ELSEVIER

European Journal of Pharmacology 330 (1997) 109–113

ejp

Mazindol and lidocaine are antinociceptives in the mouse formalin model: involvement of dopamine receptor

Alvorita L. Bittencourt, Reinaldo N. Takahashi *

Departamento de Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis SC 88015-420, Brazil

Received 19 March 1997; revised 29 April 1997; accepted 7 May 1997

Abstract

The antinociceptive potential of mazindol, an anorectic drug, and lidocaine, an amide-type local anesthetic, were investigated in the mouse formalin test with concurrent motor function assessment. In addition, the role of dopamine and opioid receptors in mediation of the antinociceptive action of these drugs was examined. The i.p. injection of mazindol (1.25–10 mg/kg) and lidocaine (10–30 mg/kg) induced significant antinociceptive responses in both phases of the test. Cocaine (20 mg/kg, i.p.), used as positive control, also inhibited the pain responses caused by formalin. Haloperidol (0.2 mg/kg, i.p.), and sulpiride (5 mg/kg, i.p.), a dopamine D_2 receptor antagonist, reduced the antinociceptive actions of mazindol and cocaine, while SCH 23390, *R*(+)-7-chloro 8-hydroxy-3methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3 benzazepine (0.03 mg/kg, i.p.), a dopamine D_1 receptor antagonist, did not affect these responses. Only the antinociception associated with mazindol was reversed by naloxone (2 mg/kg, i.p.). The same pretreatments failed to modify lidocaine-induced antinociception. The drug conditions used in this study did not reveal any motor impairment in the rotarod test. These observations suggest an involvement of dopaminergic mechanisms, mainly via dopamine D_2 receptors, in the antinociceptive action of mazindol in the formalin test, but the nature of mechanisms involved in the lidocaine responses remains unsolved. © 1997 Elsevier Science B.V.

Keywords: Formalin test; Mazindol; Lidocaine; Antinociception; Dopamine; Opioid

1. Introduction

There is growing evidence that central dopaminergic transmission is involved in the control of nociception. For instance, several studies have shown that apomorphine, a dopamine receptor agonist, induces antinociceptive effects in a variety of tests (Paalzow and Paalzow, 1983; Tulunay et al., 1975; Michaël-Titus et al., 1990). Further, cocaine, another dopamine agonist, has also been reported to possess central analgesic activity in addition to a local anesthetic effect (Lin et al., 1989, Pertovaara et al., 1990). Recently we have found that systemic administration of lidocaine and mainly dimethocaine, a local anesthetic with a great activity at the dopaminergic reuptake binding site, significantly increased both tail-flick and paw-lick latencies and dose dependently reduced the number of writhes induced by acetic acid in mice (Rigon and Takahashi, 1996). These findings confirmed and extended the literature suggesting that other local anesthetics besides cocaine have analgesic effects, presumably acting by dopaminergic mechanisms.

Mazindol is an anorectic drug extensively used in Brazil. It is known to act primarily, although not exclusively, by inhibiting dopamine uptake (Javitch et al., 1983; Pögün et al., 1991). Therefore, similarly to cocaine and d-amphetamine, mazindol increases locomotor activity, reduces food intake, induces circling, stereotypies and self-administration behavior in animals (Kruk and Zarrindast, 1976; Zambotti et al., 1976; Zanin and Takahashi, 1994; Mattei and Carlini, 1995). To the best of our knowledge, no published data exist on the antinociceptive properties of mazindol.

The formalin test is a unique model of nociception which has pharmacological characteristics that differ from those of reflex types of pain test such as the tail-flick test (Dennis and Melzack, 1980; Abbott et al., 1982; Abbott and Young, 1988). Interestingly, midbrain dopamine systems have been implicated in the mediation of analgesia in the formalin test, but not in the tail-flick test (Morgan and Franklin, 1990).

In the light of the above considerations, the present

* Corresponding author. Tel.: (55-48) 231-9764; Fax: (55-48) 222-4164.

PII S0014-2999(97)00182-9

experiments were designed to assess the antinociceptive activities of mazindol and lidocaine in the formalin test in mice. As positive control, an additional group of mice treated with cocaine was used. We also examined the possibility that dopaminergic receptors are involved in the antinociceptive effects of these drugs by co-administering them with dopamine receptor antagonists. Since it is known that there is a close functional relationship between endogenous opioid systems and dopaminergic pathways, the involvement of opioid receptors in these responses was also investigated by using naloxone pretreatment.

2. Materials and methods

2.1. Animals

Male Swiss albino mice weighing 30–35 g from our own colony were used. All animals were kept in cages, in groups of 20, with free access to laboratory food and water. They were maintained in a room with controlled a temperature (23 + 1°C) and a 12 h light cycle (lights on 7:00 h). All procedures used in the present study complied with The Brazilian Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

The drugs used were mazindol HCl (Instituto Quimica Campinas, IQC), lidocaine HCl, haloperidol HCl (Sigma), cocaine HCl (Merck), sulpiride HCl, naloxone HCl, SCH 23390 HCl (*R*(+)-7-chloro 8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine), (Research Biochemicals International). All drugs were dissolved in distilled water, except for mazindol, sulpiride and haloperidol which were dissolved in 0.025% carboxymethylcellulose and diluted with distilled water. All doses are expressed as weight of salt and were administered in a volume of 0.1 ml/10 g of body weight. The control solutions consisted of an equivalent volume of distilled water plus vehicle.

2.3. Formalin test procedure

The formalin test was carried out in an open glass cylinder, 17 cm in diameter, with a mirror placed under the floor to allow an unobstructed view of the paws. Mazindol (1.25, 2.5, 5 or 10 mg/kg), lidocaine (10, 20 or 30 mg/kg), cocaine (20 mg/kg) or control solution was injected i.p. 15 min before the formalin injection. Dopamine receptor antagonists and naloxone were given 15 min before drug treatments. Each animal was injected with 20 μl of formalin 2.5% in the intraplantar region of the right hindpaw. Mice were then observed (2 at a time) 0–5 min and 15–30 min post-formalin and the amount of time spent licking the injected paw was timed with a stopwatch. A vehicle control group was included for each compound or set of compounds evaluated.

2.4. Rotarod test

The rotarod apparatus consisted of rotating bar (2.5 cm diameter) covered with sandpaper and revolving at 7 rpm. Mice were placed upon the bar and the time spent upon the rotating bar was registered at 3 and 20 min after drug administration. Twenty-four hours before the experiment mice underwent a conditioning session. For evaluation of drug action, mice had to remain on the bar for up to 120 s.

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to analyze the influence of treatments upon antinociception and motor coordination. A Newman–Keuls test was used to compare each treatment with the corresponding control group. The accepted level of significance for all tests was $P < 0.05$.

3. Results

The antinociceptive effects of mazindol and lidocaine in the mouse formalin test are illustrated in the two panels of Fig. 1. As can be seen, mazindol (1.25–10 mg/kg, i.p.) and lidocaine (10–30 mg/kg, i.p.) dose dependently attenuated the time of paw licking during the first phase. During the later phase of pain the paw licking of control

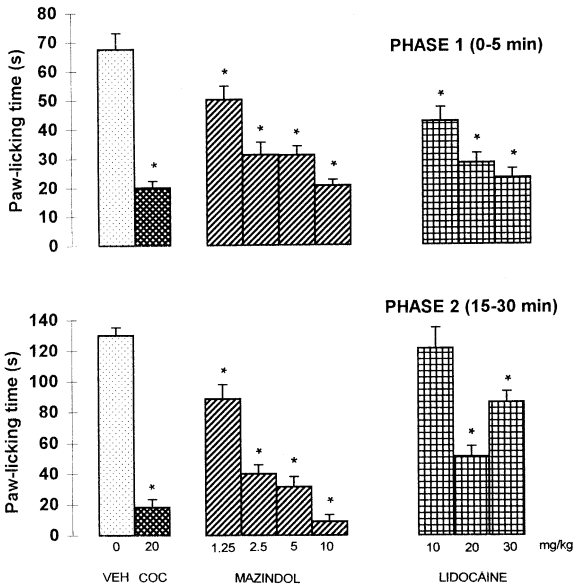

Fig. 1. Effect of mazindol, lidocaine, cocaine (COC) or vehicle (VEH), on formalin-induced paw-licking during phases 1 and 2. Treatment was given i.p. 15 min before formalin injection. Data are expressed as means ± SEM for 10 animals. * Significantly different from the control group, $P < 0.05$, Newman–Keuls test.

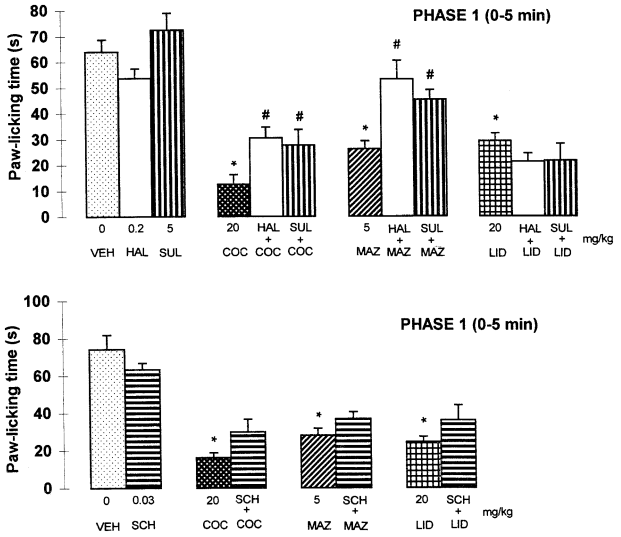

Fig. 2. Influence of dopamine receptor antagonists, haloperidol (HAL), sulpiride (SUL) and SCH 23390 (SCH), on the antinociceptive action of mazindol, lidocaine or cocaine in the first phase of the formalin test. Top panel: dopamine D_2 receptor antagonists HAL (0.2 mg/kg) and SUL (5 mg/kg). Bottom panel: dopamine D_1 receptor antagonist SCH (0.03 mg/kg). Data are expressed as means ± SEM for 10 animals. * Significantly different from group treated with vehicle, $P < 0.05$. # Significantly different from respective control group, $P < 0.05$, Newman–Keuls test.

animals became more prominent. Again mazindol significantly reduced the pain responses in a dose-related manner while only higher doses of lidocaine (20 or 30 mg/kg) showed a significant antinociceptive effect. As expected, cocaine (20 mg/kg, i.p.) produced a significant antinociceptive action in both phases of the test.

Since a qualitatively similar pattern of responses was observed during phases 1 and 2 of the formalin test, only the results of phase 1 are depicted for the following experiments.

The influence of antagonism of dopaminergic receptors on the antinociceptive effects elicited by cocaine, mazindol and lidocaine are shown in Fig. 2. Pretreatment with the preferential dopamine D_2 receptor antagonists haloperidol (0.2 mg/kg, i.p.) and sulpiride (5 mg/kg, i.p.) significantly reversed the antinociceptive effects of mazindol (5 mg/kg) and cocaine (20 mg/kg) in both phases, but not that induced by lidocaine (20 mg/kg) (Fig. 2, top panel).

Pretreatment with the dopamine D_1 receptor antagonist SCH 23390 at a dose of 0.03 mg/kg failed to reverse the antinociceptive effects induced by mazindol, lidocaine and cocaine (Fig. 2, bottom panel).

Haloperidol, sulpiride and SCH 23390 were administered at doses which per se did not affect the pain responses (Fig. 2).

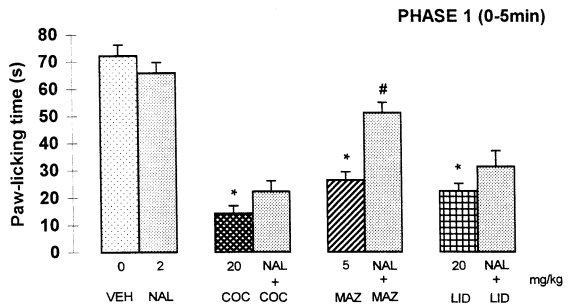

Fig. 3. Influence of an opioid antagonist, naloxone (NAL), on the antinociceptive action of mazindol, lidocaine or cocaine in the first phase of the formalin test. Data are expressed as means ± SEM for 10 animals. * Significantly different from group treated with vehicle, $P < 0.05$. # Significantly different from respective control group, $P < 0.05$, Newman–Keuls test.

Table 1
Influence of cocaine, mazindol, lidocaine, the dopamine receptor antagonists, haloperidol, sulpiride and SCH 23390, and the opioid antagonist, naloxone, upon the ability of mice to remain on the rotarod

Treatment	Dose (mg/kg, i.p.)	Rotarod performance [a]	
		3 [b]	20 [b]
Vehicle (VEH)	—	119.6 ± 0.3	119.5 ± 0.5
Cocaine (COC)	20	119.0 ± 0.6	120.0 ± 0.0
Mazindol (MAZ)	1.25	119.6 ± 0.3	120.0 ± 0.0
MAZ	2.5	119.0 ± 0.6	119.0 ± 0.6
MAZ	5	118.6 ± 1.3	119.3 ± 0.4
MAZ	10	118.6 ± 0.9	119.6 ± 0.3
Lidocaine (LID)	10	120.0 ± 0.0	120.0 ± 0.0
LID	20	119.3 ± 0.6	120.0 ± 0.0
LID	30	120.0 ± 0.0	120.0 ± 0.0
Haloperidol (HAL)	0.2	118.6 ± 0.6	118.6 ± 0.6
HAL + COC	0.2 + 20	119.6 ± 0.3	120.0 ± 0.0
HAL + MAZ	0.2 + 5	118.3 ± 0,6	117.6 ± 1.2
HAL + LID	0.2 + 20	119.3 ± 0.4	120.0 ± 0.0
Sulpiride (SUL)	5	120.0 ± 0.0	120.0 ± 0.0
SUL + COC	5 + 20	119.6 ± 0.3	120.0 ± 0.0
SUL + MAZ	5 + 5	118.6 ± 0.6	119.3 ± 0.4
SUL + LID	5 + 20	119.0 ± 0.6	120.0 ± 0.0
SCH 23390 (SCH)	0.03	119.3 ± 0.6	119.6 ± 0.3
SCH + COC	0.03 + 20	120.0 ± 0.0	120.0 ± 0.0
SCH + MAZ	0.03 + 5	119.3 ± 0.6	120.0 ± 0.0
SCH + LID	0.03 + 20	120.0 ± 0.0	119.6 ± 0.3
Naloxone (NAL)	2	119.3 ± 0.4	120.0 ± 0.0
NAL + COC	2 + 20	119.3 ± 0.6	120.0 ± 0.0
NAL + MAZ	2 + 5	118.6 ± 0.6	120.0 ± 0.0
NAL + LID	2 + 20	120.0 ± 0.0	120.0 ± 0.0

[a] Time (s) that the mouse remained on rotarod.
[b] Min after formalin injection.

Haloperidol, sulpiride and SCH 23390 were administered at doses which per se did not affect the pain responses (Fig. 2).

Fig. 3 illustrates the influence of the opioid antagonist naloxone on the pain responses attenuated by mazindol, lidocaine or cocaine in the formalin test. As can be seen only the significant antinociceptive effect of mazindol was sensitive to opioid receptor blockade by 2 mg/kg of naloxone.

The effects of all drug conditions on motor coordination are shown in Table 1. None of the drug regimens at the dosage range used in this study caused motor incoordination as measured in the rotarod apparatus.

4. Discussion

Consistent with our previous study using other pain models, systemic administration of lidocaine significantly

reduced the pain-licking responses in the mouse formalin test, thus confirming that it possesses analgesic properties (Rigon and Takahashi, 1996). Moreover, to our knowledge the present study is the first to document that mazindol, an anorectic drug, induces strong and dose-dependent antinociception in the formalin test, an effect that is dissociated from motor dysfunction and which is probably mediated by central mechanisms. Indeed, the analgesic doses of mazindol required were approximately 4–8-fold lower than those of cocaine tested as positive control.

We previously hypothesized that the antinociceptive effects of lidocaine and mazindol, in a similar way to that induced by cocaine, could involve, at least in part, dopaminergic mechanisms. The present results show that haloperidol and sulpiride, preferential dopamine D_2 receptor antagonists, significantly attenuated the antinociceptive effects of mazindol and cocaine in the formalin test. However, pretreatment with SCH 23390, a dopamine D_1 receptor antagonist, did not modify the responses induced by these drugs. These findings confirm and extend other studies showing that dopamine agonists such as d-amphetamine, cocaine and apomorphine produce analgesia mediated by dopaminergic mechanisms (Lin et al., 1989; Morgan and Franklin, 1990).

One somewhat surprising result from these experiments was that naloxone, an μ opioid antagonist, attenuated the response induced by mazindol, while it failed to alter the antinociceptive effects caused by cocaine. There is sufficient evidence that the dopaminergic and opioid systems are interconnected (Michaël-Titus et al., 1990; Suaudeau and Costentin, 1995). Therefore, we expected that naloxone might also attenuate the action of cocaine, as observed in other experimental models (Pertovaara et al., 1990; Gerrits et al., 1995).

Although at variance with the results of Lin et al. (1989) showing that cocaine produces antinociception in rats which is reversed by dopamine D_1 and D_2 receptor antagonists, our study provides evidence indicating that dopamine D_2 receptors play a critical role in the antinociceptive actions of mazindol and cocaine in the mouse formalin test.

It must be conceded that only one dose of antagonist was used in the present study. However, the observation that the reduced nociceptive responses elicited by mazindol were reversed both by the dopamine D_2 receptor antagonists and the reference opioid antagonist, naloxone, strongly supports the contention that the stimulation of dopamine D_2 receptors by agonists triggers the release of an opioid substance which mediates the analgesic effects induced by dopamine receptor agonists (Suaudeau and Costentin, 1995). Additional support for this hypothesis comes from our experiments showing the additive antinociceptive effect following the co-administration of mazindol plus morphine in mice (unpublished data).

Another finding of interest is that lidocaine caused an antinociceptive response in the formalin test, a response which was unaffected by dopaminergic and opioid antagonists. It is interesting to note that Graham et al. (1995) recently reported that local anesthetics belonging to the amide class, such as lidocaine, do not affect the dopaminergic system. However, the possibility that the antinociceptive action of lidocaine involves other mechanisms should also be considered. Thus, it has been shown that lidocaine below the dose range used in the present study suppresses C-fiber-evoked polysynaptic reflexes in rats (Woolf and Wiesenfeld-Hallin, 1985). In addition, there have been reports of the central inhibitory effects of lidocaine mediated by spinal strychnine-sensitive glycine receptors well before blockade of conduction is achieved (Biella et al., 1993; Biella and Sotgiu, 1993). Also, a recent report has show that local anesthetics can inhibit tachykinin receptors, which are important for the neurotransmission of nociceptive signals (Li et al., 1995).

In summary, the present results indicate that mazindol, an anorectic drug, and lidocaine, an amide-type local anesthetic, cause a significant antinociceptive effect in the mouse formalin test. This antinociceptive effect of mazindol appears to be mediated by D_2 dopaminergic and opioidergic mechanisms, while lidocaine's action was unaffected by these systems. In addition, these results show that drugs available for distinct therapeutic purposes, like mazindol and lidocaine, may possess potential use as antinociceptive or adjunct agents for pain management.

Acknowledgements

The authors thank Carlos R. Tonussi for his helpful comments on the manuscript. This work was supported, in part, by CNPq-Brasil.

References

Abbott, F.V., Young, S.N., 1988. Effect of 5-hydroxytryptamine precursors on morphine analgesia in the formalin test. Pharmacol. Biochem. Behav. 31, 855–860.

Abbott, F.V., Melzack, R., Samuel, C., 1982. Morphine analgesia in the tail-flick and formalin pain tests is mediated by different neural systems. Exp. Neurol. 75, 644–651.

Biella, G., Sotgiu, M.L., 1993. Central effects of systemic lidocaine mediated by glycine spinal receptors: An iontophoretic study in the rat spinal cord. Brain Res. 603, 201–206.

Biella, G., Lacerenza, M., Marchettini, P., Sotgiu, M.L., 1993. Diverse modulation by systemic lidocaine of iontophoretic NMDA and quisqualic acid induced excitations on rat dorsal horn neurons. Neurosci. Lett. 157, 207–210.

Dennis, S.G., Melzack, R., 1980. Pain modulation by 5-hydroxytryptaminergic agents and morphine as measured by three pain tests. Exp. Neurol. 69, 260–270.

Gerrits, M.A.F.M., Zwartouw, N.P.E.E., Van Ree, J.M., 1995. Opioid blockade attenuates acquisition and expression of cocaine-induced place preference conditioning in rats. Psychopharmacology 119, 92–96.

Graham, J.H., Maher, J.R., Robinson, S.E., 1995. The effect of cocaine

and other local anesthetics on central dopaminergic neurotransmission. J. Pharmacol. Exp. Ther. 27, 707–717.

Javitch, J.A., Blaustein, R.O., Snyder, S.H., 1983. [^{3}H] Mazindol binding associated with neuronal dopamine uptake sites in corpus striatum membranes. Eur. J. Pharmacol. 90, 461–462.

Kruk, Z.L., Zarrindast, M.R., 1976. Mazindol anorexia is mediated by activation of dopaminergic mechanisms. Br. J. Pharmacol. 58, 367–372.

Li, Y.M., Wingrove, D.E., Too, H.P., Marnerakis, M., Stimson, E.R., Strichard, G.R., Maggio, J.E., 1995. Local anesthetics inhibit substance P binding and evoked increases in intracellular Ca^{2+}. Anesthesiology 82, 166–173.

Lin, Y., Morrow, T.J., Kiritsy-Roy, J.A., Terry, L.C., Casey, K.L., 1989. Cocaine: evidence for supraspinal, dopamine mediated, non opiate analgesia. Brain Res. 479, 306–312.

Mattei, R., Carlini, E.A., 1995. Mazindol: Anorectic and behavioral effects in female rats. Arch. Int. Pharmacodyn. Ther. 330, 279–281.

Michaël-Titus, A., Bousselmames, R., Costentin, J., 1990. Stimulation of dopamine D_2 receptors induces an analgesia involving an opioidergic but non enkephalinergic link. Eur. J. Pharmacol. 187, 201–207.

Morgan, M.J., Franklin, K.B.J., 1990. 6-Hydroxydopamine lesions of the ventral tegmentum abolish d-amphetamine and morphine analgesia in the formalin test but not in the tail-flick test. Brain Res. 519, 144–149.

Paalzow, G.H.M., Paalzow, L.K., 1983. Opposing effects of apomorphine on pain in rats. Evaluation of the dose response curve. Eur. J. Pharmacol. 88, 27–35.

Pertovaara, A., Kauppila, T., Tukeva, T., 1990. The effect of medetomidine, an α_2-adrenoceptor agonist, in various pain tests. Eur. J. Pharmacol. 179, 323–328.

Pögün, S., Scheffel, U., Kuhar, M.J., 1991. Cocaine displaces [^{3}H]WIN35,428 binding to dopamine uptake sites in vivo more rapidly than mazindol or GRB 12909. Eur. J. Pharmacol. 198, 203–205.

Rigon, A.R., Takahashi, R.N., 1996. The effects of systemic procaine, lidocaine and dimethocaine on nociception in mice. Gen. Pharmacol. 27, 647–650.

Suaudeau, C., Costentin, J., 1995. Analgesic effect of the direct D_2 dopamine receptor agonist RU 24926 and cross tolerance with morphine. Fundam. Clin. Pharmacol. 9, 147–152.

Tulunay, F.C., Sparber, S.B., Takemori, A.E., 1975. The effect of dopaminergic stimulation and blockade on the nociceptive and antinociceptive responses of mice. Eur. J. Pharmacol. 33, 65–70.

Woolf, C.J., Wiesenfeld-Hallin, Z., 1985. The systemic administration of local anaesthetics produces a selective depression of C-afferent fibre evoked activity in the spinal cord. Pain 23, 361–374.

Zambotti, F., Carruba, M.O., Barzaghi, F., Vicentini, L., Groppetti, A., Mantegazza, P., 1976. Behavioral effects of a new nonphenylethylamine anorexigenic agent: Mazindol. Eur. J. Pharmacol. 36, 405–412.

Zanin, M., Takahashi, R.N., 1994. Sex difference in sensitization to the locomotor effects of mazindol in rats. Brain Res. Bull. 34, 385–387.