

Effect of interaction between acute administration of morphine and cannabinoid compounds on spontaneous excitatory and inhibitory postsynaptic currents of magnocellular neurons of supraoptic nucleus

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ABSTRACT

Objective(s): Opioids and cannabinoids are two important compounds that have been shown to influence the activity of magnocellular neurons (MCNs) of supraoptic nucleus (SON). The interaction between opioidergic and cannabinoidergic systems in various structures of the brain and spinal cord is now well established, but not in the MCNs of SON.

Materials and methods: In this study, whole cell patch clamp recording of neurons in rat brain slice was used to investigate the effect of acute morphine and cannabinoid administration on spontaneous inhibitory and excitatory postsynaptic currents (sIPSCs and sEPSCs) in MCNs.

Results: Bath application of morphine produced an increase in sEPSCs frequency and a decrease in sIPSCs frequency. In contrast, bath application of URB597 (fatty acid amide hydrolase (FAAH) inhibitor) produced a decrease in sEPSCs frequency but an increase in sIPSCs frequency. WIN55212-2 (cannabinoid receptor agonist) decreased both sIPSCs and sEPSCs frequencies of MCNs. Co-application of morphine and URB597 attenuated the effect of morphine on MCNs.

Conclusion: Taken together, these data indicated that at the cellular level, pharmacological augmentation of endocannabinoids could attenuate morphine effects on MCNs.

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Introduction

The magnocellular neurons (MCNs) of the supraoptic nucleus (SON) play a crucial hormonal role due to the vasopressin (AVP) that they synthesize. AVP is a potent vasopressor and neurotransmitter (1) that has several important physiological functions, mediated by different G-protein-coupled receptors. AVP mediates vasoconstriction, regulation of urine output, regulation of cardiovascular system (1), regulation of renin-angiotensin-aldosterone system (2), and stimulation of ACTH secretion (3). Thus, plasma vasopressin concentration may be an accurate marker for pathophysiological conditions and useful to guide treatment for them.

Both intrinsic conductance and a variety of excitatory and inhibitory inputs can regulate the activity of MCNs. The important excitatory afferents in the hypothalamus are glutamatergic (4, 5), and inhibitory afferents are largely GABAergic (6, 7).

Also, there is a wide variety of transmitters that can modulate the efficacy of afferent transmission into these cells (8, 9). Opioids and cannabinoids are two important transmitters that have been shown to influence MCN neuronal activity (10-12). It has been shown that endogenous cannabinoids are released as retrograde messengers in the SON by MCNs and suppress synaptic GABA and glutamate release (11, 12). Therefore, it seems that endogenous cannabinoids can regulate MCNs activity by modulation of both excitatory and inhibitory postsynaptic currents (13).

On the other hand, the SON receives opioid innervation from other brain regions and contains high levels of μ and κ opioid receptor binding sites. Both *in vivo* and *in vitro* extra and intracellular recordings have shown that opioids could affect MCN activity (14). During perfusion of the slice with opioid compounds, some supraoptic cells were profoundly inhibited, the others showed no response

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or were excited (15). In addition, opioids can change the content of AVP in the MCN (12). The effects of morphine on plasma AVP levels are somewhat confusing. Some reports showed that morphine stimulated AVP secretion (16, 17) while others did not suggest a significant increase in plasma AVP level (18, 19).

Opioids and cannabinoids share a similar pharmacological profile, both induce analgesia, hypothermia, sedation, hypotension, inhibition of intestinal motility and locomotor activity, changes in mood, and finally depression of the immune function (20, 21). Receptors for these transmitters are colocalized in the same neurons in the spinal cord and various brain areas such as SON (22).

Since in the previous study we showed the excitatory effects of acute morphine administration on MCNs activity (23), we performed the present study with the aim of investigating the effect of the interaction of morphine and cannabinoid systems on spontaneous inhibitory and excitatory postsynaptic currents (sIPSCs and sEPSCs) in MCNs of SON.

Materials and Methods

All experiments in the present study were conducted in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23) revised 1996. Male Wistar rats (70–100 g, 3–4 weeks old) were purchased from Pasteur institute (Tehran, Iran). Animals were kept in a room with 12 hr/12 hr light/dark cycle (lights on at 07:00 AM) and controlled temperature (22 ± 2 °C) before conducting experiments.

Chemicals

The drugs in this study were WIN55212-2 (cannabinoid receptor agonist, Sigma, St. Louis, MO, USA), AM-251 (cannabinoid receptor CB1 antagonist, Sigma, St. Louis, MO, USA), URB597 (fatty acid amide hydrolase (FAAH) inhibitor, Cayman chemical, Ann Arbor, MI, USA), morphine sulphate (Temad CO., Tehran, Iran), naloxone (morphine receptor antagonist, Sigma, St. Louis, MO, USA), bicuculline (GABA receptor antagonist, Fluka, Switzerland), AP-5 (glutamate receptor antagonist, Sigma, St. Louis, MO, USA), CNQX (glutamate receptor antagonist, Ascent scientific, UK) and TTX (voltage-gated Na channel inhibitor; Sigma, St. Louis, MO, USA).

Electrophysiological recording from SON neurons

Slice preparation

Animals were anesthetized with ether and decapitated. The brain was quickly removed and placed in ice-cold (0–2 °C) slicing solution containing (in mM) 87 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 7 MgCl_2 , 0.5 CaCl_2 , 25 NaHCO_3 , 25 glucose, and 75 sucrose, saturated with 95% O_2 and 5% CO_2 . Coronal slices (250 μm) were cut with a vibratome (Campden

instruments Co. UK) from a block of tissue containing the hypothalamus. Slices including the SON were hemisected along the midline and allowed to recover for at least 1 hr in 32–34 °C. The slice was then transferred into a recording chamber in which it was submerged and continuously perfused with ACSF (0.5 ml/min). The composition of the ACSF was as follows (in mM): 126 NaCl, 2.5 KCl, 1.2 Na_2HPO_4 , 18 NaHCO_3 , 1.2 MgCl_2 , 2.4 CaCl_2 , and 11 glucose, pH 7.4 (295 mOsm/kg).

Drug application

All drugs were bath applied. Appropriate stock solutions with defined concentration were made and diluted with ACSF just before application. Drug applications were performed with a constant rate of 0.5 ml/min for a period of 10 min and then plain ACSF was substituted during the rest of the recording.

Whole-cell patch-clamp recording

To characterize the rapid membrane effects of morphine, URB597, and WIN55212-2 on MCNs, we performed whole cell patch clamp recordings in neurons of the SON in acutely prepared hypothalamic slices. MCNs were identified visually by their relatively large somatic size and position in the SON using infrared differential interference contrast (IR-DIC) microscopy (BX51WI Olympus, Tokyo, Japan). Patch-clamp recording pipettes (3–7 M Ω) were filled with a solution containing the following (in mM): 130 CsCl (for IPSC) or 130 potassium gluconate (for EPSC), 10 HEPES, 1 CaCl_2 , 1 MgCl_2 , 5 EGTA, 1 NaCl, 2 $\text{Na}_2\text{-ATP}$ and pH adjusted to 7.2 with CsOH (for IPSC) or KOH (for EPSC). To prevent evoked potentials and currents, the cells were recorded in the presence of TTX (2 μM) at 32 ± 2 °C. Spontaneous EPSCs were recorded as inward synaptic currents at a holding potential of -70 mV (28) in the presence of the GABA_A receptor antagonist bicuculline (30 μM) and were blocked by the ionotropic glutamate receptor antagonists AP-5 (50 μM) and CNQX (20 μM). Spontaneous IPSCs were recorded with cesium-containing electrodes as outward synaptic currents at a holding potential of +30 mV (24) in the presence of the AP-5 (50 μM) and CNQX (20 μM) and were blocked by the GABA_A receptor antagonist bicuculline (30 μM). Data were collected only after a 15–20 min baseline recording during which a stable amplitude and frequency of synaptic currents were observed. For each cell, an epoch of 5 min immediately before drug administration was considered as the control value and the rest of the recordings were compared with these pre-treatment control values. Membrane currents were recorded using an amplifier (Axopatch 200B, Molecular device, USA), low-pass filtered at 2 kHz and digitized using the Digidata 1322A (Axon instrument, USA). Series resistance (up to 20 M Ω) was monitored online during the recording

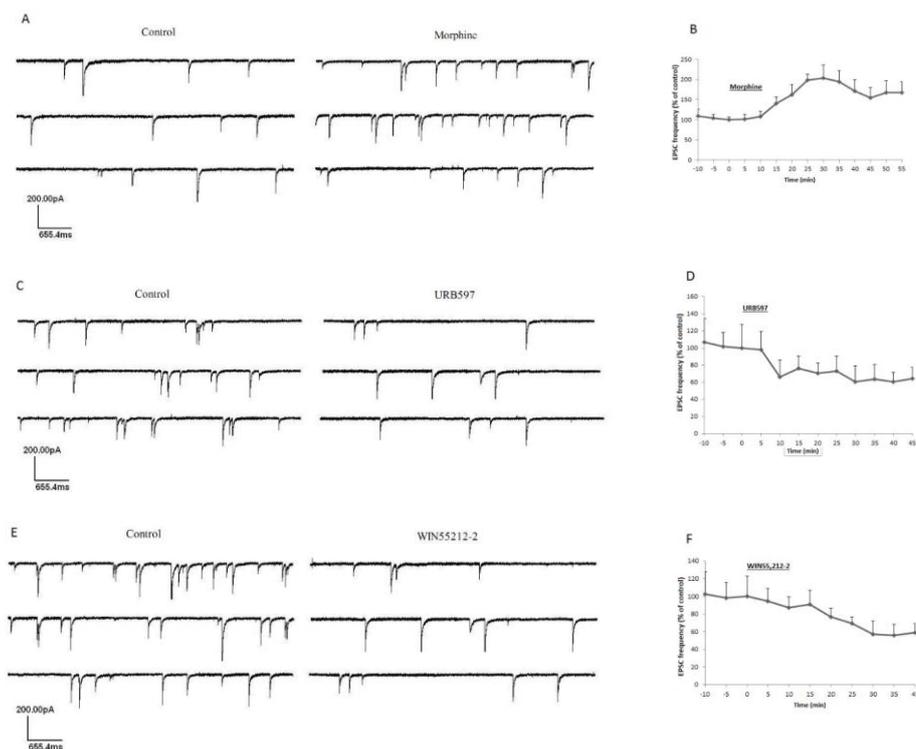


Figure 1. The effects of morphine, WIN55212-2, and URB597 on EPSCs. A) The representative trace and B) the line chart shows the frequency of sEPSCs before and after morphine administration, C & D) The frequency of sEPSCs before and after URB597 administration and E & F) The frequency of sEPSCs before and after WIN55212-2 administration. Morphine increased the frequency of sEPSCs, but WIN55212-2 and URB597 had inhibitory effects on sEPSCs

and cells were excluded from data analysis if more than 15% change occurred during the course of the experiment. No whole cell series resistance compensation was made during the recording of spontaneous events. Spontaneous events were detected with the threshold levels of 3-times the baseline noise using the Mini Analysis Program (Synaptosoft Inc., NJ, USA). The amplitude of the synaptic current was calculated from the baseline to the peak of each response.

Data analysis

Data are presented as mean \pm SEM. Data were analyzed using one-way ANOVA followed by Dunnett's post-test, paired *t*-test or unpaired *t*-test as appropriate. *P*-values of less than 0.05 were considered statistically significant.

Results

Spontaneous synaptic currents were recorded from 156 supraoptic neurons. Both sEPSCs and sIPSCs were observed without any stimulus.

Morphine-induced excitation of MCNs

Figures (1A, B) and (2A, B) show one neuron recorded excitatory and inhibitory currents as a trace and during patch clamp recording. Application of morphine in the bath perfusion suppressed GABAergic synaptic activity in MCNs but increased

glutamatergic synaptic activity in these neurons. Bath application of the lowest effective dose of morphine (25 μ M) for 10 min caused a significant decrease (32% \pm 10.55) in the frequency of sIPSCs (P <0.001, n =7, 1 neuron/1 slice, Figures 2A & B, 3) and a significant increase (120% \pm 2.18) in the frequency of sEPSCs (P <0.001, n =7, 1 neuron/1 slice, Figures 1A & B, 3) but had no effect on amplitudes of either sEPSCs or sIPSCs compared to control values (Figures 3A & B). Thus, morphine had a net excitatory effect on spontaneous synaptic currents of MCNs.

URB597 inhibition of MCNs

Figures (1C, D) and (2C, D) show one neuron recorded excitatory and inhibitory currents as a trace and during patch clamp recording affected by URB597. Administration of URB597 increased GABAergic synaptic activity but decreased glutamatergic synaptic activity in MCNs. Bath application of URB597 (100 nM) for 10 min caused a 64% \pm 7.16 increase in the frequency of sIPSCs (P <0.01, n =6, 1 neuron/1 slice, Figures 2C & D, 3) and a 30% \pm 1.18 decrease in the frequency of sEPSCs (P <0.05, n = 6, 1 neuron/1 slice, Figures 1C & D, 3) without significant effect on amplitude of sIPSCs and sEPSCs (Figures 3B & D). Thus, URB597 had a net inhibitory effect on spontaneous synaptic currents of MCNs.

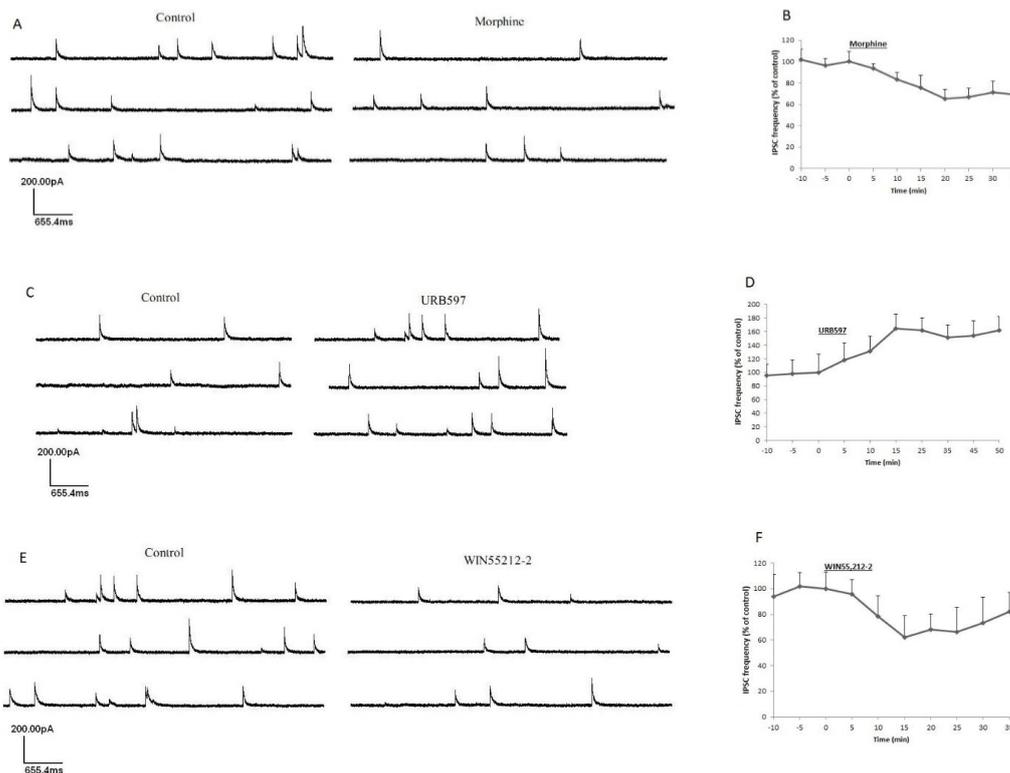


Figure 2. The effects of morphine, WIN55212-2, and URB597 on IPSCs. A) The representative trace and B) The line chart shows the frequency of sIPSCs before and after morphine administration, C & D) The frequency of sIPSCs before and after URB597 administration and E & F) The frequency of sIPSCs before and after WIN55212-2 administration. Morphine and WIN55212-2 had inhibitory effects on the frequency of sIPSCs, but URB597 increased sIPSCs

WIN55212-2 inhibition of both sIPSCs and sEPSCs

Figures (1E, F) and (2E, F) show one neuron recorded excitatory and inhibitory currents as a trace and during patch clamp recording. WIN55212-2 induced a significant inhibition of sIPSCs and sEPSC in MCNs. Bath application of WIN55212-2 (500 nM) for 10 min produced significant decrease both in

sIPSCs frequency ($36\% \pm 3.78$) ($P < 0.01$, $n = 7$; 1 neuron/1 slice, Figures 2E & F, 3) and in sEPSCs ($33\% \pm 2.92$) ($P < 0.05$, $n = 7$; 1 neuron/1 slice, Figures 1E & F, 3) without significant changes in amplitudes (Figures 3B & D). Thus, it seems WIN55212-2 alone had any net effect on spontaneous synaptic currents of MCNs.

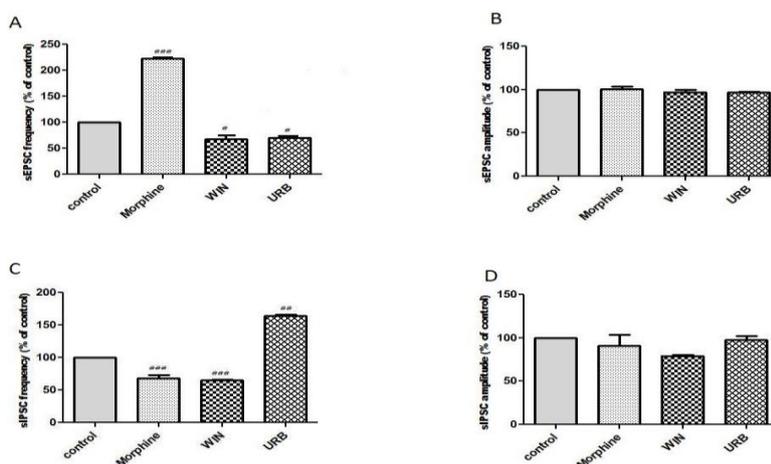


Figure 3. The comparison of the effects of morphine, WIN55212-2, and URB597 on IPSCs and EPSCs. A) Bath application of morphine (25 μ M) elicited an increase in the frequency of sEPSCs, but WIN55212-2 (500 nM) and URB597 (100 nM) decreased the frequency of sEPSCs. B) In all cases the amplitude of sEPSCs remains unchanged ($n = 7$). C) Bath application of morphine (25 μ M) and WIN (500 nM) elicited a reduction in the frequency of sIPSCs, but URB597 (100 nM) increased the frequency of sIPSC. D) In all cases, the amplitude of sIPSCs remains unchanged ($n = 7$). Each bar represents mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ with respect to control in this and subsequent figures

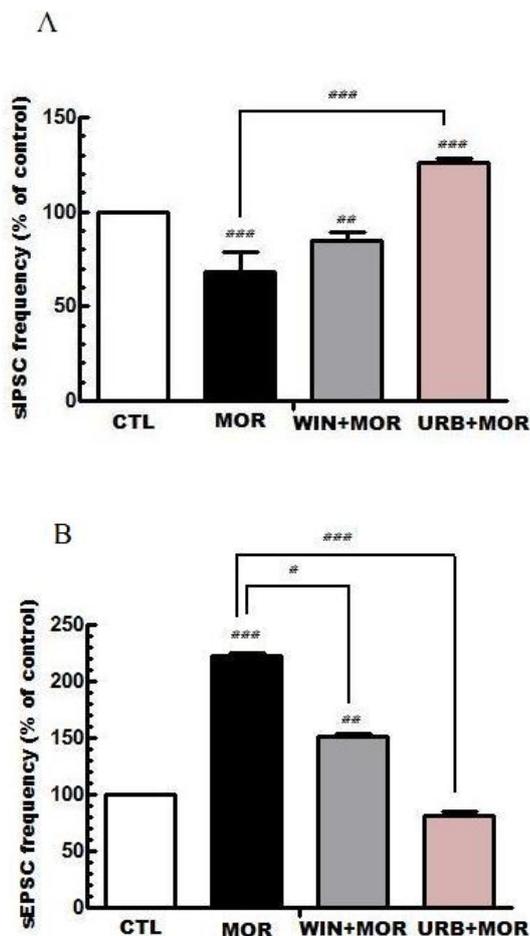


Figure 4. The effect of morphine on sIPSC and sEPSC frequency and amplitude of magnocellular neurons of supraoptic nucleus in the presence of URB597 and WIN55,212-2. A) Mean changes in the sIPSC frequency. Co-administration of morphine (25 μ M) and URB597 (100 nM) increased the frequency of sIPSCs and co-administration of morphine (25 μ M) and WIN55,212-2 (500 nM) decreased the frequency of sIPSCs. B) Mean changes in the sEPSC frequency. Co-administration of morphine (25 μ M) and URB597 (100 nM) decreased the frequency of sEPSCs and co-administration of morphine (25 μ M) and WIN55,212-2 (500 nM) increased the frequency of sEPSCs. Each bar represents mean \pm SEM (n=6)

Effect of interaction between morphine and cannabinoids on sEPSC and sIPSC

In order to evaluate the interaction between morphine and cannabinoids, bath application of either WIN55212-2 or URB597 for 10 min was followed by bath application of morphine for 10 min. While co-application of WIN55212-2 (500 nM) and morphine (25 μ M) produced a significant decrease in sIPSCs frequency ($P < 0.01$) (Figure 4A), co-administration of URB597 (100 nM) and morphine (25 μ M) significantly increased ($P < 0.01$) sIPSCs frequencies compared to control values (Figure 4A). Moreover, co-application of WIN55212-2 (500 nM) and morphine (25 μ M) significantly increased sEPSCs frequencies ($P < 0.001$), but co-administration of URB597 (100 nM) and morphine (25 μ M)

produced no significant effect on sEPSCs compared to control values (Figure 4B). Co-administration of morphine and cannabinoids had no effect on amplitudes of either sEPSCs or sIPSCs compared to control values. Thus, the results of co-administration of morphine and cannabinoid compounds in this study showed that cannabinoids could modulate the morphine effects on spontaneous synaptic currents of MCNs.

Discussion

In the present study bath application of morphine (25 μ M) reduced the frequency of sIPSCs and increased the frequency of sEPSCs without alterations in their amplitude. In addition, our *in vivo* data showed that after acute injection of morphine, there was an increase in plasma AVP levels compared to the control group (23). The previous studies have shown that morphine could have either inhibitory or excitatory effects, depending on the protocol of morphine administration (acute or chronic). Some studies indicated that acute morphine administration increased expression of AVP in SON (25). Electrophysiological studies also showed that μ and κ opioidergic inputs decreased and increased excitability of MCNs, respectively, through modulation of voltage-dependent potassium currents (26). In our study in agreement with some investigations, acute administration of morphine stimulated MCNs of SON. Also, different effects of morphine on excitatory or inhibitory synapses can be related to the specificity of the type of opioid receptors expressed on terminals or activation of different signaling pathways.

It has been shown that MCNs of the SON synthesize and release endocannabinoids (13, 27). The early observations suggested the inhibitory effects of cannabinoids in the SON (13). The endocannabinoids act as retrograde messengers in the SON, and CB1 receptors are localized on presynaptic terminals (13). Several studies have shown that the endocannabinoids anandamide (AEA) and 2-arachidoyl-glycerol (2-AG) differ in their synthetic pathways (28) and may be produced under distinct physiological conditions or in distinct brain regions (29, 30). Some studies indicated that both AEA and 2-AG were produced in SON and affected the synaptic activity in this region (11, 12).

In our study, bath application of exogenous cannabinoid receptor agonist WIN55212-2 decreased the frequency of both sIPSCs and sEPSCs in MCNs, which is consistent with the results of previous studies (13). This effect may be due to direct action of CB receptor agonist on presynaptic CB1 receptors and subsequent prevention of transmitter release (13). Moreover, bath application of URB597 (FAAH enzyme inhibitor) produced a net inhibitory effect on MCNs through both attenuating sEPSCs and increasing sIPSCs frequencies. The effects of WIN55212-2 on synaptic transmission

differed from those induced by URB597, which may be due to different mechanisms of action of these two cannabinoid compounds.

It has been shown that WIN55212-2 administration decreased AEA release and increased 2-AG release in hypothalamus whereas URB597 administration produced opposite effects on AEA and 2-AG release (31). These results suggest that CB receptors are able to control the local release of endocannabinoids in the hypothalamus and strengthen the view that AEA and 2-AG have distinct physiological roles in brain functioning (31). AEA appears to be involved in neurotransmission, whereas 2-AG appears to have mostly housekeeping functions (32). It is plausible that the effects mediated by different patterns of endogenous cannabinoid release by URB597 and WIN55212-2 are acting on an as-yet-unknown AM251 sensitive CB receptor (11, 12). Studies on CB1 and CB2 receptor knockout mice have provided evidence for a non-CB1, non-CB2 receptor (i.e. CBx receptor) in the hippocampus (33) and hypothalamus (34). It seems that the CBx receptors in the hypothalamus are activated by AEA (34). Also, there are other receptor systems involved in pharmacological effects of cannabinoids such as the orphan G protein-coupled receptor GPR55, which is expressed in several tissues including some brain regions (35). It has been reported that several endocannabinoids (including AEA and 2-AG) bind to GPR55 and are potent stimulants, whereas some exogenous cannabinoids including WIN55212-2 exhibit weak receptor stimulation (36). Moreover, WIN55212-2 may induce its effect by binding to other brain-expressed GPCR known by some as a CB3 receptor (37). In addition, it was shown that endocannabinoids and exogenous cannabinoids activated different G proteins (38). Another possible explanation is that the endocannabinoid increased by URB597 instead of acting at presynaptic GABA terminals; it directly activates CB1 receptors on upstream axo-axonic GABA synapses onto presynaptic GABA terminals and is reducing GABA release and effectively disinhibiting the GABA release onto the MCNs (11, 12). Still another possibility is that endocannabinoids interact with other neurotransmitters to modulate GABA release, as there is a large body of evidence for endocannabinoid interaction with several other neurotransmitter systems (11, 12).

In regard to known different mechanisms of cannabinoid effects, in this study the inhibitory effects of WIN55212-2 and URB597 on excitatory or inhibitory synaptic currents may be due to direct inhibition of presynaptic terminals, while the excitatory effects of URB597 on inhibitory currents may be created by CB receptors activation on upstream axo-axonic GABA synapses onto presynaptic GABA terminals (11-13).

In the present study, co-administration of morphine and WIN55212-2 produced a significant decrease in sIPSCs. Moreover, an increase in sEPSCs was observed after co-administration of morphine and WIN55212-2. Considering the opposite effects of WIN55212-2 and morphine on sEPSCs, it seems that morphine actions on sEPSCs are more dominant than WIN55212-2 in MCNs.

Co-application of morphine and URB597 modulated their effects on sEPSCs. Considering the increasing effect of co-administration of URB597 and morphine on sIPSCs, it seems that the enhancement of endocannabinoid activity is likely more dominant than direct activation of opioid receptors in MCNs.

An impressive amount of data has demonstrated the existence of strong interactions between the endogenous cannabinoid and opioid systems (20, 39). There have been direct demonstrations of THC-induced release of endogenous opioids in brain areas (40, 41). Thus, blocking opioid receptors would block the effects of the cannabinoid-induced released opioids (32). After the cloning of the cannabinoid CB1 receptor (42), it was shown that pharmacological blockade or genetic ablation of cannabinoid CB1 receptors decreased many of the effects of opioids (43-48). Brain levels of endogenous cannabinoids have been reported to be increased, decreased or unchanged after the administration of opioids, depending on which endocannabinoid (AEA or 2-AG) was measured and the protocol of opioid administration (i.e. acute or chronic) (49-51). It seems that modulation of opioid effects is most likely mediated by changes in AEA rather than 2-AG levels. It has been shown that acute administration of morphine increases the levels of AEA, but not 2-AG, in brain areas such as the nucleus accumbens, the caudate putamen and the hippocampus (51). Thus, release of AEA may play a role in the effect of opioids, and blocking cannabinoid receptors would block the effects of the released AEA. Cannabinoid and opioid receptors, especially μ receptors, show similar brain distributions and have been shown to have at least a partial degree of co-localization in some brain areas (52-54), also they share similar second messenger cascades (37, 55). These facts suggest that cannabinoid and opioid receptors interact at the level of the cell membrane or at the level of signaling pathways. Some studies suggested the existence of cross-talk between cannabinoids and opiates on the sensitization to morphine and the implication of endocannabinoid system in the process of sensitization to opiates (56). Whereas, the others showed that CB1 receptors as well as morphine receptors interacted with Gz proteins and both receptors regulated Gz proteins which were implicated in their desensitization and cross-desensitization (38).

This study similar to other investigations on co-administration of opioids and cannabinoid

compounds in other areas of CNS showed that co-administration of morphine and cannabinoids modulated the morphine effects on MCNs in SON.

Conclusion

Considering the recognized interactions between the effects of cannabinoid and opioid systems, progress towards understanding the molecular basis of these interactions and the degree to which the endogenous opioid and cannabinoid systems interact still has far to go. Further advances may lead to the use of the cannabinoid system to develop new therapeutic protocols.

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