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Mechanism of the antinociceptive action of mesaconitine: participation of brain stem and lumbar enlargement

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1 The antinociceptive action of mesaconitine (MA) microinjected into the nucleus reticularis gigantocellularis (NRGC), the nucleus reticularis paragigantocellularis (NRPG), the periaqueductal gray (PAG) or the lumbar enlargement was investigated in rats by use of the tail immersion test. In addition, the effects of β-adrenoceptor antagonists and an α-adrenoceptor antagonist administered intrathecally (i.t.) on the antinociceptive action of MA given into the NRPG were also examined by the tail immersion test.

2 MA (50, 100 ng per rat) microinjected into the NRGC, the NRPG, the PAG and the lumbar enlargement increased the response latency in rats in a dose-dependent fashion. MA (50 ng per rat) microinjected into neighbouring sites, the nucleus reticularis parvocellularis, the nucleus originis nervi abducentis and the fasciculus longitudinalis medialis, elicited no significant effect.

3 Intrathecally administered propranolol (1 and 5 μg per rat), atenolol (1 and 5 μg per rat) and IPS-339 (1 and 5 μg per rat) remarkably inhibited the increase of the response latency induced by MA (50 ng per rat) given into the NRPG.

4 Intrathecally administered phenoxybenzamine (1 and 5 μg per rat) inhibited the increase of the response latency induced by MA (50 ng per rat) injected into the NRPG but to a lesser extent than the β-adrenoceptor antagonists.

5 It is concluded that the NRGC, the NRPG, the PAG and the lumbar enlargement are involved in the sites of the antinociceptive action of MA and that the antinociceptive effect of MA administered into NRPG is elicited by activation of the inhibitory noradrenergic neurones from the NRPG in particularly via β-receptor-mediated effects of noradrenaline.

Introduction

In a previous paper, we demonstrated that the sites of antinociceptive action of mesaconitine (MA) were present in the central nervous system (Murayama *et al.*, 1984). MA-induced antinociception can be potentiated by β-adrenoceptor stimulation and reduced by β-adrenoceptor inhibition (Murayama & Hikino, 1985). However, the sites at which the antinociceptive action of MA are produced in the central nervous system have not been elucidated.

In order to clarify the active sites in the central nervous system, MA was microinjected into the nucleus reticularis gigantocellularis (NRGC) and the nucleus reticularis paragigantocellularis (NRPG) in the lower brain stem and the periaqueductal gray (PAG) in the upper brain stem of rats. In addition MA was injected into the lumbar enlargement in the spinal cord through a catheter inserted into the spinal subarachnoid space. The antinociceptive activity of MA after these injections was measured by means of the tail immersion test in rats.

It has been reported that the inhibitory neurones descending from the NRPG, which include noradrenergic neurones, project to lamina V of the lumbar enlargement and modulate the transmission of the noxious stimuli (Meessen & Olszewski, 1949; Takagi *et al.*, 1975; 1976). Hence, we also investigated the role of the descending noradrenergic neurones in the antinociceptive action of MA by the combined administration of MA into the NRPG with adrenoceptor antagonists into the lumbar enlargement in rats.

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Methods

Male rats of the Sprague-Dawley strain, weighing 200–250 g, were used. All rats were maintained at 24–25°C on a 12 h light-dark cycle and were given food and water *ad libitum*.

Microinjection into the brain stem

Rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). The stainless steel guide cannula (23 gauge, 6 mm long) with stylet unit was stereotaxically implanted according to the atlas of Fifková & Maršala (1967) and fixed to the skull with dental cement. The target sites were the NRGC, the NRPG and the PAG. Each rat was kept in a separate cage and at least 7 days was allowed for recovery from surgery before microinjection of the drug. An injection cannula of 30 gauge stainless steel tube was attached to a 10 μl Hamilton syringe by a PE-10 polyethylene tube and drug solution (0.5 μl) was injected over an interval of approx. 60 s.

Intrathecal administration of drug

The insertion of the catheter (9 cm) into the spinal subarachnoid space of the lumbar enlargement was carried out according to the method of Yaksh & Rudy (1976). The catheter was flushed with physiological saline solution and the animal was permitted to recover for 7 days before experimentation. Only animals without motor deficiencies were used. Drug solution (5 μl) was injected and the catheter (volume: 6–8 μl) was cleared by injection of physiological saline solution (10 μl).

Measurement of antinociceptive activity

Nociceptive response latency was determined by the tail immersion test in rats (Ben-Bassat *et al.*, 1959). Briefly, the test involves the immersion of the whole tail in water maintained at 58°C for assessment of antinociception. The nociceptive end point was taken as the time when the animal responded either by a violent jerk of the tail or total body flick. A cut off time of 10 s was imposed on all animals during the measurement of the response latency in order to avoid local tissue damage. Antinociceptive activity of a drug is described as a percentage of the response time before drug-treatment. At the end of experiment, the location of the injection cannula tip was marked by injection of methylene blue dye solution (0.5 μl) just before decapitation. The brain was then removed and fixed in 10% neutral formalin for 4 to 5 days and sectioned. Microinjection sites were observed microscopically.

The antinociceptive effect of MA (1, 3, 9, 27, 81 μg kg^{-1}) administered intravenously was measured 40 min after administration and the 50% effective dose (ED_{50}) and 95% confidence limits were calculated according to Litchfield & Wilcoxon (1949).

Drugs

Mesaconitine hydrobromide (MA) was prepared from *Aconitum* roots in our laboratory (Sato *et al.*, 1979). The other drugs used were atenolol (Sigma), phenoxybenzamine hydrochloride (Tokyo Kasei) and propranolol hydrochloride (Sumitomo Chemical). IPS-339 ((t-butyl-amino-3-ol-2-propyl)-oximino-9-fluorene) was a gift from Dr Leclerc (Université Louis Pasteur).

Statistical analysis of data

Data are expressed as mean ± s.e.mean. One-way analysis of variance was used to evaluate the results in all the experiments.

Results

Antinociceptive effect of mesaconitine administered into the nucleus reticularis gigantocellularis, the nucleus reticularis paragigantocellularis and neighbouring sites

Saline administration into the NRGC or the NRPG resulted in no changes of the response latency in the tail immersion test. MA microinjected into the NRGC or the NRPG caused a dose-dependent increase of the response latency. MA administration of 50 and 100 ng per rat into the NRPG or 50 ng per rat into the NRGC showed biphasic changes of the response latency of rats. The first phase was found as a slight increase of the response latency at 5 to 10 min after MA administration, and the second phase was found as a greater increase of the response latency at 20 to 120 min after MA administration (Figure 1a,b). Staggering gait was found in rats injected with MA at 5 to 20 min after administration. Hyperreactivity in response to noise or air puff was not observed.

The effect of MA (50 ng per rat) administered into the sites neighbouring the NRGC and the NRPG, the nucleus reticularis parvocellularis, the nucleus originis nervi abducentis and the fasciculus longitudinalis medialis, was not significant. The maximum response latency in these sites was observed 60, 30 or 45 min respectively after MA administration but was only 1.3 to 1.4 times that of the control.

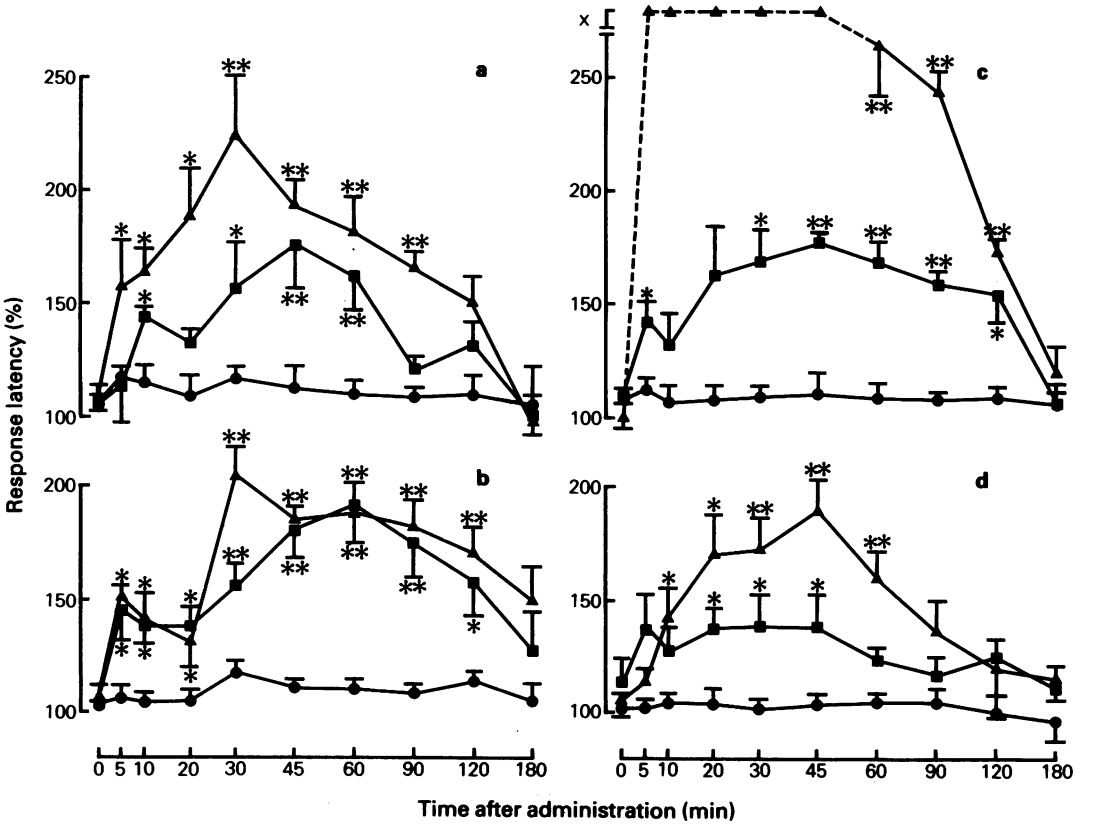

Figure 1 Antinociceptive effect of mesaconitine injected into the nucleus reticularis gigantocellularis (a), the nucleus reticularis paragigantocellularis (b), the periaqueductal gray (c) and the lumbar enlargement (d) in rats. Symbols indicate the following: in (a), (b) and (c), control ($n = 6$, ●), MA ($n = 5$, 50 ng per rat, ■) and MA ($n = 5$, 100 ng per rat, ▲), and in (d), control ($n = 7$, ●), MA ($n = 7$, 0.5 μg per rat, ■) and MA ($n = 7$, 1.0 μg per rat, ▲). (x) in (c) represents the response latency of cut off time. The mean response time (s) of the control was 2.1 ± 0.1. Significantly different from the control: $^{*}P < 0.05$ or $^{**}P < 0.01$.

Antinociceptive effect of mesaconitine administered into the periaqueductal gray

Saline administered into the PAG exhibited no effect on the response latency of rats. MA administration (50 ng per rat) into the PAG caused biphasic changes in the response latency. Thus, the potentiation of the response latency was found at 5 min and at 20 to 120 min after MA administration. MA (100 ng per rat) injected into the PAG increased the response latency at 5 to 120 min after administration, the effect of 100 ng per rat being greater than that seen with 50 ng per rat (Figure 1c). Running was observed in the MA-injected rats immediately after MA administration, in particular at 100 ng per rat, and continued for about 30 min.

Antinociceptive effect of mesaconitine administered into the lumbar enlargement

MA (0.5 and 1.0 μg per rat) administered into the subarachnoid space of the lumbar enlargement caused dose-dependent increases of the response latency at 10 to 60 min after administration (Figure 1d).

Antinociceptive effect of mesaconitine administered intravenously

The ED_{50} value of the antinociceptive effect of MA administered intravenously was 9.1 μg kg^{-1} (95% confidence limits: 5.1–16.2). The i.v. dose of MA required to cause 180% of the response latency was 12.0 μg kg^{-1} (95% confidence limits: 6.6–21.0) which was equivalent to the ED_{55}.

Table 1 Effect of intrathecally administered propranolol, atenolol, IPS-339 and phenoxybenzamine on the antinociception induced by mesaconitine injected into the nucleus reticularis paragigantocellularis in rats

Drug (μg per rat, i.t.)	*Dose of mesaconitine* (ng per rat)	*Response latency* (%) *0*	*5*	*10*	*20*	*30*	*60*	*90*	*120* (min)
Saline	0	107 ± 6	102 ± 3	110 ± 5	99 ± 9	106 ± 8	108 ± 5	98 ± 7	105 ± 4
Saline	50	105 ± 2	140 ± 7	138 ± 7	137 ± 10	151 ± 7	183 ± 15	162 ± 14	144 ± 12
Propranolol									
1	0	97 ± 4	103 ± 12	108 ± 6	110 ± 3	101 ± 6	108 ± 5	105 ± 6	118 ± 11
5	0	99 ± 4	101 ± 15	112 ± 10	100 ± 3	115 ± 3	115 ± 8	107 ± 5	104 ± 5
1	50	98 ± 6	135 ± 7	129 ± 7	111 ± 7	124 ± 10	127 ± 3	134 ± 3	132 ± 1
5	50	108 ± 8	139 ± 10	125 ± 15	115 ± 8	106 ± 5**	105 ± 3**	111 ± 8*	110 ± 5
Atenolol									
1	0	89 ± 3	103 ± 15	114 ± 7	106 ± 14	118 ± 15	117 ± 9	108 ± 5	111 ± 5
5	0	90 ± 3	84 ± 9	107 ± 7	104 ± 10	113 ± 12	98 ± 15	90 ± 6	96 ± 2
1	50	109 ± 9	134 ± 7	125 ± 16	104 ± 10	115 ± 17**	156 ± 12	153 ± 9	126 ± 6
5	50	106 ± 4	129 ± 9	131 ± 4	126 ± 4	125 ± 5*	126 ± 5*	114 ± 5*	113 ± 5
IPS-339									
1	0	103 ± 5	110 ± 5	122 ± 2	106 ± 6	114 ± 15	124 ± 10	107 ± 9	118 ± 6
5	0	101 ± 14	101 ± 4	102 ± 8	113 ± 10	98 ± 2	113 ± 6	98 ± 7	97 ± 11
1	50	105 ± 5	138 ± 5	135 ± 10	129 ± 11	118 ± 11*	126 ± 5	122 ± 14	116 ± 6
5	50	104 ± 4	148 ± 13	124 ± 16	105 ± 8	110 ± 7**	109 ± 10**	112 ± 12	120 ± 6
Phenoxybenzamine									
1	0	119 ± 7	117 ± 8	116 ± 4	106 ± 6	111 ± 5	117 ± 7	101 ± 5	114 ± 7
5	0	97 ± 5	104 ± 5	96 ± 5	88 ± 8	95 ± 11	80 ± 4	88 ± 9	91 ± 5
1	50	109 ± 2	138 ± 7	130 ± 9	127 ± 8	151 ± 6	157 ± 5	142 ± 3	129 ± 12
5	50	115 ± 7	134 ± 11	142 ± 6	126 ± 12	143 ± 14	141 ± 9	140 ± 6	131 ± 11

$n = 6$. Propranolol, atenolol, IPS-339 and phenoxybenzamine were administered at − 15 min, and mesaconitine was given at 0 min. The mean response time (s) of the control was 2.0 ± 0.1. Significantly different from the mesaconitine control group at each time: $*P < 0.05$ or $**P < 0.01$.

Effect of adrenoceptor antagonists administered into the lumbar enlargement on antinociception produced by mesaconitine microinjected into the nucleus reticularis paragigantocellularis

Intrathecally administered propranolol, atenolol and IPS-339 (1 and 5 μg per rat) elicited no effect on the response latency in rats. The increase of the response latency induced by MA (50 ng per rat) administered into the NRPG was significantly diminished in a dose-dependent manner by the i.t. administration of propranolol, atenolol and IPS-339. Phenoxybenzamine (1 and 5 μg per rat, i.t.) produced a slight hyperalgesia and reduced MA-induced potentiation of the response latency (Table 1).

Discussion

The NRGC, the NRPG, the PAG and the lumbar enlargement are known to be the very important sites correlating with the transmission of nociceptive information (Tsou & Yang, 1964; Takagi *et al.*, 1975; 1977). In the present study, the participation of these sites in the antinociceptive action of MA was first investigated.

MA administered into the NRGC and the NRPG increased the response latency of rats dose-dependently. The time courses of the increase of the response latency were similar in both cases, showing biphasic patterns composed of the first phase, a transient increase of the response latency at 5 to 20 min after MA administration, and the second phase, a greater increase of the response latency after 30 min of MA administration. Judging from the results that (1) the second phase was found without any behavioural changes and (2) MA administered into the sites neighbouring the NRGC and the NRPG exerted no significant effect, among the lower brain stem structures, the NRGC and the NRPG are now concluded to be sensitive regions with respect to the antinociception induced by MA. Although the mechanism of the first phase, a slight increase of the response latency with staggering gait of the rats, is not well understood, it may presumably originate from the behavioural changes in addition to the antinociceptive effect of MA.

In the PAG, MA at the dose of 50 ng per rat exhibited a biphasic antinociceptive action similar to that described above and at the dose of 100 ng per rat

caused a very potent increase of the response latency. It is now known that descending neurones as well as ascending neurones are present in the PAG and that the descending inhibition from the PAG correlates with neural mechanism of analgesia (Carstens *et al.*, 1979). Therefore, the antinociceptive action of MA may occur by the activation of descending neurones from the PAG.

Next, the effect of MA administered i.t. on the response latency in rats was examined. The dose-dependent increase of the response latency was found also in this case, indicating that the lumbar enlargement is one of the sites of the antinociceptive action of MA.

Now, it was possible that MA injected into brain regions exerted its effect at the local sites, or MA injected locally escaped into the blood stream and acted at some other sites. The doses of MA injected into brain regions (50 ng per rat ($200-250\,ng\,kg^{-1}$) into the NRGC, the NRPG and the PAG, 1 μg per rat ($4-5\,\mu g\,kg^{-1}$) into the lumbar enlargement, producing 180% of the response latency) were significantly less than the i.v. dose of MA required for the same degree of antinociception ($12\,\mu g\,kg^{-1}$). Additionally, in the sites neighbouring the NRGC and the NRPG, MA had no significant effect. Based on the above evidence, it is concluded that the action of MA injected into the brain regions is the local one.

There are a number of reports that the NRGC, the NRPG, the PAG and the lumbar enlargement are involved in the sites of the antinociceptive action of morphine (Reynolds, 1969; Takagi *et al.*, 1975; Duggan *et al.*, 1977; Takagi *et al.*, 1977; Calvillo *et al.*, 1979). In this study, MA was now found to cause the antinociceptive effect also in the same sites. However, it is known that the mechanism of the antinociceptive action of MA differs from that of morphine because the antinociceptive effect of MA was not antagonized by levallorphan and the influence of monoamine-related substances on the antinociception of MA was different from that of morphine (Murayama *et al.*, 1984).

Recently, it was demonstrated that the inhibitory neurones, including noradrenergic neurones, project from the NRPG to lamina V of the lumbar enlargement (Takagi *et al.*, 1975; Takagi *et al.*, 1976; Sato, 1981). We demonstrated that MA antinociception is potentiated by noradrenaline or isoprenaline administered intracerebroventicularly (i.c.v.), inhibited by propranolol i.c.v. and not affected by phenoxybenzamine i.c.v., and that MA increased the turnover rate of noradrenaline in the medulla oblongata as well as pons and spinal cord (Murayama *et al.*, 1984; Murayama & Hikino, 1985). Based on the above evidence, it is postulated that MA antinociception can be elicited via noradrenergic neurones in brain stem and spinal cord. Therefore, the effects of several adrenoceptor antagonist given into the subarachnoid space of the lumbar enlargement on the antinociceptive action of MA microinjected into the NRPG were examined. Adrenoceptor antagonists used were propranolol, a nonselective β-antagonist, atenolol, a β_1-selective antagonist, IPS-339, a β_2-selective antagonist, and phenoxybenzamine, a nonselective α-antagonist. Propranolol, atenolol and IPS-339 administered i.t. at the doses used brought about no changes of the response latency but significantly reduced the second phase increase of the response latency produced by MA given into the NRPG. Although phenoxybenzamine also inhibited the potentiation of the response latency by MA, its activity was less than that of the β-blocking agents. It is probable that, besides its α-blocking activity, its hyperalgesic activity may also participate in its inhibitory effect on the antinociceptive action of MA, because phenoxybenzamine alone induced a slight degree of hyperalgesia. Based on the above evidence, it is postulated that MA administered into the NRPG promotes the noradrenergic inhibition of the transmission of noxious stimuli, via β-adrenoceptors rather than via α-adrenoceptors.

In summary, the NRGC, the NRPG, the PAG and the lumbar enlargement are involved in the sites of the antinociceptive action of MA. Further, it is revealed that the antinociceptive effect of MA administered into the NRPG is mediated by activation of the inhibitory noradrenergic neurones contained in descending neurones from the NRPG particularly via β-adrenoceptor mediated effects of noradrenaline.

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