Introduction

Cannabinoids affect many regulatory processes controlled by the hypothalamus (Habaye et al. 2002; Harrod and Williams 2003). For example, cannabinoids stimulate appetite, food-seeking behavior and energy intake (Harrod and Williams 2003; Horvath 2003). Energy homeostasis is regulated in large part by the hypothalamic feeding circuitry comprising the proopiomelanocortin (POMC), neuropeptide Y (NPY), and agouti-related peptide (AGRP)-containing neurons in the arcuate nucleus (ARC) (Cowley et al. 2003; Marks and Cone 2003; Olczewski et al. 2003) and orexin and melanin-concentrating hormone (MCH)-containing neurons in the lateral hypothalamus (DiLeone et al. 2003) as well as input from the ventromedial nucleus of the hypothalamus (Varma et al. 2000). A likely substrate on which cannabinoids act to stimulate hypothalamic levels of endogenous cannabinoids such as anandamide and 2-arachidonoylglycerol (Di Marzo et al. 2001). Cannabinoids can alter neuronal excitability via presynaptic inhibition of neurotransmission by amino acid neurotransmitters such as γ-aminobutyric acid (GABA) (Vaughan et al. 1999) and glutamate (Shen et al. 1996). Cannabinoids can also inhibit neuronal activity by activating postsynaptic K⁺ currents. For example, cannabinoids activate an inwardly rectifying K⁺ current in AtT20 cells transfected with CB1 receptor (Mackie et al. 1995) and in oocytes co-expressing the CB1 receptor and the G-protein-gated, inwardly rectifying K⁺ channel GIRK1 (Henry and Chavkin 1995). In hippocampal pyramidal neurons, cannabinoids also positively modulate the depolarization-activated, A-type K⁺ current (Deadwyler et al. 1995). Despite recent advances in our understanding of how cannabinoids affect hypothalamic function, the cellular mechanisms through which they regulate processes such as energy homeostasis are largely unknown. This study endeavored to examine the cannabinoid modulation of A-type K⁺ currents (Iₐ) in hypothalamic neurons from the ARC in which POMC neurons can be found. All animal procedures described in this study are in accordance with institutional guidelines based on National Institutes of Health standards.

Female and male Topeka guinea pigs (280–405 g) were obtained from Elm Hill Breeding Labs (Chelmsford, MA), kept under controlled temperature (69–73°F) and light (12 h on:12 h off), and provided with food and water ad libitum. They were castrated under ketamine/xylazine anesthesia (33 and 6 mg/kg, respectively, sc) 4–10 days prior to experimentation.

Tetrodotoxin (TTX) with citrate was dissolved in Ultrapure H2O to a stock concentration of 1 mM. Tetraethylammonium chloride (TEA) was dissolved in Ultrapure H2O to a stock concentration of 500 mM. 4-Aminopyridine (4-AP) and nickel chloride hexahydrate (NiCl2) were dissolved in UltraPure H₂O to a stock concentration of 100 mM. Arachidonyl-2′-chloroethylamide (ACEA) was dissolved in ethanol to a stock concentration of 1 mM. cis-4-[Phosphomethyl]-2-piperidinecarboxylic acid (CGS 19755; 10 mM) was dissolved in 0.1 N NaOH and then diluted to the final volume with Ultrapure H₂O. WIN-55,212–2, AM251 and NBQX were dissolved in dimethyl sulfoxide to stock concentrations of 10 mM. Stock solutions of SR 95531, ω-conotoxin MVIIIC, and rHeteropo-
dotoxin-2 (rHptx2; 10 mM, 10 μM, and 100 μM, respectively) were prepared with UltraPure H₂O.

Whole cell patch recordings in hypothalamic slices were performed as previously described (Ibrahim et al. 2003; Wagner et al. 2000). Neurons exhibiting transient outward tail currents evoked immediately after a hyperpolarizing voltage command (≥20 mV) from rest were selected for further analysis. The cells were perfused for 6–7 min with 25 mM TEA, 100 μM 4-AP, and 1 μM TTX to block other depolarization-activated K⁺ channels [except for the IA, which is resistant to TEA and to low concentrations of 4-AP (Storm 1988)], and to isolate the cells from synaptic input impinging on it. Cells were then subjected to baseline activation and inactivation protocols. The activation of the IA was evaluated by holding the membrane potential at −110 mV, giving 10-mV depolarizing steps (500 ms) starting from −110 up to −10 mV and measuring the resultant peak current that appears at the onset of the various voltage commands. The inactivation of the IA was evaluated by holding the membrane potential at −60 mV and giving 10-mV prepulses (500 ms) from −110 to −40 mV with each pulse followed by a depolarizing test command to −10 mV. The resultant outward current elicited by the depolarizing test command was measured for each of the prepulse potentials. After collecting the baseline measurements, slices were perfused with one of the two CB1 receptor agonists WIN 55,212–2 (1 μM) or ACEA (1 μM) in the presence of TEA, 4-AP, and TTX for 7–8 min, and then the activation and inactivation protocols were run again. We assessed CB1 receptor specificity by testing the effect of WIN 55,212–2 on the IA protocols in the presence of the CB1 receptor antagonist AM251 (1 μM). To assess the sensitivity of the IA to antagonism by 4-AP or rHptx2, the protocols occasionally were executed in the presence of higher (3 or 10 mM) concentrations of 4-AP or 100 nM rHptx2. In some experiments, we tested whether cannabinoids presynaptically modulate the IA by blocking the actions of spontaneously released amino acid neurotransmitters at N-methyl-D-aspartate (NMDA), non-NMDA, and GABA_A receptors with CGS 197755 (10 μM), NBQX (3 μM), and SR 95531 (10 μM), respectively, in conjunction with blockade of synaptic transmission with TTX. The amplitude and voltage dependence of the IA were analyzed using p-Clamp and SigmaPlot 8.0 software. We obtained estimates of the half-maximal voltage (V½) and maximal peak current (I_max) from the activation and inactivation curves generated by fitting the data (peak current vs. membrane voltage) to the Boltzmann equation (Deadwyler et al. 1995). If we encountered confounding Ca²⁺ currents that were ≥10% of the I_max then we performed the experiments in the presence of 300 μM NiCl₂ and 100 nM ω-conotoxin MVIC (as well as TEA, 4-AP, and TTX) to block T-, N-, and P/Q-type Ca²⁺ channels. After recording, some slices were processed for immunohistochemistry described previously (Ronneklev et al. 1990).

Variance homogeneity was evaluated using Cochran’s C test. Comparisons between treatment groups were performed using the one-way or two-way ANOVA followed by the least significant difference (LSD) test. Evaluation of the frequency of occurrence was accomplished using the χ² contingency test in conjunction with Freeman-Tukey deviate (FTD). Differences were considered statistically significant if the probability of error was <5%.

Forty neurons from the ARC of castrated female and male guinea pigs exhibited transient outward currents like those shown in Figs. 1–3. We observed this transient outward current in an equivalent percentage of ARC neurons from female (24 of 62 neurons; 39%) and male (16 of 39 neurons; 41%) animals. Eight of these neurons were subjected to immunohistochemistry, and all eight neurons were immunopositive for the Kv4.2 channel subtype (not shown). By contrast, only one of four (25%) neurons not displaying a transient outward current expressed the Kv4.2 channel subtype (not shown). The somas of seven of these neurons extended into adjacent slides, and this enabled us to subsequently test for β-endorphin immunoreactivity. Sixty-two percent (5 of 8) of these neurons also were immunopositive for β-endorphin, suggesting that all POMC neurons exhibiting the transient outward current also expressed the Kv4.2 channel subtype. In addition, both 4-AP and the Kv4.2 channel blocker rHptx2 markedly attenuated these currents (Fig. 1). Figure 2, A and B, show membrane current traces observed during whole cell patch-clamp recordings from castrated female and male guinea pigs used to generate inactivation curves (Fig. 2C) from which the voltage dependence of the inactivation of the transient outward current is assessed. Bath application of the CB1 receptor agonist WIN 55,212–2 (1 μM) produced a rightward shift in the inactivation curve for the transient outward current observed in neurons from ovariecetomized females. This was manifest by an increase in the V½ over 10 mV (control: −83.8 ± 2.7 mV vs. WIN 55,212–2: −71.1 ± 3.2 mV; P < 0.05; Fig. 2) with no discernable effect on the I_max (708.9 ± 98.6 vs. 564.4 ± 108.5 pA). This effect was completely blocked by the CB1 receptor antagonist AM251 (1 μM; Fig. 2C; n = 4) and was not associated with a change in the holding current or slope conductance (not shown). We observed a similar rightward shift in the IA inactivation curve with the anandamide analog

![Image](https://via.placeholder.com/150)
FIG. 2. CB1 receptor activation alters the voltage dependence of the \(I_A\) inactivation in a sex-dependent fashion. A: \(I_A\) evoked under baseline control conditions in hypothalamic neurons from female (top) and male (bottom) guinea pigs during the inactivation protocol. The \(I_A\) is observed as a transient outward current immediately following delivery of the test pulse (denoted by arrow). B: \(I_A\) evoked in the presence of WIN 55,212–2 (1 \(\mu\)M) in the same neurons using the same inactivation protocol as in A. C: composite inactivation curves for the \(I_A\) derived from recordings of female (top) and male (bottom) guinea pig hypothalamic neurons. The Boltzmann equation fits the curves to the corresponding data points. Symbols and accompanying vertical lines represent means \(\pm\) 1 SE of peak currents normalized to the \(I_{max}\) that were observed at the test pulse following a given prepulse. The horizontal dashed lines seen in both graphs represent \(I_{max} = 0.5 = V_{1/2}\). Top graph: 3 vertical dotted lines intersect the abscissa at different points; representing the \(V_{1/2}\) observed under baseline control conditions (center), in the presence of \(1 \mu\)M WIN 55,212–2 (right) and in the presence of WIN 55,212–2 and the CB1 receptor antagonist AM251 (left). Bottom graph: 2 vertical dotted lines intersect the abscissa; the one on the right represents the \(V_{1/2}\) observed under baseline control conditions, and the one on the left represents the \(V_{1/2}\) observed in the presence of \(1 \mu\)M WIN 55,212–2. Asterisk, the estimated \(V_{1/2}\) derived from hypothalamic neurons in the presence of WIN 55,212–2 that is significantly different \((P < 0.05; 1\text{-way ANOVA/LSD})\) than that observed under baseline control conditions and in the presence of the antagonist.

ACEA (control: \(-78.2 \pm 4.4 \text{ mV}\) vs. \(1 \mu\)M ACEA: \(-69.3 \pm 3.3 \text{ mV}\); \(n = 5\)). Antagonism of miniature synaptic currents with CGS 19755 (10 \(\mu\)M), NBQX (3 \(\mu\)M), and SR 95531 (10 \(\mu\)M), in conjunction with the blockade of synaptic transmission by TTX, did not alter this modulatory action of cannabinoids. By contrast, WIN 55,212–2 exerted no such shift in the inactivation curve derived from neurons in orchidectomized male guinea pigs (\(-76.8 \pm 3.5 \text{ vs. } -79.4 \pm 4.4 \text{ mV}\); Fig. 2) and also did not influence the \(I_{max}\) (811.5 \(\pm\) 154.1 vs. 807.9 \(\pm\) 183.8 pA).

Figure 3, A and B, show membrane current traces, also obtained during recordings in neurons from castrated female and male guinea pigs, used to derive the activation curves (Fig. 3C) from which we assessed the voltage dependence of the activation of this current. Contrary to the agonist-induced depolarizing shift observed for the inactivation curve in neurons from ovariectomized females (Fig. 2), WIN 55,212–2 was without effect on either the \(V_{1/2}\) or the \(I_{max}\) of the activation curve (\(-34.3 \pm 1.9 \text{ vs. } -30.3 \pm 1.7 \text{ mV}\); 804.0 \(\pm\) 144.7 vs. 664.5 \(\pm\) 121.6 pA; Fig. 3, B and C). In addition, the indices of activation of the transient outward current observed in neurons from orchidectomized male guinea pigs were likewise unaffected (\(-29.4 \pm 2.0 \text{ vs. } -26.7 \pm 3.4 \text{ mV}\); 879.5 \(\pm\) 285.3 vs. 905.2 \(\pm\) 305.9 pA; Fig. 3).

Taken together, these data indicate that POMC neurons express an \(I_A\) and that there is a sex difference in the positive modulation of this current by cannabinoids. These conclusions are based on the following observations: I) guinea pig ARC
neurons, including those immunopositive for β-endorphin and/or the Kv 4.2 channel subunit, express a transient outward current that is sensitive to antagonism by 4-AP and hRptx2 and 2) the CB1 receptor agonists WIN 55,212–2 and ACEA induce a rightward shift in the inactivation curve for this current in female but not male animals. This IA was prominently displayed in 40% (40 of 101) of ARC neurons, which is slightly above the 30% incidence that we previously reported for neurons in the guinea pig preoptic area (Wagner et al. 2000).

Essentially, the cannabinoid-induced rightward shift in the inactivation curve for the IA means that less of a hyperpolarizing stimulus is required to remove the ion channel from inactivation. Therefore more of the channels would be available at subthreshold membrane voltages to provide a transient outward current that would help offset subsequent depolarizing stimuli, and thereby lower neuronal firing rate. Indeed, the IA promotes membrane repolarization during an action potential and increases the interspike interval between action potentials, thereby reducing action potential duration and firing frequency (Rudy 1988). Future current-clamp studies will test the ability of cannabinoids to increase spike latency and to decrease spike duration and firing frequency, in response to positive, depolarizing test current pulses that immediately follow a series of hyperpolarizing prepulses that vary in magnitude. Furthermore, this cannabinoid-induced shift in the voltage dependence of the IA inactivation is consistent with that previously reported for cultured hippocampal neurons, a process that was attributed to a reduction in intracellular cAMP levels (Deadwyler et al. 1995).

A cannabinoid-induced increase in the IA and the corresponding decrease in the firing rate of POMC neurons would decrease the release of the anorexigenic peptides α-MSH, β-endorphin and CART co-expressed in these cells. This would account, in part, for the prominent hyperphagia elicited by cannabinoids (Harrold and Williams 2003; Horvath 2003). Moreover, the observed sex difference in the CB1 receptor coupling to the IA in cells integral to hypotalamic feeding circuitry provides some insight into the cellular determinant(s) that may ultimately lead to gender differences in feeding behavior. Indeed, the ability of centrally administered CB1 receptor agonists like CP 55,940 to stimulate consumption of highly palatable foodstuffs is sexually differentiated (Miller et al. 2004). Finally, the sex difference in CB1 receptor/IA coupling observed in the present study is also consistent with reported sex differences in cannabinoid-induced antinociception (Tseng and Craft 2001), locomotion (Tseng and Craft 2001; Wiley 2003) and postural syncope (Mathew et al. 2003).

In conclusion, these results reveal a sex difference in the cannabinoid modulation of the IA in ARC neurons, including POMC neurons. They also impart some insight into the mechanism(s) by which cannabinoids alter energy homeostasis.

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**References**


