or MOR by i.c.v., i.cist. and i.t. injection were almost equipotent. Though in the tail pinch and acetic acid-induced writhing test, i.t. injection showed more potent antinociceptive activity than that of i.c.v. and i.cist. injection. On the other hand, systemically administered LA was not antagonized by NLX²⁾ but in the present tail pinch test, supraspinally and spinally administered LA were partially antagonized by NLX. In contrast to LA, supraspinal and spinal antinociception of MOR were significantly antagonized by systemic NLX administration.

The dorsal root ganglion cell and the nociceptive primary afferent fiber contain neuropeptides, such as substance P or cholecystokinin.⁶⁾ It has been recently reported that noxious mechanical or thermal stimuli specifically increase the release of substance P or somatostatin, respectively,⁷⁾ and MOR or opioid peptides had NLX-reversible suppressive action on the substance P and somatostatin release.^{8,9)} On the other hand, endogenous peptides, e.g. neurotensin and vasopressin, exhibit NLX-resistant analgesic activity.¹⁰⁻¹²⁾ From these facts and the results in this experiment, it is conceivable that the central site of the antinociceptive effect of LA may be located at the spinal level and may involve release of NLX-sensitive endogenous opioid peptides and/or NLX-resistant endogenous analgesic substance.

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