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Short communication

The neuropeptide FF analogue, 1DMe, acts as a functional opioid autoreceptor antagonist in the rat spinal cord

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Abstract

We assessed the possible influence of a neuropeptide FF analogue, 1DMe ([D-Tyr^1,(NMe)Phe^3]neuropeptide FF), on the inhibitory action of endogenous and exogenous ∂-opioid receptor agonists on K^+-evoked [Met^5]-enkephalin release from superfused rat spinal cord slices. 1DMe (0.1–10 μM) dose-dependently enhanced the increase in superfusate [Met^5]-enkephalin content due to the peptidase inhibitors thiorphan (1 μM) and bestatin (20 μM), and prevented the reduction in [Met^5]-enkephalin release due to stimulation of ∂ receptors by 1 μM deltorphin I. Because it had the same effects as ∂-opioid receptor antagonists, 1DMe might act through the functional blockade of presynaptically located ∂-opioid autoreceptors.

Keywords: [Met^5] enkephalin; In vitro release; Neuropeptide FF; Spinal cord; Opioid receptor ligand; Peptidase inhibitor

1. Introduction

In rodents, neuropeptide FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2) can exert either anti-opioid or opioid-like effects when it is injected intracerebroventricularly or intrathecally (i.t.), respectively (Roumy and Zajac, 1998). Thus, through the latter route of injection, neuropeptide FF as well as 1DMe ([D-Tyr^1,(NMe)Phe^3]neuropeptide FF), a high affinity analogue partially protected from enzymatic breakdown (Gicquel et al., 1992), mimic the antinociceptive action of opioids in rats. Although neuropeptide FF and its analogues do not bind to opioid receptors, their antinociceptive action can be markedly reduced by opioid receptor antagonists (Roumy and Zajac, 1998). Indeed, 1DMe-induced antinociception very likely occurs through an induced release of [Met^5]-enkephalin within the spinal cord (Ballet et al., 1999).

Mechanisms underlying the stimulatory effect of 1DMe on [Met^5]-enkephalin release remain to be determined. Two-thirds of the terminals showing [Met^5]-enkephalin-like immunoreactivity in the superficial layers of the spinal cord are endowed with ∂-opioid receptor-like immunoreactivity (Cheng et al., 1995). [Met^5]-enkephalin very likely reduces its own release by acting at these receptors, whose blockade, as expected, leads to an enhanced release of [Met^5]-enkephalin (Cesselin et al., 1999). Neuropeptide FF-induced inhibition of synaptic transmission could be attributable to a reduced probability of presynaptic release rather than to a depression of postsynaptic sensitivity (Chen et al., 2000). The aim of the present study was to directly assess the hypothesis that stimulation of spinal neuropeptide FF receptors could modulate [Met^5]-enkephalin release under the control of presynaptically located opioid receptors.

2. Materials and methods

Male Sprague–Dawley rats (Centre d'élevage R. Janvier, Le Genest-Saint Isle, France) of 280–340 g body weight were kept under controlled environmental conditions (22 °C, 12 h alternate light–dark cycles, 60% humidity, food and water ad libitum) for at least 1 week before being used for the experiments.

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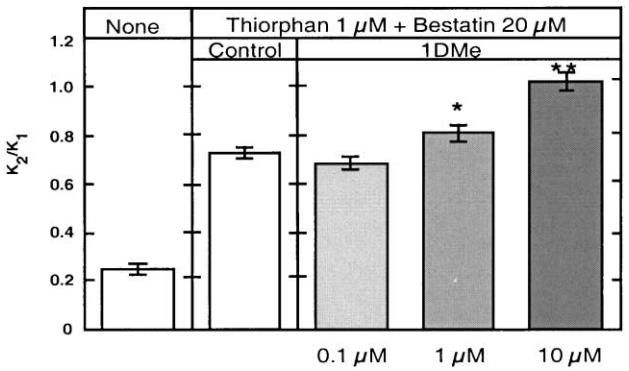

Fig. 1. Effects of 1DMe on [Met^5]-enkephalin overflow from the dorsal zone of the lumbar enlargement due to superfusion with 1 μM thiorphan plus 20 μM bestatin. Slices of the dorsal zone of the lumbar enlargement were depolarised twice (K1, K2, fractions 3–4 and 10–11, respectively) by 30 mM K^+ in the course of continuous superfusion with ACSF at a flow rate of 0.25 ml/min. Thirteen fractions (1 ml) were collected for each experiment and drugs were added to the ACSF from the beginning of fraction 8 up to the end of the experiment. The drugs-induced changes in the K^+-evoked [Met^5]-enkephalin release were assessed from the K2/K1 ratio (*y* axis) as described in Materials and methods. Each bar is the mean ± S.E.M. of values calculated from at least eight independent experiments. All values in the presence of thiorphan + bestatin were significantly higher ($P < 0.001$) than the value of K2/K1 in superfusion experiments where the second K^+ pulse was applied without drugs (None). $^*P < 0.05$, $^{**}P < 0.001$ compared to K2/K1 value in the presence of 1 μM thiorphan plus 20 μM bestatin (Control).

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive #87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions #6228 to S.B. and #0313 to F.C.).

Superfusion of slices of the dorsal zone of the lumbar enlargement with an artificial cerebrospinal fluid (ACSF) was performed as described in detail elsewhere (Cesselin et al., 1984).Tissues were depolarised twice by 30 mM K^+. The effects of compounds to be tested on the release of [Met^5]-enkephalin-like material were investigated under control and depolarising conditions. Since the ratio of K^+-induced [Met^5]-enkephalin-like material overflow during the second depolarisation (K2) over that during the first (K1) one was constant in the absence of drugs, any change in this ratio in the presence of a given substance for the second depolarisation could be ascribed to the effect of this particular substance on the Ca^{2+}-dependent release of [Met^5]-enkephalin-like material (see Cesselin et al., 1984).

[Met^5]-enkephalin-like material content of each superfusate fraction was determined using a specific radioimmunoassay (Cesselin et al., 1984). Statistical analyses were carried out using analysis of variance followed by Fischer's protected least significant difference test. When the *P* value was higher than 0.05, a difference was considered to be non-significant.

[^{125}I] [Met^5]-enkephalin, specific activity: 80 TBq/mmol, was from New England Nuclear (Boston, MA, USA). Other compounds were: deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2), bestatin, naltrindole (Sigma) and thiorphan (Bachem). 1DMe ([D-Tyr^1,(NMe)Phe^3]neuropeptide FF) was synthesised by solid phase methods as described (Gicquel et al., 1992). All other compounds were of the best purity available (Merck, Prolabo).

3. Results

The outflow of [Met^5]-enkephalin-like material remained essentially stable under control conditions ([K^+] = 5.4 mM) during the whole collection period. K^+-induced depolarisation enhanced [Met^5]-enkephalin-like material release, and the K2/K1 ratio, remarkably constant from one perfusion chamber to another, was close to 0.25 (see Figs. 1 and 2).

Under control conditions, except thiorphan plus bestatin, none of the drugs presently tested affected the spontaneous outflow of [Met^5]-enkephalin-like material from spinal cord slices (not shown). In the presence of thiorphan (1 μM) plus bestatin (20 μM), the [Met^5]-enkephalin-like material content of superfusate fractions was significantly higher ($+135 \pm 18\%$, $P < 0.001$, $n = 19$) than under basal conditions. In addition, when 1 μM thiorphan and 20 μM bestatin were added to the ACSF, the K^+-evoked overflow of [Met^5]-enkephalin-like material was also markedly increased, as indicated by the K2/K1 ratio which reached ~ 290% of the control value (Fig. 1). The increase in the K2/K1 ratio due to the peptidase inhibitors was dose-de-

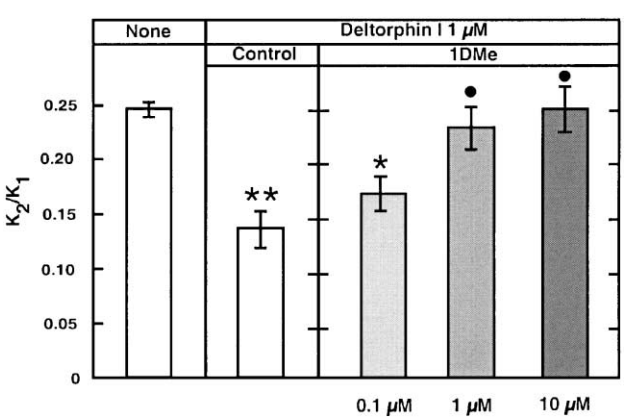

Fig. 2. Reversal by 1DMe of the inhibitory effect of 1 μM deltorphin I on the K^+-evoked release of [Met^5]-enkephalin from the dorsal zone of the lumbar enlargement. The experimental protocol and the expression of results were the same as those described in the legend to Fig. 1. Each bar is the mean ± S.E.M. of values calculated from at least eight independent experiments. $^*P < 0.01$, $^{**}P < 0.001$ compared to K2/K1 value in superfusion experiments where the second K^+ pulse was applied without drugs (None). ● $P < 0.001$ compared to K2/K1 value in the presence of 1 μM deltorphin I alone (Control).

pendently enhanced by adding 1DMe (0.1–10 μM) into the superfusing fluid (Fig. 1). However, 1DMe (0.01–10 μM) alone did not affect the K^+-evoked release of [Met^5]-enkephalin-like material from spinal cord slices (not shown). In contrast to thiorphan plus bestatin, deltorphin I (0.01–1.0 μM) dose-dependently reduced the K^+-evoked release of the peptide (not shown). Fig. 2 shows that 1DMe dose-dependently prevented the inhibitory effect of 1 μM deltorphin I on the K^+-evoked release of [Met^5]-enkephalin-like material. In this respect, 1DMe mimicked the effect of the ∂-opioid receptor antagonist naltrindole, and indeed the K2/K1 value determined in the presence of deltorphin I (1 μM) plus 1DMe (10 μM) and naltrindole (1 μM) did not differ from that found in the presence of the former two compounds only (not shown).

4. Discussion

Although previous investigations indicated that 1DMe, when applied i.t., dose-dependently increased the release of [Met^5]-enkephalin-like material from the spinal cord of anaesthetised rats (Ballet et al., 1999), this compound was presently found to exert no effect on the spontaneous outflow of the peptide from spinal cord slices. In fact, this result is not surprising since the spontaneous [Met^5]-enkephalin-like material outflow in vitro was shown to be only partly dependent on extracellular Ca^{2+} (Cesselin et al., 1984). The K^+-evoked overflow of [Met^5]-enkephalin-like material from spinal cord slices, which can be completely prevented by the removal of Ca^{2+} from the superfusing ACSF (Cesselin et al., 1984), remained also unchanged in the presence of 1DMe at a concentration as high as 10 μM. As this concentration was shown to be sufficient to trigger a significant enhancement (+43% over the basal value) of spinal [Met^5]-enkephalin-like material outflow when the drug was applied i.t. in anaesthetised animals (Ballet et al., 1999), it can be hypothesised that the stimulatory effect of 1DMe on [Met^5]-enkephalin-like material release from the rat spinal cord in vivo involves mechanism(s) that were lost in the in vitro preparation under basal conditions. Obviously, in spinal cord slices, [Met^5]-enkephalin-containing neurones are no longer under the control of the neuronal network that normally modulates their activity in vivo. Nevertheless, the in vitro preparation offers the advantage of allowing the study of mechanisms responsible for the local control of the release of neurotransmitters, e.g. [Met^5]-enkephalin.

This in vitro approach was presently used for assessing further the feedback inhibition of [Met^5]-enkephalin release that was previously observed in vivo (Cesselin et al., 1999). Our data confirmed that the concomitant blockade of aminopeptidases by bestatin and of "enkephalinase" (E.C. 3.4.24.11) by thiorphan (see Roques et al., 1993) protects extracellular [Met^5]-enkephalin from enzymatic degradation (Cesselin et al., 1999). As expected from the occurrence of a phasic inhibitory control exerted by endogenous opioids acting at presynaptic ∂-opioid receptors when the extracellular levels of [Met^5]-enkephalin-like material are increased, the blockade of ∂-, but not μ-, opioid receptors was reported to markedly increase the spinal overflow of [Met^5]-enkephalin-like material due to peptidases inhibition (Cesselin et al., 1999). These endogenous opioids could very likely be [Met^5]-enkephalin itself because this peptide is a preferential ligand at ∂-opioid receptors. Interestingly, the increase in K^+-evoked [Met^5]-enkephalin-like material overflow caused by the blockade of peptidases could also be further enhanced by addition to the superfusing fluid of the neuropeptide FF receptor agonist 1DMe. Accordingly, stimulation of spinal neuropeptide FF receptors had the same effect as the blockade of ∂-opioid receptors.

In agreement with previous data (Cesselin et al., 1999), and very likely for the same reasons as those evoked above about 1DMe, deltorphin I did not affect the spontaneous outflow of [Met^5]-enkephalin-like material. However, this selective ∂-opioid receptor agonist significantly diminished the K^+-evoked overflow of the peptide. Acting again like a ∂-opioid receptor antagonist, 1DMe was found to prevent the inhibitory effect of 1 μ deltorphin I on the K^+-evoked release of [Met^5]-enkephalin-like material. Furthermore, as expected from such an action, the prevention of deltorphin-induced decrease in K^+-evoked [Met^5]-enkephalin-like material overflow by 1DMe yielded an increase in the K2/K1 value which was similar to and not additive with that caused by naltrindole, a selective ∂-opioid receptor antagonist.

In conclusion, the present findings indicate that one of the possible mechanisms responsible for the enhancement of spinal [Met^5]-enkephalin-like material release in response to spinal neuropeptide FF receptor stimulation by 1DMe (Ballet et al., 1999) could be related to its ∂-opioid autoreceptor antagonist-like properties. Whether neuropeptide FF receptors and ∂-opioid receptors are, or are not, located on the same [Met^5]-enkephalin-containing neurones deserves further investigations. However, the fact that neuropeptide FF receptors within the dorsal horn are mostly expressed by intrinsic neurones (Gouardères et al., 2000) is compatible with such a possibility. The potent antinociceptive action of i.t. administered 1DMe, which can be blocked by opioid receptor antagonists (Roumy and Zajac, 1998), suggests that neuropeptide FF receptor agonists as well as ∂-opioid receptor antagonists acting selectively at the spinal presynaptic autoreceptors responsible for the negative control of [Met^5]-enkephalin-containing neurones would offer a novel opportunity for alleviating pain by activating spinal enkephalinergic neurotransmission. Indeed, such treatments would enhance [Met^5]-enkephalin extracellular levels allowing the peptide to reduce the spinal transmission of nociceptive messages at both pre- and postsynaptic sites (Cesselin et al., 1999).

Acknowledgements

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