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Short communication

The partial agonist properties of levocabastine in neurotensin-induced analgesia

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Abstract

The antihistaminic drug levocabastine is a ligand for the low affinity neurotensin receptor (NTS2). Its intracerebroventricular administration to mice induced a significant analgesia in the writhing test but not in the hot plate test. In the writhing test, levocabastine decreased neurotensin-induced analgesia to a level not significantly different from the effects of levocabastine alone. In the hot plate test, levocabastine had no analgesic effect but completely reversed the neurotensin-induced analgesia. Mepyramine, another antihistaminic drug, did not share these levocabastine effects. Neither levocabastine nor mepyramine modified the colonic temperature or reversed the neurotensin-induced hypothermia. Thus, levocabastine behaves as a partial agonist at neurotensin NTS2 receptors, which are involved in visceral nociception, but not at yet unidentified neurotensin receptors involved in hypothermia.

Keywords: Levocabastine; Neurotensin; Analgesia; Neurotensin low affinity receptor; Hypothermia

1. Introduction

The neuropeptide neurotensin is widely distributed in the central nervous system and peripheral tissues. When injected in the central nervous system, it induces several effects, including a hypothermia and a naloxone-insensitive analgesia (Bissette et al., 1976; Clineschmidt et al., 1979). Two types of neurotensin receptors, previously distinguished by their affinity for neurotensin and their sensitivity to the antihistaminic drug levocabastine (Schotte et al., 1986), have been recently cloned. The high-affinity, levocabastine-insensitive, neurotensin receptor (NTS1) has been the first to be cloned (Tanaka et al., 1990; Vita et al., 1993). Then in 1996, the low-affinity levocabastine-sensitive neurotensin receptor (NTS2) was cloned (Chalon et al., 1996; Mazella et al., 1996). Recently, a third neurotensin receptor has been cloned (Mazella et al., 1998) and identified as gp95/sortilin, a non G-protein-coupled receptor. There are evidences which argue against the involvement of neurotensin NTS1 receptor in the analgesic and hypothermic effects of neurotensin: (i) These effects are not antagonized by SR48692, a neurotensin receptor antagonist which displays a rather good specificity for neurotensin NTS1 receptor (Dubuc et al., 1994); (ii) the binding affinity of various pseudopeptide neurotensin analogues for neurotensin NTS1 receptor is not correlated with their analgesic and hypothermic potencies (Labbé-Jullié et al., 1994); (iii) the repeated intracerebroventricular (i.c.v.) administration of antisense oligodeoxynucleotides targeted to the mRNA encoding the neurotensin NTS1 receptor does not reduce neurotensin analgesic effect in the writhing test in mice (Dubuc et al., 1999). This latter result is in contrast with that of Tyler et al. (1998a) who recently demonstrated that the inhibition of neurotensin NTS1 receptor synthesis after gene blockade by antisense peptide nucleic acids injected into the periaqueductal grey resulted in a loss of both hypothermic and analgesic responses to neurotensin. However, these authors underlined that the synthesis of another neurotensin receptor, other than neurotensin NTS1 receptor, could be decreased, because the antisense peptide nucleic acids was only a 12-mer, which could be conserved in another neurotensin receptor type. Moreover, the same authors (Tyler et al., 1998b) have shown that levocabastine, injected into the periaqueductal grey of rats, induced neither hypothermia nor antinocicep-

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tion on the hot plate test but antagonized these neurotensin effects. In contrast, neurotensin NTS2 receptor appears a good candidate for mediating the analgesic effect of neurotensin: the blockade of its central expression by repeated i.c.v. injections of antisense oligodeoxynucleotides resulted in a marked reduction in the neurotensin-induced analgesia (Dubuc et al., 1999).

These results prompted us to investigate whether levocabastine displays hypothermic and especially analgesic effects in mice or modifies the neurotensin-induced analgesia. In order to distinguish the effects of levocabastine from those of a pure histamine H1 receptor antagonist, we also compared the effects of levocabastine with those elicited by mepyramine.

2. Materials and methods

Male Swiss albino mice (CD1, Charles River, St Aubin lès Elbeuf, France), weighing 20–22 g, were used throughout the study. The animals were tested only once and sacrificed immediately thereafter by decapitation.

Intracerebroventricular injections (10 μl) were performed, according to Haley and Mc Cormick (1957).

Colonic temperature was measured with a thermistor probe (Thermalert TH5, Physitemp, Cliffton, USA), inserted 2 cm into the rectum, immediately before and at various intervals after the i.c.v. injection.

Analgesic activity was evaluated using the writhing test and the hot plate test.

The writhing test was performed according to Koster et al. (1959). Mice were i.c.v. injected with solvent or the drugs to be tested, 20 min before the intraperitoneal (i.p.) administration of an acetic acid solution (0.5%). The number of body stretches was counted over a 5-min period, beginning 5 min after the i.p. injection.

The hot plate test was derived from that of Eddy and Leimbach (1953). The plate was maintained at 55 ± 0.5°C. The jump latency was measured 20 min after the i.c.v. injection of the solvent or the drugs to be tested. A cut-off time of 240 s was used to avoid injury.

Neurotensin (Neosystem, Strasbourg, France) and mepyramine (Sigma, St. Louis, MO, USA) were dissolved in saline; levocabastine (a generous gift of Janssen Pharmaceutica, Beerse, Belgium) was dissolved in dimethylsulfoxide (DMSO) and diluted with saline and DMSO, for a final concentration of 10% of this latter. In order to avoid the administration of solutions containing more than 10% of DMSO and due to its low solubility, the maximal injected dose of levocabastine was 1350 pmol. Vehicle control was 10% DMSO.

Results were expressed as means ± S.E.M. from 8–16 animals. Statistics were conducted using a two-way analysis of variance (ANOVA) followed, when appropriate, by Tukey's test.

3. Results

In the writhing test, levocabastine had no significant effect at the 16.5 pmol dose (not shown); however, it significantly reduced the number of writhes in the range of 50–1350 pmol doses. This analgesic effect was dose-dependent and reached a plateau, corresponding approximately to a 40% reduction in the number of writhes. When levocabastine, at 150, 450 and 1350 pmol doses was opposed to the 24 pmol dose of neurotensin which reduced intrinsically by about 85% the number of writhes, it reduced this neurotensin-induced analgesia, in a dose-dependent manner, up to its own analgesic effect (reduction of 40% of writhes as previously indicated) (Fig. 1, upper panel). In similar conditions, mepyramine, for the same equimolar doses, was devoid of intrinsic analgesic effect and did not reverse the neurotensin-induced reduction of writhes (Fig. 1, lower panel).

In the hot plate test, levocabastine, i.c.v. administered at 450 pmol and 1350 pmol doses, did not modify the jump latency but completely reversed the analgesic effect elicited by neurotensin 115 pmol (Fig. 2, upper panel). In similar conditions, mepyramine, used at doses equimolar to those of levocabastine, had no intrinsic analgesic effect and did not reverse the neurotensin-induced increase in jump latency (Fig. 2, lower panel).

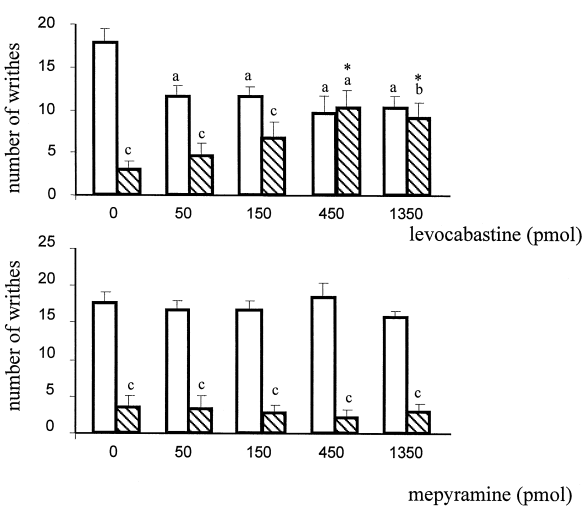

Fig. 1. Intrinsic effects of levocabastine and mepyramine and their interaction with neurotensin in the writhing test. Vehicle, levocabastine or mepyramine alone (white columns) or associated with neurotensin (24 pmol) (hatched columns) were injected i.c.v. 20 min before the i.p. injection of acetic acid solution (0.5 %). Means ± S.E.M. of 12–16 mice per group. Two-way ANOVA followed by Tukey's test indicates an interaction between neurotensin and levocabastine ($F(4.13) = 12.81$; $P < 0.001$), a significant effect of neurotensin ($P < 0.001$), a significant effect of levocabastine ($P < 0.05$ at 50 and 150 pmol, $P < 0.02$ at 450 and 1350 pmol); a lack of interaction between neurotensin and mepyramine and a lack of intrinsic effect of mepyramine. (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$ as compared to vehicle injected animals. $^*P < 0.02$.

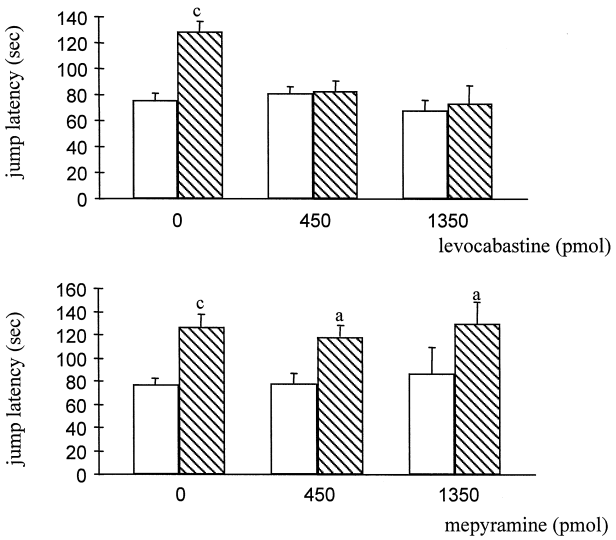

Fig. 2. Intrinsic effects of levocabastine and mepyramine and their interaction with neurotensin in the hot plate test. Vehicle, levocabastine or mepyramine alone (white columns) or associated with neurotensin (115 pmol) (hatched columns) were injected i.c.v. 20 min before the hot plate test. Means ± S.E.M. of 12–16 mice per group. Two-way ANOVA followed by Tukey's test indicates an interaction between neurotensin and levocabastine ($F(2.95) = 7.28$; $P < 0.001$), a significant effect of neurotensin ($P < 0.001$); a lack of interaction between neurotensin and mepyramine and a lack of intrinsic effect of mepyramine. (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$ as compared to vehicle injected animals.

Neither levocabastine nor mepyramine displayed (from 50 up to 1350 pmol i.c.v. administered) hypothermic effect. The same equimolar doses of these two substances did not modify the hypothermia induced by neurotensin (24 pmol) simultaneously i.c.v. administered (not shown).

4. Discussion

The administration of levocabastine and mepyramine by i.c.v. route has been chosen for the following reasons. (i) Their central availability after systemic administration is uncertain; (ii) interactions of peripherally administered H1 antihistaminic drugs with the writhing test have been reported (Abacioglu et al., 1993); (iii) the comparisons between drugs effects on an equimolar basis are more easily performed.

We evidenced that levocabastine displays an antinociceptive effect in the writhing test. However, this effect was of a limited intensity since from 150 pmol it reduced the number of writhes by only 40% when neurotensin was able to abolish them (Coquerel et al., 1988). This weak analgesic effect of levocabastine was not additive to that of neurotensin which, at the 24 pmol dose, reduced by about 85% the number of writhes. On the contrary, levocabastine decreased the strong analgesic effect of neurotensin to a level not significantly different from the effects of levocabastine alone. This looks like a competitive dualism, characteristic of the interaction between a full agonist (neurotensin) and a partial agonist (levocabastine).

By considering the licking latency in the hot plate test, Tyler et al. (1998b) did not report an analgesic effect of levocabastine injected, at high doses, into the periaqueductal grey. When the jump latency was considered in the same test, we neither found analgesic effect of levocabastine. One may observe that the effective doses of neurotensin are obviously lower (about 10-fold) in the writhing test than in the hot plate test. Nevertheless, in the hot plate test, levocabastine reversed the neurotensin-induced analgesia. These effects of levocabastine did not depend on its antagonist activity at histamine H1 receptors since they were not shared, in the present study as well as in that of Tyler et al. (1998b), by the specific histamine H1 receptor antagonist mepyramine, which is devoid of any affinity for neurotensin receptors (Schotte and Leysen, 1989). Our present data are in accordance with those obtained in Xenopus oocytes injected with the neurotensin NTS2 receptor mRNA, where levocabastine displayed the same intrinsic activity as neurotensin, since it triggered a Cl^- inward current (Mazella et al., 1996).

The analgesia induced by neurotensin in the writhing test or in the hot plate test was reversed by levocabastine whereas the hypothermia induced by neurotensin was not. These data indicate that neurotensin receptors involved in hypothermia differ from those involved in analgesia, which is a confirmation, under different experimental conditions, of the data reported by Tyler et al. (1998b).

In conclusion, the present study shows that levocabastine displays an analgesic effect in the writhing test but not in the hot plate test. This effect seems to depend on a low intrinsic activity at neurotensin low-affinity receptors NTS2, unless another pharmacological property, not yet identified, accounts for this analgesic effect. Moreover, it may be concluded that receptors which mediate neurotensin-induced hypothermia differ from those involved in its analgesic effect.

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