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Inhibitory effect of anandamide on resiniferatoxin-induced sensory neuropeptide release in vivo and neuropathic hyperalgesia in the rat

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Abstract

Anandamide (AEA) is an endogenous cannabinoid ligand acting predominantly on the cannabinoid 1 (CB_1) receptor, but it is also an agonist on the capsaicin VR_1/$TRPV_1$ receptor. In the present study we examined the effects of AEA and the naturally occuring cannabinoid 2 (CB_2) receptor agonist palmitylethanolamide (PEA) on basal and resiniferatoxin (RTX)-induced release of calcitonin gene-related peptide (CGRP) and somatostatin in vivo. Since these sensory neuropeptides play important role in the development of neuropathic hyperalgesia, the effect of AEA and PEA was also examined on mechanonociceptive threshold changes after partial ligation of the sciatic nerve. Neither AEA nor PEA affected basal plasma peptide concentrations, but both of them inhibited RTX-induced release. The inhibitory effect of AEA was prevented by the CB_1 receptor antagonist SR141716A. AEA abolished and PEA significantly decreased neuropathic mechanical hyperalgesia 7 days after unilateral sciatic nerve ligation, which was antagonized by SR141716A and the CB_2 receptor antagonist SR144528, respectively. Both SR141716A and SR144528 increased hyperalgesia, indicating that endogenous cannabinoids acting on CB_1 and peripheral CB_2–like receptors play substantial role in neuropathic conditions to diminish hyperalgesia. AEA and PEA exert inhibitory effect on mechanonociceptive

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hyperalgesia and sensory neuropeptide release in vivo suggesting their potential therapeutical use to treat chronic neuropathic pain.

Keywords: Anandamide; Palmitylethanolamide; Resiniferatoxin; SR141716A; SR144528; Somatostatin; Calcitonin gene-related peptide; Neuropathic hyperalgesia; Capsaicin-sensitive nerve terminals; Radioimmunoassay

Introduction

Most of the central and peripheral pharmacological actions of the endogenous cannabinoid ligand anandamide (AEA) are mediated via cannabinoid 1 receptor (CB_1) activation: it inhibits adenylate-cyclase and voltage-sensitive Ca^{2+} channels (Vaughan et al., 2000), therefore it has potential presynaptical counteraction with depolarization-induced release of neurotransmitters. Richardson et al. (1998a) were the first who demonstrated that AEA inhibits CGRP release from isolated skin and dorsal horn. Experimental data obtained by Calignano et al. (1998) pointed out that AEA attenuates pain behaviour produced by chemical damage to cutaneous tissue by interaction with CB_1 receptors located in the periphery. They found similar effect with palmitylethanolamide (PEA), which is released together with AEA from a common phospholipid precursor, by acting on peripheral cannabinoid 2 (CB_2)-like receptors (Calignano et al., 1998).

On the other hand, since AEA is structurally related to vanilloid compounds (capsaicin and olvanil), it has been described to be an agonist on the cloned vanilloid subtype 1 capsaicin receptor (VR_1/$TRPV_1$; Caterina et al., 1997; Pertwee, 1997) similarly to resiniferatoxin (RTX), a potent irritant of plant origin (Szállási and Blumberg, 1999). Therefore, AEA has some vanilloid-like effects as well, like induction of vasodilatation by activating VR_1 on peripheral sensory nerves and causing release of CGRP (Zygmunt et al., 1999). In patch-clamp experiments on VR_1-transfected cells it causes capsazepine-sensitive currents (Smart et al., 2000). Accumulating evidence suggest that AEA exerts potent anti-nociceptive action in different acute pain models like formalin (Jaggar et al., 1998), acetic acid, kaolin, $MgSO_4$, capsaicin and hot plate (Calignano et al., 2001) and tail flick tests (Richardson et al., 1998b). It has also been demonstrated to be an effective anti-hyperalgesic compound under inflammatory pain conditions, it inhibits carrageenin-induced thermal hyperalgesia (Richardson et al., 1998b), turpentine-evoked viscero-visceral hyper-reflexia of the urinary bladder (Jaggar et al., 1998) and mechano-nociceptive hyperalgesia in Freund's complete adjuvant-induced arthritis (Smith et al., 1998). The other endogenous fatty acid ethanolamide, PEA, a selective agonist on peripheral CB_2-like receptors, has been demonstrated to inhibit carrageenin-induced mechanical hyperalgesia (Mazzari et al., 1996) and formalin—(Jaggar et al., 1998), acetic acid-, kaolin- and $MgSO_4$-induced acute pain, but it has no effect on capsaicin-evoked and thermal nociception (Calignano et al., 2001).

Based on these data, the aim of the present study was to analyse the effect of AEA and PEA on basal and RTX-induced plasma concentrations of CGRP and somatostatin to obtain data in vivo for the role of inhibitory cannabinoid and excitatory capsaicin receptors in the action of AEA on the release of these peptides. Since these sensory neuropeptides play significant role in the development of neuropathic hyperalgesia (Hökfelt et al., 1994; Rittenhouse et al., 1996; Stanton-Hicks and Salamon, 1997), the effect of AEA and PEA was also investigated on mechanonociceptive threshold changes after partial ligation of the sciatic nerve.

Methods

Measurement of plasma neuropeptide concentrations

Male Wistar rats (240–280 g) were anaesthetized with 100 mg/kg, i.p. sodium thiopentone (Trapanal) after 12 h starvation. Following anaesthesia the tail vein and the right carotid artery were cannulated for drug administration and blood sampling, respectively. A tracheal T-cannula was inserted for artificial respiration when required. Dose-response relations between systemic RTX administration (0.1, 0.3, 0.6, 1 and 3 μg /kg, i.v.) and plasma somatostatin and CGRP concentrations were determined. The applied RTX dose in the subsequent experiments was approximately the ED_{50} value (0.6 μg/kg). The effects of AEA or PEA (100 μg/kg i.v., in volume of 0.1 ml per 100 g of body weight) on basal somatostatin and CGRP levels and their influence on RTX-evoked neuropeptide release were investigated.

Pretreatment with AEA or PEA (10 and 100 μg/kg, i.v.) was performed 5 min prior to RTX injection. The action of the selective (CB_1) antagonist SR141716A and the peripheral CB_2 receptor antagonist SR144528 (100 μg/kg i.v., 10 min before the administration of the respective cannabinoid ligand) on the effect induced by AEA or PEA on RTX-evoked neuropeptide release was also examined (n = 6 per group). Arterial blood samples (4 ml per animal) were taken 5 min after drug administration into ice-cold tubes containing EDTA (8 mg) and Trasylol (1250 U). Following centrifugation (2000 r.p.m. for 10 min at 4 °C) the peptides from the plasma were extracted by addition of 3 volumes of absolute alcohol. After precipitation and a second centrifugation (3000 r.p.m. for 10 min at 4 °C) the samples were dried under nitrogen flow. Plasma somatostatin (Németh et al., 1996; Szolcsányi et al., 1998) and CGRP (Németh et al., 1998) concentrations were measured by means of specific and sensitive radioimmunoassay (RIA) methods.

Neuropathic hyperalgesia following partial sciatic nerve injury in the rat

Male Sprague-Dawley rats (180–250 g) were anaesthetised with 50 mg/kg i.p. pentobarbitone sodium (Nembutal). Unilateral common sciatic nerve was exposed high in the thigh and 1/3–1/2 of the nerve trunk was carefully separated and tightly ligated using a siliconised silk suture (Ethicone 8-0). Then the wound was closed and the animals were allowed to survive for 8 days (Seltzer et al., 1990). During this period, signs of spontaneous pain (holding the legs in elevated position) and mechano-nociceptive hyperalgesia developed. Mechano-nociception of the hindpaws was measured by Randall-Selitto test using Ugo Basile analgesimeter. Continously increasing pressure was applied on the paw of conscious rats and the threshold force which elicited withdrawal was determined. The obtained results indicate force measured on a scale calibrated in grams. Control values were measured before operation during a period of 3–4 days. Four measurements were made on each rat and the average of the last two assessments were taken as controls. Significant decrease in mechanical threshold developed 7 days after the surgery. Measurements on the 7th day were taken 1.5–2 h before and 30 min after administration of AEA or PEA (100 μg/kg, i.p.). SR141716A and/or SR144528 (3 mg/kg, i.p.) were injected 30 min prior to AEA or PEA. Solvent-treated rats served as controls in both groups. Changes of mechano-nociceptive thresholds in percentage compared to the respective preoperation values before and 30 min after drug administration were calculated.

Ethics

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical committee of Medical Faculty, University of Pécs, Hungary (BA02/2000-6/2001).

Drugs and chemicals

Sodium thiopentone (Byk Gulden, Konstanz, Germany), Trasylol (Richter-Gedeon Ltd., Budapest, Hungary), EDTA, Tween 80, dimethyl-sulfoxide (DMSO) and ethanol (Reanal, Budapest, Hungary), somatostatin-14, Tyr(1)-somatostatin-14, resiniferatoxin and pentobarbitone sodium (Sigma, St. Louis, USA), AEA (RBI, Natick, USA), PEA (ICN, Aurora, USA), rat Tyr-α-CGRP (23–37) (Bachem, Bubendorf, Switzerland) were used. ^{125}I-labelled Tyr-α-CGRP (23–37) and Tyr(1)-somatostatin-14 were prepared in our laboratory Németh et al., 2002). Somatostatin and CGRP antisera were provided by Dr. T. Görcs, University Medical School of Budapest. SR141716A and SR144528 were generous gifts from Sanofi-Synthelabo, Montpellier, France. RTX was dissolved in ethanol and further dilutions were made with saline. AEA was dissolved in absolute ethanol to obtain 25 mg/ml stock solution from which further dilutions were made (10% ethanol, 5% Tween 80 and 85% saline). In case of PEA DMSO was used instead of ethanol.

Statistical analysis

Results are presented as means ± s.e.mean. Non-parametric (Mann-Whitney test) was used for statistical evaluation of the data, $*P<0.05$, $**P<0.01$.

Results

Effect of RTX on plasma somatostatin and CGRP concentrations

Systemic RTX administration (0.1–3 μg/kg, i.v.) caused dose-dependent elevation of plasma somatostatin (from 5.02 ± 0.56 up to 113.66 ± 8.07 fmol/ml) and CGRP (from 17.5 ± 0.78 up to 90.92 ± 4.93 fmol/ml) levels. Significant increases in plasma somatostatin and CGRP concentrations were observed from doses of 0.3 and 0.6 μg/kg RTX and the maximum increase was 22.3- and 5.8-fold, respectively (Fig. 1).

Effects of AEA and PEA on basal and RTX-evoked somatostatin and CGRP plasma concentrations

Neither AEA nor PEA (100 μg/kg, i.v.) caused significant alterations in basal plasma neuropeptide levels. RTX (0.6 μg/kg, i.v.) evoked 3.1 fold elevation of CGRP, and 10.7 fold increase of somatostatin concentration. AEA injected 5 min before RTX in doses of 10 and 100 μg/kg decreased neuropeptide release induced by RTX in a dose-dependent manner. After 10 μg/kg AEA CGRP and somatostatin release were inhibited by 18.5 ± 1.7 and 48.1 ± 3.2%, respectively

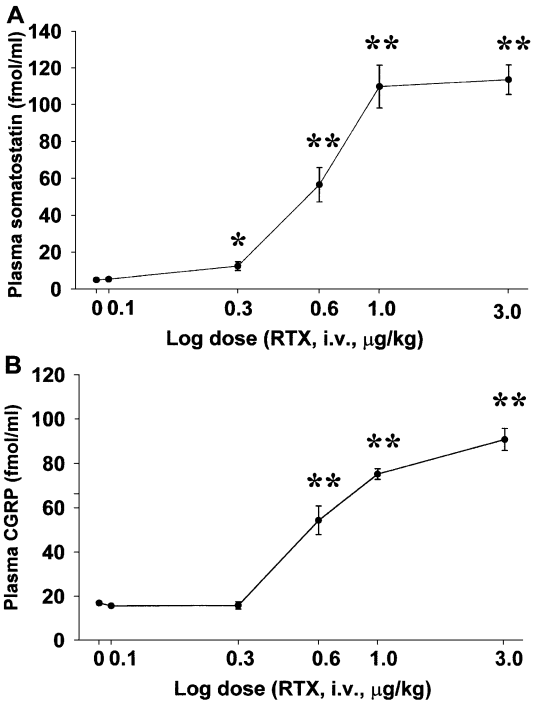

Fig. 1. Dose-dependent elevation of plasma somatostatin (A) and CGRP (B) concentrations 5 min after systemic RTX administration (0.1–3 μg/kg, i.v.). Data are expressed as means ± s.e.mean obtained from 6 experiments, *$P<0.05$, **$P<0.01$ compared to the respective basal values (Mann-Whitney U-test).

and following 100 μg/kg these inhibitions were 60.1 ± 4.6 and 76.3 ± 3.0%. Pretreatment with the CB_1 antagonist SR141716A (100 μg/kg, i.v. 10 min prior to AEA) prevented the inhibitory action of AEA on RTX-evoked release of CGRP and markedly diminished that of somatostatin. PEA caused even more effective dose-dependent decrease of RTX-induced CGRP and somatostatin release, the inhibition was 53.8 ± 2.9 and 58.2 ± 3.8% in response to 10 μg/kg, and 66.1 ± 2.7 and 82.03 ± 3.1% after 100 μg/kg i.v. dose (Fig. 2).

Effects of AEA and PEA on neuropathic hyperalgesia following partial sciatic nerve injury

Mechano-nociceptive threshold significantly decreased on the 7th day after partial ligation of the sciatic nerve (Seltzer et al., 1990), 29.7 ± 0.6% hyperalgesia developed. Pretreatment with AEA (100 μg/kg i.p.) 30 min before measurement abolished the hyperalgesia. The CB_1 receptor antagonist SR141716A (3 mg/kg i.p.) increased hyperalgesia by 37.1% indicating the anti-hyperalgesic role of endogenous cannabinoids in neuropathic pain conditions, and the inhibitory effect of AEA was completely abolished 30 min after SR141716A injection.

The other endocannabinoid, PEA (100 μg/kg i.p.) decreased hyperalgesia by 79.4%, and this action was prevented by the peripheral CB_2 receptor antagonist SR141528 (3 mg/kg i.p.) pretreatment. This

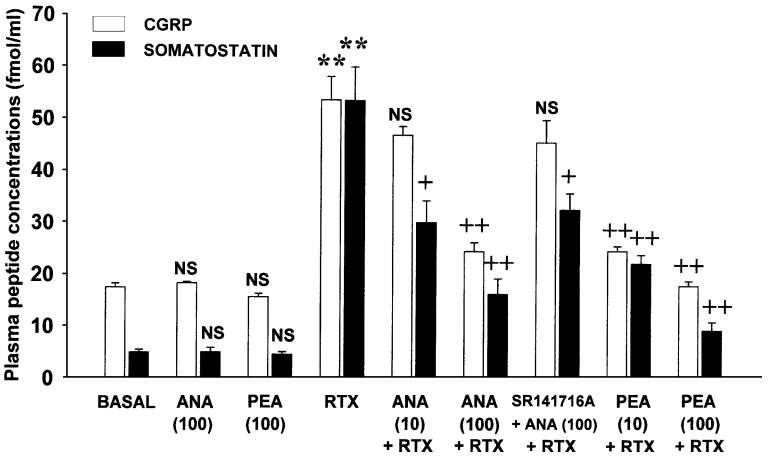

Fig. 2. Plasma concentrations of CGRP and somatostatin under basal conditions in untreated rats and in response to AEA (100 μg/kg, i.v.), PEA (100 μg/kg, i.v.) and RTX (0.6 μg/kg, i.v.). The 5th , 6th and 7th pairs of columns indicate the dose-dependent inhibitory effect of 10 and 100 μg/kg AEA on RTX-induced neuropeptide release and the antagonistic action of the selective CB_1 receptor blocking agent SR141716A. The 8th and 9th groups of bars refer to the dose-dependent inhibition induced by PEA (10 and 100 μg/kg, i.v.) on RTX-evoked release of CGRP and somatostatin. Results are shown as means ± s.e.mean of 6 experiments, $*P<0.05$, $**P<0.01$ compared to the respective basal values; $+P<0.05$, $++P<0.01$ compared to the RTX-induced peptide release (Mann-Whitney U-test).

latter compound, similarly to the effect of SR141716A, increased hyperalgesia by 47.5%, but the combination of the CB_1 and CB_2 receptor antagonists did not cause further decrease of the mechanonociceptive threshold values (Fig. 3).

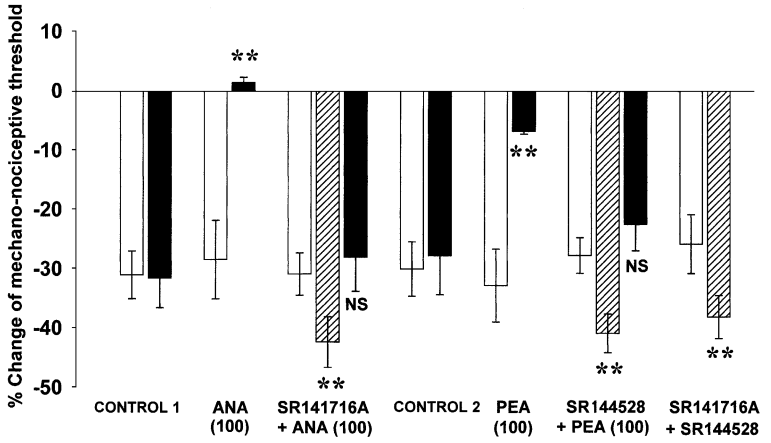

Fig. 3. Percentage changes in mechano-nociceptive threshold of the rat hindpaw compared to the pre-operation values 7 days after partial ligation of the sciatic nerve. Open coloumns indicate hyperalgesia before drug injection, hatched columns refer to the values 30 min after the CB_1 receptor antagonist SR141716A and/or the CB_2 receptor antagonist SR144528 injection (3 mg/kg, i.p.) and solid bars show the results 30 min following AEA or PEA treatment (100 μg/kg, i.p.). Data are presented as means ± s.e.mean of 6 experiments, $**P<0.01$ compared to the respective control group (control 1: solvent of AEA, control 2: solvent of PEA). Non-parametric Mann-Whitney U-test was used for statistical evaluation.

Discussion

Our data revealed that both AEA and PEA inhibited RTX-induced CGRP and somatostatin release in vivo via CB_1 and CB_2–like receptors without affecting basal neuropeptide concentrations. In the rat enhancement of plasma neuropeptide levels evoked by i.v. injection of RTX was used to assess the in vivo release of sensory neuropeptides from the capsaicin-sensitive nerve endings in response to stimulation of the VR_1/$TRPV_1$ capsaicin receptors (Caterina et al., 1997; Szállási and Blumberg, 1999). The effect of RTX was dose-dependent on the release of both neuropeptides, but it was more pronounced in the case of somatostatin. AEA in doses of 10 and 100 μg/kg i.v. did not alter basal neuropeptide concentrations, but inhibited the enhancements induced by RTX. This inhibition was partly prevented by pretreating the rats with the CB_1 cannabinoid receptor antagonist SR141716A (Pertwee, 1997). Therefore, it is concluded that the release of sensory neuropeptides in response to systemic activation of the VR_1/$TRPV_1$ receptors by RTX is inhibited by the potent CB_1 receptor agonism of AEA. The AEA-induced release of CGRP in isolated arterial preparations (Zygmunt et al., 1999) is an indication that some capsaicin-sensitive sensory fibres are probably less densely supplied by cannabinoid CB_1 receptors and in these vascular areas the effect of VR_1 receptor agonism of AEA is not suppressed by the powerful inhibition due to activation of CB_1 receptors. It has been suggested that AEA is an endogenous ligand for the VR_1/$TRPV_1$ capsaicin receptor (Zygmunt et al., 1999; Smart et al., 2000). The functional significance of this mechanism, however, was questioned owing to the highly potent inhibitory effect of AEA on CGRP release via activation of cannabinoid receptors on cutaneous nerves and in the spinal cord (Szolcsányi, 2000a,b). The issue remained unsettled since AEA activated cannabinoid receptors in several isolated organ preparations in similar concentrations as the capsaicin receptors of the isolated arterial segments (Zygmunt et al., 1999). Furthermore, on primary cultures of small trigeminal neurones 200 nM AEA elicited both an increase in intracellular calcium concentration and an inhibition of capsaicin-evoked calcium transients (Szőke et al., 2000). Our results revealed concentration-dependent dual effect of AEA on CGRP, substance P and somatostatin release from isolated rat tracheae, low concentration (10^{-5} M) inhibited, while high concentrations (5×10^{-5}–10^{-4} M) induced peptide outflow (Németh et al., 2003).

Similarly to AEA, another endocannabinoid, PEA, acting selectively on peripheral CB_2-like receptors (Jaggar et al., 1998), was also able to diminish RTX-evoked plasma somatostatin and CGRP release. The anti-nociceptive effects of PEA are antagonized by the CB_2 receptor antagonist SR 144528, however, it has no significant affinity for CB_2 receptors expressed on cultured cells or in rat spleen slices (Showalter et al., 1996; Lambert et al., 1999; Malan et al., 2003). Therefore, it has been suggested that PEA may act on a yet not characterised, possibly CB_2-like receptor (Calignano et al., 2001). This proposal was supported by the finding that PEA did not, but AM1241, a synthetic CB_2 receptor-selective agonist did inhibit thermal and capsaicin-induced nociception. Systemic administration of AM1241 also abolished nerve injury-induced tactile and thermal hypersensitivity (Malan et al., 2003).

CGRP and somatostatin, derived from the capsaicin-sensitive subpopulation of sensory afferents contribute to the development of neuropathic hyperalgesia (Rittenhouse et al., 1996; Stanton-Hicks and Salamon, 1997). The synthesis of these peptides in primary sensory neurons is reduced in painful neuropathic conditions (Hökfelt et al., 1994; Rittenhouse et al., 1996), but there are also some data demonstrating increased CGRP mRNA in the spared neurons of the dorsal root ganglia in the spinal nerve ligation model (Fukuoka et al., 1998). AEA abolished the decrease of mechanonociceptive threshold 7 days after Seltzer operation acting on CB_1 receptors, PEA reduced hyperalgesia via CB_2–

like receptors. Other synthetic CB_1 receptor agonists, like WIN55,212-2, CP-55,940 and HU210 also produce complete reversal of mechanical hyperalgesia in the sciatic nerve ligation model of the rat, their effect is likely to be mediated by an action in both the central and peripheral nervous sytems (Fox et al., 2001). In the present study both CB_1 and CB_2 antagonists applied alone or in combination diminished nociceptive thresholds, indicating a functional tonic inhibitory role of endocannabinoids in neuropathic pain, possibly by reducing sensory neuropeptide release. These results obtained in neuropathic pain conditions are in complete accordance with the data of Calignano et al. (1998), who showed that these CB_1 and CB_2 receptor antagonists prolong and enhance pain behaviour produced by tissue damage, indicating as well that peripheral cannabinoid receptors participate in the intrinsic control of pain initiation probably by locally generated AEA and PEA. Biochemical data concerning upregulation of thalamic cannabinoid receptors one day after nerve injury are in accordance with this conclusion (Siegling et al., 2001).

Conclusions

The present results demonstrate that activation of peripheral cannabinoid receptors inhibits stimulation-induced sensory neuropeptide release and reverses mechanical hypersensitivity produced by nerve injury. Neuropathic pain is particularly difficult to treat, there is no effective therapy available so far. Cannabinoid ligands, especially peripherally acting selective CB_2 receptor agonists free of central nervous system side effects, could open future perspectives for the therapy of neuropathic pain symptoms.

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