Effects of opioids, cannabinoids, and vanilloids on body temperature

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1. ABSTRACT

Cannabinoid and opioid drugs produce marked changes in body temperature. Recent findings have extended our knowledge about the thermoregulatory effects of cannabinoids and opioids, particularly as related to delta opioid receptors, endogenous systems, and transient receptor potential (TRP) channels. Although delta opioid receptors were originally thought to play only a minor role in thermoregulation compared to mu and kappa opioid receptors, their activation has been shown to produce hypothermia in multiple species. Endogenous opioids and cannabinoids also regulate body temperature. Mu and kappa opioid receptors are thought to be in tonic balance, with mu and kappa receptor activation producing hyperthermia and hypothermia, respectively. A particularly intense research focus is TRP channels, where $TRPV_1$ channel activation produces hypothermia whereas $TRPA_1$ and $TRPM_8$ channel activation causes hyperthermia. The marked hyperthermia produced by $TRPV_1$ channel antagonists suggests these warm channels tonically control body temperature. A better understanding of the roles of cannabinoid, opioid, and TRP systems in thermoregulation may have broad clinical implications and provide insights into interactions among neurotransmitter systems involved in thermoregulation.

2. INTRODUCTION

Opioids and cannabinoids produce profound changes in body temperature that are highly dependent on species, strain, age, ambient temperature, dose, administration route, and degree of restraint of the animal. This literature has been extensively reviewed and will not be addressed here. The present chapter will focus on recent developments, including but not limited to the impact of other systems (e.g. neurotransmitter, neuropeptide, second messenger) on the thermoregulatory effects of cannabinoids and opioids and the emerging role of delta opioid receptors in thermoregulation. The chapter will also devote special attention to the potential roles of peripheral opioid receptors and sigma sites in body temperature regulation and review findings related to the roles of the endogenous cannabinoid and vanilloid systems on the tonic regulation of body temperature. Finally, we will briefly discuss novel findings regarding the impact of transient receptor potential (TRP) channels on thermoregulatory processes.

3. OPIOID EFFECTS ON BODY TEMPERATURE

3.1. Kappa opioid receptors and body temperature

Three types of opioid receptors - delta, mu, and kappa opioid - mediate the thermoregulatory effects of

opioids. Kappa opioid receptor activation produces hypothermia in rats and mice (1-4). Much of the early work investigating a role for kappa opioid receptors in thermoregulation was directed at the thermoregulatory effects of morphine. The administration of morphine to rats at doses of 4 to 15 mg/kg morphine produces robust hyperthermia, but progressively higher doses induce hypothermia (2). Experiments using selective opioid receptor agonists and antagonists reveal that mu opioid receptor activation is responsible for the hyperthermic response to morphine whereas kappa opioid receptor activation mediates the hypothermic effect of morphine (4-16). The hypothermic effects of kappa opioid receptor agonists have been thoroughly (6) but little is known about the mechanism of hypothermic action and the neurotransmitter systems involved. Recent studies suggest the hypothermic effect of kappa opioid receptor agonists is modulated by a number of transmitter and second messenger systems, including but not limited to glutamate, nitric oxide, calcium channels, serotonin (5-HT), nociceptin/orphanin FQ, pertussis toxin, cholera toxin, biogenic amines, neurotensin, and G-protein-gated potassium (GIRK) channels (*e.g.*, loperamide, baclofen, oxotremorine, and ethanol are examples of drugs that produce hypothermia that is dependent on the GIRK2 gene) (3, 15, 17-24). The hypothermia induced by U50,488H is enhanced by the selective serotonin reuptake inhibitor (SSRI) fluoxetine, thus demonstrating that a 5-HT reuptake block, and subsequent elevation in extracellular 5-HT levels, increases kappa opioid receptor-evoked hypothermia (15). U50,488H-induced hypothermia is also augmented by chlorpromazine, indicating that kappa-opioid-receptor-mediated hypothermia is sensitive to the actions of biogenic amines (25). Similarly, U50,488H in combination with neurotensin produced a dose-dependent and additive hypothermia.

The hypothermic response to kappa opioid receptor activation is markedly influenced by nitric oxide, a diverse second messenger produced in both the peripheral and central nervous systems (26-30). Thermoregulation and fever, as well as body temperature changes produced by drug exposure, are sensitive to the level of nitric oxide production. Studies have produced inconsistent results, however, with some reporting that nitric oxide displays antipyretic properties and participates in the production of hypothermia (29, 31-34) whereas others suggest nitric oxide formation contributes to fever generation (35-38). With regard to kappa opioid hypothermia in rats, pharmacological evidence indicates peripheral and central nitric oxide production may be required (3). The nonspecific nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) (50 mg/kg, s.c. or 1 mg/kg, i.c.v.) prevents the hypothermic effect produced by U50,488H (10 mg/kg, s.c.) (3). The efficacy of i.c.v. L-NAME indicates the hypothermic response is dependent on nitric oxide production in the brain, a finding that is in agreement with prior work showing that central nitric oxide production is necessary for the hypothermic actions of insulin and arginine vasopressin (33-34). It should be noted that the sites of action of L-NAME are distributed ubiquitously throughout the body, including the brown

adipose tissue, where they mediate heat production, and the vascular smooth muscle, where they mediate heat conservation (39-40). Thus, it is also possible that peripheral nitric oxide production participates in the hypothermia caused by kappa opioid receptor activation.

Many researchers in the field are now directing efforts toward identifying molecular targets which mediate the hypothermic response with the ultimate goal of determining whether a final common pathway is responsible for the production of hypothermia (24). One target of great interest is the G-protein-gated potassium (GIRK) channel. These channels are modulated by a variety of G-protein-coupled neurotransmitter receptors (GPCRs), including serotonergic (5-HT$_{1A}$), GABAergic (GABA$_B$), muscarinic (M$_2$), adenosine (A$_1$), opioid, adrenergic, and dopaminergic (D$_2$) receptors (41). Their primary functions are regulation of cellular excitability and action potential duration as well as maintenance of resting membrane potential. Neuronal cells predominantly express GIRK1, GIRK2, and GIRK3 but also express GIRK4 to a lesser extent (42). GIRK channel activation typically decreases neuronal firing rate. Pharmacological activation of the same GPCRs that are known to positively modulate GIRK channels also produces hypothermia in rodents (43-48). Novel experiments conducted in GIRK2 null mutant mice indicate activation of GIRK2-containing potassium channels plays a significant role in hypothermia induced by the activation of serotonergic (5-HT$_{1A}$), GABAergic (GABA$_B$), muscarinic (M$_2$), adenosine (A$_1$), and mu, delta, and kappa opioid receptors (24). In light of these results and documented evidence that GIRKs are final common effectors for many central neurotransmitter systems, GIRK2-containing channels may act as molecular hubs to control the hypothermic response to a variety of pharmacologically and structurally distinct drugs. In addition to GIRK channels, calcium channels are also thought to play a key role in kappa opioid hypothermia, as U-50,488H-indued hypothermia is mediated through PTX-sensitive transducer G-proteins (G(i/o)) coupled to L-type Ca^{2+} channels (21-22).

The development of tolerance to kappa opioid hypothermia is a particularly interesting phenomenon which is related directly to excessive glutamate signaling. U50,488H, when administered repeatedly to rodents, loses its hypothermic efficacy (49 -51). The mechanism of tolerance is not entirely clear, but increased glutamate signaling plays a critical role (52-54). The extent to which changes in glutamate activity contribute to opioid tolerance is dependent on the class of opioid agonist and biological endpoint which is investigated. For example, development and expression of the analgesic tolerance produced by repeated morphine administration is largely due to increased NMDA receptor activation (52, 55-57). A role for NMDA receptors in tolerance to the analgesic effect of kappa opioid receptor agonists has not been consistently demonstrated, with some studies demonstrating that NMDA antagonists block tolerance and others reporting no effect (53, 58-61). Interestingly, NMDA receptor antagonism does not alter the hypothermic tolerance to NMDA receptors (59, 62).

Another element of the glutamate system, glutamate transporter subtype 1 (GLT-1), does impact kappa opioid hypothermic tolerance. Recent experiments demonstrate that GLT-1 transporter activation prevents the hypothermic tolerance normally produced by U50, 488H (51). In those experiments, a single injection of U-50,488H (20 mg/kg, s.c.) induced significant hypothermia in rats (51). Tolerance to the hypothermic effect of U50,488H was induced by injecting rats with U50,488H (20 mg/kg) twice daily for 7 days. When rats treated repeatedly with U50,588H were also exposed to ceftriaxone (200 mg/kg, i.p.), a beta-lactam antibiotic and GLT-1 transporter activator, the acute hypothermic response to U50,488H was not affected but the development of tolerance to U50,488H-induced hypothermia was completely prevented. The injection of dl-threo-β-benzyloxyaspartic acid (TBOA) (0.2 micromoles, i.c.v.), a non-transportable inhibitor of glutamate uptake, into the lateral ventricle completely blocks the ceftriaxone effect, indicating the mechanism of ceftriaxone action was increased cellular glutamate uptake. This finding identifies a functional interaction between morphine and beta-lactam antibiotics, one of the world's most widely prescribed drug classes. Other studies have also demonstrated a functional interaction between the opioid system and beta-lactam antibiotics, which are the only practical pharmaceuticals known to enhance glutamate uptake through GLT-1 transporter activation (63-71). Increased cellular glutamate uptake would be expected to reduce extracellular glutamate and produce an overall reduction in glutamate transmission which is normally produced by repeated exposure to a kappa opioid agonist. Therefore, the explanation most consistent with the current literature is that development of hypothermic tolerance to U50,488H is highly dependent on an increase in glutamate transmission, and that GLT-1 transporter activation by a beta-lactam antibiotic helps to preserve the hypothermic efficacy of U50,488H by abolishing the glutamate-dependent component of tolerance.

3.2. Delta opioid receptors and body temperature

Early work suggested only a minor role for delta opioid receptors in thermoregulation, but recent studies using selective and more pharmaceutically-friendly non-peptide delta opioid agonists suggest a more significant role for these receptors (5, 23, 48, 72, 73, 74, 75). Low doses of deltorphin-II, a delta$_2$ agonist, injected centrally produce hypothermia whereas higher doses of the agonist produce a biphasic response of hypothermia followed by hyperthermia (23). The hypothermic effect of deltorphin-II is antagonized by the delta opioid receptor antagonist naltrindole, thus indicating that delta opioid receptor activation was the mechanism of action of deltorphin-II.

Early studies attempting to link delta opioid receptors and thermoregulation relied exclusively on the use of peptidergic agonists, which had to be administered centrally to avoid inactivation by peripheral peptidase action. The latest studies rely more upon non-peptide delta opioid agonists. These compounds were developed commercially because of their improved side effect profile (i.e., less respiratory depression and constipation) relative to morphine and mu opioid agonists. Because non-peptide opioid agonists are also resistant to peripheral peptidase inactivation, they are produce quantifiable central effects following systemic administration. The non-peptide delta opioid agonist that has received the most attention is (+)-4-[(*aR*)-*a*-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80), a diphenylmethylpiperazine derivative. SNC-80 is highly selective for delta opioid receptors over mu and kappa opioid receptors, and several observations suggest that the pharmacological profile of SNC-80 differs from that of peptide agonists, such as DPDPE (delta$_1$), DADLE (mu/delta), and deltorphin-II (delta$_2$) (76-80). Although SNC-80 is 1000-fold less potent than deltorphin-II in stimulating locomotor activity in rats, the lower potency does not correlate with differences in brain penetration for SNC-80 and deltorphin-II or with the superior binding affinity and efficacy of SNC-80 at delta opioid receptors in rat brain homogenates (81). In a series of elegant experiments, Baker and Meert (2002) used delta opioid receptor antagonists to characterize the hypothermic effects of SNC-80 in mice (48). Their work revealed that SNC-80 induced dose-dependent hypothermia that was blocked by naltrindole; decreased by low doses of naltriben, a delta$_2$ antagonist; and not affected by BNTX, a delta$_1$ antagonist. Work by our own laboratory confirmed the robust hypothermic effects of SNC-80 in rats and subsequently used the versatile non-peptide agonist to identify the receptor phenotype, delta$_1$ or delta$_2$, which mediated the hypothermia. Experiments indicated that the systemic administration of SNC-80 induced dose-dependent hypothermia that was rapid in onset and persistent (74). The most interesting finding was that the hypothermic effect of SNC-80 was completely blocked by pre-treatment with naltriben but not BNTX. The ineffectiveness of BNTX, coupled with the efficacy of naltriben, suggests SNC-80 activated delta$_2$ opioid receptors to induce hypothermia. This work also revealed that SNC-80 acted in the brain to produce hypothermia because administration of naltriben into the ventricles completely blocked SNC-80-evoked hypothermia.

The specific brain site of action of SNC-80 is unknown. A likely candidate is the preoptic anterior hypothalamus (POAH), a major central site of thermoregulation (82). Delta opioid receptor binding has been detected in the POAH but is sparse compared to kappa and mu opioid receptor labeling (83-84). Central delta opioid receptors produce a significant hypothermic response in rats and mice upon activation. Experiments conducted in rats demonstrated that the hypothermic response to SNC-80 is not affected by pretreatment with the peripherally restricted opioid antagonist methlynaltrexone, thus indicating the hypothermic mechanism of action of SNC-80 is entirely independent of peripheral opioid receptor activation (74). A lack of involvement of peripheral opioid receptors in rats contrasts with results in mice, where SNC-80 induces hypothermia which is partially dependent on peripheral opioid receptor activation (48). In addition, loperamide, a peripherally restricted opioid agonist, produces hypothermia in mice that is attenuated by both naltrindole and naltriben (48). Still another study indicates the peripherally restricted

kappa opioid agonist (RS)-[3-[1-[[(3,4-Dichlorophenyl)acetyl]methylamino]-2-(1-pyrrolidinyl)ethyl]phenoxy]acetic acid hydrochloride (ICI 204448) (2.5, 5, and 10 mg/kg, s.c.) produces hypothermia in rats exposed to cold ambient temperatures (85). One surprising finding is that the direct injection of deltorphin-II into the POAH produces hyperthermia, instead of hypothermia (86) . This outcome underscores how the thermoregulatory effects of delta opioid agonists, akin to agonists at kappa and mu opioid receptors, vary widely with the administration route and indicates delta opioid agonists act at both hypothalamic and extra-hypothalamic $delta_2$ receptors to produce changes in body temperature (87).

A significant role for $delta_2$ opioid receptors in thermoregulation has been demonstrated, but the impact of $delta_1$ opioid receptors is less clear. Experiments conducted in rats reveal that pretreatment with BNTX does not affect the hypothermic response to SNC-80, thus indicating that $delta_1$ opioid receptors are not a primary mediator of the hypothermia produced by SNC-80 in rats (74). BNTX also failed to reverse SNC-80-induced hypothermia in mice when it was tested in a different experimental design, one in which the $delta_1$ opioid receptor antagonist was administered 15 min following SNC-80 administration (48). It should be noted, however, that a role for $delta_1$ opioid receptors in other aspects of thermoregulation has been demonstrated (4, 5, 10). Doses of BNTX that were ineffective versus SNC-80 antagonized morphine-evoked hypothermia, suggesting that $delta_1$ receptors help to maintain the hypothermia initiated by mu receptor activation (48). Thus, the role of $delta_1$ receptors may change during the progression of opioid-evoked effects, including hypothermia.

Limited information is available regarding endogenous systems which modulate delta opioid receptor-induced hypothermia, but nitric oxide and serotonin are clearly involved. Experiments indicate the hypothermic response to delta opioid, as well as kappa opioid (3), receptor agonists require nitric oxide production (88). One potential mechanism is that the hypothermic response is linked directly to increased nitric oxide production (89). A functional link between nitric oxide and delta receptors exists in opioid-induced gastric protection (92), peripheral antinociception (90-91), and immunological modulation (89). Joint evidence that inhibition of nitric oxide synthase inhibits the hypothermia produced by both kappa and delta opioid receptor activation (3, 88) suggests that nitric oxide production might represent a final common pathway for the production of hypothermia, particularly that hypothermia mediated by opioid receptors. Another primary modulator of delta opioid receptor-induced hypothermia is serotonin. Pretreatment of rats with the $5-HT_{1A}$ antagonist WAY 100635 (1 mg/kg, s.c.) attenuated the hypothermic effect of SNC-80 (35 mg/kg, i.p.). Administration of SNC-80 (35 mg/kg, i.p.) with non-hypothermic doses (2.5, 5 and 10 mg/kg, i.p.) of the selective serotonin reuptake inhibitor fluoxetine resulted in an enhancement of the hypothermic response (75). These data indicate that delta opioid receptor-induced hypothermia requires active $5-HT_{1A}$

receptors and is highly sensitive to the rate of 5-HT reuptake. It is known that $5-HT_{1A}$ receptor activation causes hypothermia in rats and mice because WAY 100635 abolishes hypothermia produced by 5-HT agonists (93-95). Because WAY 100635 also blocks a significant proportion of SNC-80-induced hypothermia, the hypothermic response to delta opioid receptor activation may produce downstream activation of $5-HT_{1A}$ transmission, possibly through elevating extracellular 5-HT levels, which then contributes to the overall hypothermic effect of SNC-80. *In vivo* microdialysis data obtained from rats indicate that the level of extracellular 5-HT in the brains of conscious rats is increased by delta opioid receptor activation (96) and 5-HT reuptake block by fluoxetine (97, 98).

3.3. Mu opioid receptors and body temperature
In contrast to the hypothermic effect produced by kappa and delta opioid receptor activation, mu opioid receptor activation results in hyperthermia (1, 2, 4, 6, 99). The majority of early work investigating mu opioid receptor effects on body temperature used morphine. In experiments conducted in rats, morphine administered systemically at doses of 5-16 mg/kg produced significant hyperthermia whereas higher doses of morphine produced a biphasic response comprised of a brief hyperthermia followed by a pronounced and enduring hypothermia (1, 2, 6). Pretreatment of rats with naltrexone completely blocks the hyperthermic response to morphine, indicating that low doses of morphine produce hyperthermia through activation of mu opioid receptors (2, 4, 12, 99). The importance of mu opioid receptors in the production of hyperthermia was further validated by experiments showing that the mu opioid agonists DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) and PLO17 ([N-MePhe3,D-Pro4]morphiceptin), injected i.c.v. or directly into the POAH, induce hyperthermia that is blocked by the selective mu opioid receptor antagonists CTAP and beta-funaltrexamine (4-6, 99-101).

More recent studies have used knockout mice to further investigate the role of mu opioid receptors in thermoregulation (102). Results from these experiments reveal that genetic deletion of the mu opioid receptor does not impact basal body temperature or circadian body temperature rhythm because both wild type and mu opioid receptor knockout mice displayed similar baseline body temperature and diurnal/nocturnal fluctuations. However, the hyperthermia produced by a low dose of morphine (1 mg/kg) was significantly greater in wildtype mice than in mu opioid receptor knockout mice (102). Genetic deletion of mu opioid receptor also significantly inhibited the hyperthermia produced by DAMGO.

Although the effects of morphine on thermoregulation are not entirely the same in rats and mice, the trends are similar in both species. For example, low doses of morphine produce hyperthermia and higher doses produce hypothermia (48). The hyperthermia results from mu opioid receptor activation, but the mechanism underlying the hypothermia remains unresolved. Experiments conducted in rats reveal that antisense oligonucleotides directed against kappa opioid receptors

significantly attenuate the hypothermia induced by high morphine doses (12, 16). However, neither morphine- nor fentanyl-induced hypothermia in mice is affected by pretreatment with the selective kappa opioid receptor antagonist nor-binaltorphimine (48). The non-selective opioid receptor antagonist naloxone does antagonize morphine- and fentanyl-induced hypothermia, indicating an opioid receptor mechanism, but evidence that the selective mu opioid receptor antagonist naloxonazine produces similar effects suggests that the effects of naloxone cannot be entirely attributed to antagonism. It is interesting to note that kappa opioid receptor antagonism cannot reverse a pre-existing hypothermia induced by morphine or fentanyl. If it is accepted that mu opioid receptors mediate hyperthermia and kappa opioid receptors mediate hypothermia (16), then it is likely that the modulatory roles of mu and kappa opioid receptors change during prolonged hypothermia. These changes may be part of a dynamic balance of opioid receptor occupation and the effect on receptor function of body temperature itself. The success of mu opioid, but not kappa opioid, receptor antagonism to reverse morphine- and fentanyl-induced hypothermia may also result from sudden receptor blockade producing rebound effects and upsetting this balance (48). Systemic mu opioid receptor blockade following morphine exposure produces a withdrawal-like stress response (103), an effect which may alter opioid regulation of body temperature. Naloxonazine, which blocks morphine- and fentanyl-evoked hypothermia as discussed above, binds selectively to mu_1 opioid receptors, suggesting that these receptors are primarily responsible for the mu opioid effect. One final point worth mentioning is that doses of morphine which produce hyperthermia in unrestrained rats produce hypothermia in rats that are restrained (104). The exact mechanism underlying the influence of restraint stress is not clear, but it may be related to disruption of postural mechanisms which normally reduce heat loss (105) or interference with GABA transmission (106).

A number of endogenous neurotransmitters modulate the thermoregulatory responses produced by mu opioid receptor activation. One recently identified messenger which impacts mu opioid responses is agmatine, a biogenic amine synthesized from arginine by the enzyme arginine decarboxylase and hydrolyzed by the enzyme agmatinase (107). Agmatine is widely expressed throughout the brain, with the greatest concentrations detected in the midbrain (e.g. periaqueductal gray, locus coeruleus), cerebral cortex, hippocampus, amygdala, thalamus, striatum, and hypothalamic nuclei (108). Agmatine has been proposed to act as a neurotransmitter, and its brain concentrations correlate with that of other established neurotransmitters (108, 109, 110). Agmatine interacts with multiple endogenous targets, including imidazoline sites, α_2-adrenoceptors, nicotinic receptors, 5-HT_3 receptors, NMDA receptors, and nitric oxide synthase (111-116). Changes in body temperature are dependent on the level of endogenous agmatine because exogenous agmatine administration blocks the hyperthermia produced by a bacterial endotoxoin or restrain stress (117). It was subsequently shown that agmatine administration (50 mg/kg) also inhibited a significant proportion of morphine-

induced hyperthermia in rats (17). Pretreatment of rats with idazoxan (5 mg/kg, i.p.), a mixed imidazoline/alpha$_2$-adrenoeceptor antagonist, completely abolished the ability of agmatine to reduce morphine-evoked hyperthermia. In contrast, pretreatment with yohimbine, a selective alpha$_2$-adrenoeceptor antagonist, was ineffective. The ability of idazoxan to antagonize the attenuation of morphine-evoked hyperthermia by agmatine, coupled with the ineffectiveness of yohimbine, suggests imidazoline site activation mediated the effects of agmatine. Although agmatine efficacy versus morphine-induced hyperthermia appears to be highly dependent on imidazoline site activation, downstream increases in NMDA receptor activity or nitric oxide signaling cannot be excluded from the mechanism. The hyperthermic effect of morphine is reduced by pharmacological antagonism of NMDA receptors, indicating that active NMDA receptors are required for the response (99). Agmatine acts as a competitive antagonist at NMDA receptors, and the systemic administration of agmatine at doses which block morphine-evoked hyperthermia also inhibit seizure-induced extracellular glutamate levels in frontal cortex of rats (118).

Mu opioid receptors within the POAH have been found to be involved in the fever induced by interleukin-6 (IL-6) (3), as the pretreatment with the selective mu-opioid receptor antagonist, cyclic D-phe-Cys-Try-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP), significantly blocked the IL-6-induced fever. Mu opioid receptors are also implicated in the pathogenesis of the bacterial LPS fever. Pretreatment with the selective mu-opioid antagonist (CTAP), into the preoptic anterior hypothalamus, reduced the fever induced by lipopolysaccharide (LPS) (38). Moreover, LPS failed to produce fever in mice lacking the mu opioid receptor (119). The i.c.v. injection of LPS produced a dose-dependent, significant elevation in body temperature in WT (102). One study has suggested that transfer of LPS from the brain into the periphery in significant amounts is what accounts for the observed effects of i.c.v. LPS (120). It is highly unlikely that the small amount of LPS (100 ng) injected via the i.c.v. route evoked fever through its leakage into the systemic compartment, because the same amount of LPS, when injected peripherally, did not evoke fever, even at a dose 10 times higher (102).

4. SIGMA SITES AND BODY TEMPERATURE

Sigma receptors were originally proposed based upon the actions of the benzomorphan opiate, (±) SKF 10,047 (121). The observation that (±) SKF 10,047 and phencyclidine (PCP) produced similar behavioral responses in several species, and that benzomorphans inhibit the binding of [^{3}H]PCP, led to the proposition that sigma ligands exerted their effects by acting at a singular sigma/PCP site (122). Today, sites defined as sigma are not opioid or PCP (123). Sigma sites have gained heightened acceptance as unique binding sites with a specific pattern of drug selectivity and distinctive distribution throughout the body (124). Two subtypes of sigma sites are now known to exist, and they can be discriminated by their enantioselectivity for benzomorphans. Sigma$_1$ sites are sensitive to (+)-opiates and have high affinity for

haloperidol and (+)-pentazocine (125). Sigma$_2$ sites are more sensitive to (−)-opiates than sigma$_1$ sites, but the interaction is naloxone insensitive, distinguishing it from a classical opiate interaction (126). Endogenous ligands for these sites appear to exist, and the existence of a tonically active sigma system has been proposed, especially in relation to analgesia (127).

Hypothermia is produced by ligands that activate sigma sites, but delineation of a specific functional role for sigma sites in the production of the hypothermia has been slowed by a lack of selective ligands for the sites (128, 129, 130, 131, 132). Further insight into the role of sigma sites in the production of hypothermia has been provided through the use of 1,3,-di-o-tolylguanidine (DTG), which displays a high affinity for sigma receptors that is several hundredfold greater than for PCP and NMDA receptors (133-135). DTG produces behaviors that are dissimilar to PCP-evoked behaviors and physiologic effects that are inconsistent with a PCP or NMDA receptor locus (136-138). In thermoregulation studies, systemic administration of DTG induces hypothermia in rats at doses that do not produce gross alterations in behavior (130, 139, 140). The hypothermia is rapid in onset, peaks at about 60-min post-administration, and endures for 3-4 hours.

The hypothermic effect of DTG is prevented by pretreatment with the selective sigma site antagonist N-[2-(3,4-dichlorophenyl) ethyl]-N-methyl-2-(dimethylamino) ethylamine (BD 1047) (140). BD 1047 has a high affinity for sigma$_1$ and sigma$_2$ sites (141) and is several hundredfold more selective for sigma binding sites versus opioid, PCP, muscarinic, dopamine or serotonin sites (141, 142). Unlike earlier purported sigma site antagonists such as rimcazole and BMY 14802, BD 1047 does not produce *in vivo* effects by interacting with other receptor systems and displays much greater affinity and selectivity for sigma sites (142, 143). This is evident from the observation that heroic doses (greater than 25 mg/kg) of rimcazole are required for sigma site inhibition (130). The different pharmacological profiles of BD 1047 and rimcazole may be the reason for their dissimilar effects versus DTG-evoked hypothermia, with BD 1047 producing inhibition and rimcazole lacking any significant effect (128, 130). The availability and use of more selective ligands such as BD 1047 suggests that sigma sites do contribute to the production of hypothermia, but very little beyond this is known regarding the mechanism of action. Sigma sites do not tonically regulate body temperature because the injection of BD 1047 by itself did not alter body temperature.

5. CANNABINOIDS AND BODY TEMPERATURE

5. 1. Cannabinoid ligands and receptors

Ever since the identification of tetrahydrocannabinol (delta^9-THC) as the primary psychoactive constituent of marijuana in 1967 (144), a number of cannabinoid agonists and antagonists such as the aminoalkylindole (R)-(+)-[2,3-Dihydro-5-methyl-3-(4 morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone (WIN 55212-2); 2-

[(1S,2R,5S)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP 55,940); and (6aR,10aR)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol (HU-210), have been now been designed, synthesized, and tested in animals (145-149). Cannabinoids produce an array of pharmacological effects in animals, including but not limited to hypothermia, sedation, catalepsy, analgesia, motor impairment, and cognitive dysfunction (150).

The most exciting development in cannabinoid research over the past two decades is the discovery of an endogenous cannabinoid system. This system is comprised of three components: (1) cannabinoid receptors, called cannabinoid CB$_1$ and cannabinoid CB$_2$, which mediate the pharmacological actions of cannabinoid agonists; (2) endogenously produced cannabinoid-like substances which mimic the actions of exogenously administered cannabinoid agonists; and (3) hydrolytic enzymes which inactivate endogenous cannabinoids (151, 152). Both CB$_1$ and CB$_2$ receptors inhibit adenylyl cylcase activity and mitogen-activated protein kinases (MAPK) through coupling to inhibitory G-proteins (Gi/o) (153). The early belief that CB$_1$ and CB$_2$ receptors were expressed almost exclusively in the brain and periphery, respectively, has changed now changed. CB$_1$ receptors, in addition to being expressed at high levels in the brain, are also expressed in peripheral organs such as the liver (154). The existence of CB$_2$ receptors in the brain was initially discounted except for inflammatory conditions. It is now known that CB$_2$ receptors, in addition to being located on peripheral mast cells, are expressed in the brain under both normal and inflammatory conditions(155, 156, 157). Endogenous cannabinoids include anandamide (N-arachidonoyl-ethanloamine), 2-arachidonoyl-glycerol (2-AG), 2-arachidonyl-glycerol ether (noladin ether), N-arachidonoyl-dopamine (NADA), and virodhamine (152, 158-161). Anandamide and 2-AG display high affinity for CB$_1$ and CB$_2$ receptors, but the pharmacological activities of the more recently proposed endogenous cannabinoids is yet to be determined. In addition to the endogenous substances which activate cannabinoid receptors, the first endogenous CB$_1$ antagonist, hemopressin, was discovered recently (162). Degradation of endogenous cannabinoids which leads to inactivation of their biological effects is mediated by two different classes of enzymes called fatty acid amide hydrolase (FAAH) and monacylglycerol lipase (MAGL) (163, 164). Discovery of the endogenous cannabinoid system has prompted development and characterization of pharmacological probes to better investigate cannabinoid pharmacology. These probes include endogenous cannabinoid uptake inhibitors (e.g. AM404, VDM11, OMDM-1); FAAH inhibitors; MAGL inhibitors; anandamide analogues (e.g. methanandamide) that are more resistant to enzymatic hydrolysis than anandamide; mixed CB$_1$/CB$_2$ agonists (e.g. WIN 55212-2, HU-210); selective CB$_1$ antagonists/inverse agonists (e.g. SR 141716A, AM251); neutral CB$_1$ antagonists; and selective CB$_2$ antagonists/inverse agonists (e.g. SR 144528) (165).

Cannabinoid receptors are not limited to CB$_1$ and CB$_2$. The orphan G-protein coupled receptor (GPR55) has

attracted much attention as another member of the cannabinoid family, potentially explaining physiological effects that are not mediated by CB_1 and CB_2 receptors (166, 167,). *In vivo* pharmacological evidence suggests that development of antagonists targeting the novel receptor may result in medications to treat osteoporosis, inflammation, and neuropathic pain (168,169). At this point, however, at least to our knowledge, there is no published evidence that GPR55 receptor activation or antagonism produces physiologically significant changes in body temperature. It is worth noting, nevertheless, that GPR55 receptor expression has been detected in subdivisions of the hypothalamus that regulate body temperature, although the highest levels of expression are found in the hippocampus and striatum (171). The localization of GPR55 receptors in thermoregulatory centers, as well as their involvement in the inflammatory response, does raise the possibility that they may play a role in thermoregulation, perhaps at it relates to the febrile response.

5.2. Cannabinoid agonists produce hypothermia

Delta^9-THC administration induces profound hypothermia in animals (171, 172, 173). The hypothermia produced by Delta^9-THC is accompanied by a reduction in oxygen consumption, which indicates reduced heat production, as opposed to increased heat loss, is primarily responsible for the hypothermic response (175). The hypothermic effect of delta^9-THC is mimicked by cannabinoid agonists. A wide variety of species, including rats, mice, and primates, display hypothermia following acute administration of a cannabinoid agonist (148, 176-179). The hypothermic response to WIN 55212-2 has been particularly well characterized. Following systemic administration to rats and mice, WIN 55212-2 (1, 2.5, 5, or 10 mg/kg) produces a hypothermia that is rapid in onset, persistent for 3-4 hours, dose-dependent, and completely prevented by CB_1 receptor antagonism (75, 148, 179, 180, 181). Cannabinoid agonists do not evoke hypothermia in mice lacking CB_1 receptors, a finding which underscores the widely held belief that CB_1 receptor activation is the primary mechanism by which cannabinoid agonists decrease body temperature (182, 183). The hypothermic effect of WIN 55212-2 and other cannabinoids is not affected by CB_2 receptor antagonists, a finding that is entirely consistent with the tenet that CB_2 receptors play little, if any, role in the cannabinoid-induced hypothermia (178, 184-186).

Multiple brain sites contribute to the hypothermic effect produced by systemically administered cannabinoid agonists. One region that plays a critical role is the POAH (16). The POAH contains a large population of thermosensitive neurons which receive and integrate information from central and peripheral sensors and then transmit the modulating signal to thermoregulatory effectors that maintain body temperature around a set-point temperature. A consistent finding across multiple research groups is that POAH activation is absolutely required for cannabinoid agonists to produce hypothermia. The most obvious evidence is that a rapid and dose-dependent hypothermia is detected following the direct injection of

cannabinoid agonists into the POAH. The hypothermic response is independent of the chemical structure of the cannabinoid because the effect is produced by three structurally different cannabinoids - delta^9-THC, WIN 55212-2 , and HU-210 (175,187, 188). The hypothermia produced following the direct injection of WIN 55212-2 into the POAH is prevented by the systemic administration of the selective CB_1 antagonist SR 141716A (179). The effectiveness of SR 141716A indicates CB_1 receptors in the POAH are responsible for the effect, an interpretation supported by binding and immunohistochemical data which indicate that CB_1 receptor density in the POAH is among the highest of any brain region (189-193).

An even closer inspection of how cannabinoid-induced hypothermia varies with administration route provides clues about the anatomic sites which mediate cannabinoid-induced hypothermia. Both systemic and intra-POAH administration of WIN 55212-2 produces hypothermia (179). A notable difference between the administration routes is the duration of hypothermia. Systemically administered WIN 55212-2 produces hypothermia that endures for 3-4 hours whereas the intra-POAH injection of WIN 55212-2 induced hypothermia which persists for only about 60 min. Another difference between injection routes is the magnitude of the hypothermia, which is much greater following systemic administration. One final feature of the hypothermia that is sensitive to administration route is the time point at which maximal hypothermia is detected. Systemic injection of WIN 55212-2 produced maximal hypothermia 60-90 min post-administration whereas intra-POAH WIN 55212 produces peak hypothermia 30 min post-injection (179). Evidence that three aspects (duration, magnitude, maximal effect) of WIN 55212-2-induced hypothermia are sensitive to administration route indicates that other brain regions, in addition to the POAH, contribute to the hypothermia. One possibility is that different brain regions contribute to different stages of the hypothermic response. For example, CB_1 receptor activation in the POAH may be the primary trigger for initiating the hypothermic response whereas recruitment of extra-hypothalamic areas may underlie maintenance of the hypothermia. A related question is whether or not CB_1 receptor-containing neurons in the POAH are intrinsic or extrinsic to the region. CB_1 receptors are densely expressed in extrahypothalamic sites where cannabinoid systems produce hypothermia and interact with been neurotransmitter systems which regulate body temperature (175, 189,194, 195). The most likely conclusion is that cannabinoid-induced hypothermia is dependent on processes in hypothalamic and extrahypothalamic substrates.

5.3. Endogenous cannabinoid and vanilloid effects on body temperature

Unlike the opioid system, where mu and kappa opioid receptors are in tonic balance and mediate hyperthermia and hypothermia, respectively (16), a role for the endogenous cannabinoid system in the tonic regulation of body temperature has not been clearly defined. It is a consistent finding across different research groups that selective CB_1 or CB_2 receptor antagonists, even when

administered at high doses, do not produce significant changes in body temperature in rodents (149, 179, 184, 185). These findings are in direct contrast to the effects of opioid receptor antagonists and transient receptor potential channel (TRP) antagonists (i.e., $TRPV_1$), both of which produce changes in body temperature across a wide variety of species, including humans (196). The ineffectiveness of cannabinoid antagonists does not support a critical role for CB_1 or CB_2 receptors in the tonic regulation of body temperature regulation, in spite of evidence that several other biological endpoints, such as inflammatory hyperalgesia and antinociception, are impacted by an endocannabinoid tone (198, 199). Based on the widespread belief that CB_2 receptors have little, in any, role in thermoregulation, it is not unexpected that CB_2 receptor antagonism does not alter basal body temperature. It is more challenging to explain the ineffectiveness of CB_1 receptor antagonists considering the profound hypothermia that exogenously administered cannabinoid agonists produce through CB_1 receptor activation (149, 179). The majority of thermoregulatory studies have used cannabinoid receptor antagonists (e.g. SR 141716A and AM 251) which also possess inverse agonist properties. It is possible that detection of tonic body temperature regulation by CB_1 receptors was masked by the inverse agonist properties of these antagonists or by the sensitivity of the body temperature assay itself. Experiments with neutral, selective CB_1 receptor antagonists (e.g. AM 4113) which lack inverse agonist properties may be useful in addressing this possibility. Such neutral antagonists may be more useful than inverse agonists as pharmacological tools because they would produce effects only in the presence of elevated endocannabinoid levels (200, 201).

Cannabinoid receptor antagonists are not the only pharmacological tools used to investigate endocannabinoid effects on body temperature. Compounds that block endocannabinoid uptake and hydrolysis are equally attractive agents (202-204). One of the earliest examples of such a compound is AM-404 [N-(4-hydroxyphenyl)-arachidonylethanolamide], which blocks endogenous cannabinoid transport across cell membranes and enhances CB_1 receptor-mediated effects by increasing the extracellular concentration of endocannabinoids (205-207). Despite the impact of AM 404 on endocannabinoid signaling, it is now apparent that the biological effects produced by this pharmacologically rich agent are not exclusively confined to the endocannabinoid system. Sites of action of AM-404 include $TRPV_1$ receptors, calcium channels, sodium channels, cannabinoid receptors, and a novel N-acyl fatty acid-sensitive receptor (208- 214). Body temperature experiments reveal that AM 404 (1, 5, 10 and 20 mg/kg, i.p.) produces robust hypothermia in rats following peripheral administration. The hypothermia is completely abolished by pretreatment with two structurally distinct $TRPV_1$ receptor antagonists, capsazepine (30 mg/kg, i.p.) and SB 366791 (2 mg/kg, i.p.). Pretreatment with SR 141716A or a fatty acid amide hydrolase (FAAH) inhibitor, AA-5-HT, does not affect AM 404-induced hypothermia (215). Those results indicate AM 404-induced hypothermia is dependent on $TRPV_1$ receptor activation but not cannabinoid CB_1 receptor activation. A $TRPV_1$ receptor

mechanism of action for AM 404 is supported by evidences that AM 404 acts as a full agonist at both rat and human recombinant $TRPV_1$ receptors (216, 217) and that $TRPV_1$ receptor activation by capsaicin induces pronounced hypothermia in rodents (215, 218-220).

An exact mechanism is difficult to identify due to the pharmacological diversity of AM 404, but the most conceivable explanation is that AM-404 acts through a capsaicin-like process to produce hypothermia by directly activating $TRPV_1$ receptors. The same two antagonists, capsazepine and SB 366791, which block AM 404-induced hypothermia also inhibit capsaicin-evoked hypothermia (221, 202). It cannot be completely discounted that AM 404 acts as an indirect $TRPV_1$ receptor agonist by preventing anandamide uptake and increasing the extracellular concentration of anandamide, but this mechanism is unlikely because: (1) the binding site for $TRPV_1$ ligands is intracellular (223), indicating that an uptake block produced by AM 404 would lead to an increase in anandamide levels on side of the membrane (extracellular face) opposite to that of the $TRPV_1$ binding site (intracellular); (2) inhibition of the anandamide plasma membrane transporter prevents, rather than enhances, anandamide efficacy at $TRPV_1$ receptors (223); and (3) SR 141716A prevents anandamide-induced hypothermia in rats but is ineffective versus AM 404-induced hypothermia, suggesting the two compounds (anandamide and AM 404) produce hypothermia through mechanisms which are not entirely the same (24, 215).

Although anandamide can activate both cannabinoid and $TRPV_1$ receptors, its hypothermic effect appears to be mediated by CB_1 receptor activation (24, 215). This conclusion is based on evidence that anandamide (20 mg/kg, i.p.) produces hypothermia in rats which is abolished by pretreatment with SR 141716A (5 mg/kg, i.p.) but not by pretreatment with SB 366791 (2 mg/kg, i.p.) (215). Because higher anandamide concentrations are required to activate $TRPV_1$ receptors compared to CB_1 receptors (208), it is possible that anandamide-induced hypothermia would display sensitivity to $TRPV_1$ receptor blockade in a different experimental paradigm, such as one in which higher systemic doses of anandamide (e.g. 40 mg/kg) were tested or one in which anandamide was administered in combination with a FAAH inhibitor to prolong its half-life and preserve its biological activity. Nonetheless, evidence that the hypothermic effect of anandamide is mediated by CB_1 receptor activation and that the hypothermic effect of AM 404 is mediated by $TRPV_1$ receptor activation suggests the two agents produce hypothermia by different mechanisms of action.

$TRPV_1$ receptors have garnered much recent attention for their key role in tonic body temperature regulation (196). Unlike CB_1 receptor antagonists (146, 179), $TRPV_1$ receptor antagonists produce hyperthermia in multiple species (rats, dogs, and monkeys), including humans, humans following their systemic administration (196, 197, 224). These findings indicate $TRPV_1$ function in thermoregulation is conserved from rodents to primates.

Peripheral restriction of the $TRPV_1$ antagonists did not eliminate their ability to produce hyperthermia, thus indicating that their site of action is predominantly outside of the blood-brain barrier. $TRPV_1$ antagonists that are ineffective against proton activation also caused hyperthermia, indicating that blocking capsaicin and heat activation of $TRPV_1$ is sufficient to produce hyperthermia. These novel findings have led to the widespread belief that body temperature maintenance is the primary function of $TRPV_1$ receptors (196).

The mechanism of tonic $TRPV_1$ receptor activation is unclear, but enhanced phosphorylation of certain intracellular residues on the $TRPV_1$ receptor produced by the combined actions of multiple endogenous $TRPV_1$ agonists, including oleoyldopamine, NADA, and low pH may play a role (225). Other transient receptor potential (TRP) channels, such as $TRPA_1$, $TRPM_8$, $TRPV_3$, and $TRPV_4$, may also tonically regulate body temperature. The $TRPA_1$ and $TRPM_8$ agonist icilin produces dose-dependent hyperthermia in rats following systemic administration (226). The hyperthermia produced following $TRPA_1$/$TRPM_8$ activation is directly opposite to the hypothermia produced following $TRPV_1$ receptor activation by capsaicin (222, 218, 219), findings that are consistent with the classification of $TRPA_1$ and $TRPM_8$ receptors as cold channels and $TRPV_1$ receptors as warm channels. Events triggered by capsaicin include both heat loss and heat production mechanisms and involve a number of endogenous substances such as glutamate, monoamines and substance P (227). Icilin, on the other hand, activates TRP channels (TRPM8 and TRPA1) that respond to cold. The hyperthermic effect of icilin produces a dose-related hyperthermia is consistent with the effects of menthol, another cold channel agonist which also causes hyperthermia in rats (228). The most obvious difference in the hyperthermic effects of icilin and menthol is that icilin produces a hyperthermia that is greater in magnitude and persistence than menthol, perhaps related to evidence that icilin activates both $TRPM_8$ and $TRPA_1$ receptors whereas menthol only activates $TRPM_8$ receptors (229-232).

Evidence also indicates that icilin acts outside of the brain to produce hyperthermia because icilin injected into the ventricles does not affect body temperature (226). A peripheral site of action is consistent with data showing that icilin acts outside of the brain to evoke wet dog shakes and that $TRPM_8$ and $TRPA_1$ channels are located outside of the brain, primarily on sensory neurons that project to dorsal root ganglia neurons in the spinal cord and trigeminal neurons in the brain (223, 229-231, 234-237). One interpretation is that stimulation of peripheral cold-activated channels by icilin provides a signal to thermoregulatory centers located in the brain. This signal then is capable of triggering an elevation in body temperature, presumably through alterations in glutamate- and nitric oxide-based mechanisms that regulate heat production and heat loss (226). This explanation is consistent with the view that $TRPM_8$-deficient mice have a specific impairment in sensing cold temperatures which is manifested as a reduced avoidance of cold temperatures relative to wild-type mice (238). Another hallmark effect of

icilin is the production of wet-dog shakes (WDS), and the relationship between icilin-induced hyperthermia and wet-dog shaking is still not clear (239). The critical question is whether WDS occurs as a result of the hyperthermia or vice versa. Both effects of icilin are sensitive to administration route. Hyperthermia is detected following intraperitoneal or intramuscular injection whereas shaking is produced only following intraperitoneal administration (226, 239-241). This data indicates that hyperthermia is produced in the absence of shaking but that shaking is detected only when hyperthermia is present. It is possible that hyperthermia triggers the shaking through a "heat gain" or shivering response (239). Another possibility is that a metabolite of the parent compound icilin, formed following its intraperitoneal administration, causes the shaking whereas icilin itself produces the hyperthermia. Alternatively, the shaking could be the result of a local effect of icilin within the peritoneum. Future studies will need to delineate the relationship between the two hallmark responses to better determine the mechanism of action of icilin and the roles of $TRPA_1$ and $TRPM_8$ receptors in thermoregulation.

5.4. Cannabinoid interactions and body temperature

Thermoregulation is a multifactorial process involving interactions between multiple endogenous systems. It is not surprising that cannabinoid-induced hypothermia is influenced by multiple neurotransmitters and messengers, including but not limited to glutamate, nitric oxide, GABA, agmatine, serotonin, opioids, chemokines and nociceptin (173, 179,187 242-244). Glutamate is the major excitatory neurotransmitter in the mammalian brain and plays a key role in cannabinoid-induced hypothermia (187), as well as other pharmacologial effects of cannabinoids such as antinociception and catalepsy (245; 246, 247). Glutamate administered by itself causes hyperthermia (248-250). Antagonists that block NMDA receptors can produce hypothermia (62, 251-252) or hyperthermia depending on the dose of antagonist tested.

For combination studies with cannabinoids, NMDA receptor antagonists enhance hypothermia produced by the cannabinoid agonist WIN 55212-2 (187). The effect on WIN 55212-2 is produced by a noncompetitive (dextromethorphan) and competitive [(-)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate]] ((LY 235959) NMDA antagonist. Both dextromethorphan (5-75 mg/kg i.m.) and LY 235959 1-4 mg/kg i.m.) produce dose-related hypothermia in rats. However, what is most interesting is that a non-hypothermic dose of dextromethorphan (10 mg/kg) augmented the hypothermia produced by WIN 55212-2 (1, 2.5, or 5 mg/kg). The enhancement produced by dextromethorphan was pronounced, and joint-action analysis indicated that the drug-drug interaction between dextromethorphan and WIN 55212-2 was strongly synergistic. That is, the relative potency of WIN 55212-2 was enhanced about 3-fold when administered in combination with dextromethorphan. A non-hypothermic dose of LY 235959 (1 mg/kg) produced a similar enhancement in WIN 55212-2-induced hypothermia, thus confirming the hypothermic synergy. The results with LY

235959, a water-soluble compound with much greater selectivity for NMDA receptors than dextromethorphan, also indicate that receptor level interactions between CB_1 and NMDA receptors underlie the synergy.

Despite the chemical and pharmacological differences between dextromethorphan and LY 235959, their qualitatively and quantitatively similar enhancements of cannabinoid-induced hypothermia suggest a common mechanism – NMDA receptor activation –mediates the synergy. One aspect of the NMDA-CB_1 receptor synergy which remains unclear is the exact mechanism. A likely explanation is that WIN 55212-2 produces hypothermia by decreasing the level of extracellular glutamate in one or more brain regions (e.g. hypothalamus) which control body temperature. Because glutamate administration itself produces hyperthermia, presumably by increasing endogenous glutamate levels and enhancing glutamate transmission at NMDA receptors, it can be predicted that a reduction in extracellular glutamate produced by the systemic administration of WIN 55212-2 would produce the exact opposite effect, a reduction in glutamate transmission at NMDA receptors. In the case in which WIN 55212-2 is administered by itself, the reduced NMDA receptor activity would remove the hyperthermic tone normally mediated by endogenous glutamate. When WIN 55212-2 is administered in the presence of a NMDA receptor antagonist, its hypothermic effect may be further enhanced due to the combination of reduced extracellular glutamate (produced by WIN 55212-2) and direct NMDA receptor blockade (produced by LY 235959 or dextromethorphan). This interpretation is consistent with the widely held tenet that CB_1 receptor activation inhibits glutamate transmission in the brain (253, 254, 255, 256).

Nitric oxide also impacts cannabinoid-induced hypothermia. Considering the direct relation between nitric oxide production and NMDA receptor activation and the aforementioned role of NMDA receptors in cannabinoid-evoked hypothermia, it is not surprising that the nitric oxide system also plays such a critical role. NMDA receptor stimulation increases nitric oxide production through a mechanism involving increased calcium ion influx into the cell and a subsequent elevation in intracellular calcium ion concentration, which in turns leads to activation of calcium-dependent nitric oxide synthases. Akin to NMDA receptor antagonism, inhibition of nitric oxide production during WIN 55212-2 administration enhances cannabinoid-induced hypothermia (180). A non-hypothermic dose of L-NAME (50 mg/kg) augments the hypothermia produced by progressively increasing doses of WIN 55212-2 (0.5-5 mg/kg). Joint-action analysis indicates the relative potency of WIN 55212-2 is enhanced 2.5-fold when administered in the presence of L-NAME, thus revealing that the drug-drug interaction between L-NAME and WIN 55212-2 is synergistic rather than simply additive (257). The hypothermic synergy is not produced by co-administration of L-NAME and the inactive enantiomer of WIN 55212-2, WIN 55212-3 [S-(-)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-napthanlenyl) methanone mesylate] (5 mg/kg, i.m.), which confirms the synergistic interaction is due to cannabinoid receptor activation as opposed to a non-specific effect of WIN 55212-2.

The mechanism and site underlying the hypothermic synergy has not yet been identified. A central site of action is likely due to the overwhelming evidence that cannabinoids act in the brain, through a CB_1 receptor mechanism, to induce hypothermia (148, 175,176,179, 188, 258). Furthermore, CB_1 receptor-expressing neurons located in thermosensitive brain regions express high levels of nitric oxide synthase (26, 259). One possibility is that the marked hypothermia produced by WIN 55212-2 caused a compensatory increase in nitric oxide production, perhaps as a mechanism to counter the pronounced decline in body temperature. In the case in which the cannabinoid agonist is administered with a nitric oxide synthase inhibitor (L-NAME), this normal compensatory response resulting from an increase in nitric oxide production is blocked, leading to an exaggerated hypothermia. This mechanism is entirely dependent on two factors. One is that that cannabinoid agonists increase the endogenous concentration of nitric oxide, either by increasing its synthesis or slowing its degradation. Endogenous cannabinoids do stimulate nitric oxide release in rat kidneys, invertebrate nerve ganglia, and human immune tissue (260-262).

The second requirement is that an elevation in nitric oxide levels is indeed capable of counteracting cannabinoid-induced hypothermia, either by producing a hyperthermia that simply opposes the hypothermia or by inhibiting downstream signaling triggered by CB_1 receptor activation. Nitric oxide production has been shown to produce hyperthermia, but the results are inconsistent. Some studies have demonstrated that nitric oxide participates in fever generation (35-36, 263-264) whereas others have shown that nitric oxide displays antipyretic properties and participates in the production of hypothermia (3, 29, 31-34).

It is important to note that enhancement of cannabinoid responses by inhibition of nitric oxide production has been demonstrated previously, as L-NAME potentiates inhibition of contractile responses in rats produced by endogenous cannabinoids (265). Because both NMDA receptor antagonism and nitric oxide synthase inhibition enhance cannabinoid-induced hypothermia, it is tempting to speculate that inhibition of the NMDA/nitric oxide signaling pathway underlies the enhancement. If this is true, then cannabinoid agonists would be expected to produce qualitatively similar effects on NMDA receptor and nitric oxide synthase activity. It is a consensus across more than a decade of research that cannabinoid agonists inhibit glutamate release. Such an effect might be predicted to a reduction in extracellular glutamate and excitatory drive onto NMDA receptors, leading to a reduction in NMDA receptor activity and downstream nitric oxide production. WIN 55212-2 has been shown to suppress potassium-evoked neuronal nitric oxide synthase activity (266).

Considering the impact of excitatory transmission on the hypothermic response to cannabinoids,

it is not surprising that GABA-mediated inhibitory pathways also play a role (181). Glutamate and GABA systems oppositely modulate cannabinoid-induced hypothermia, with increased glutamate activity opposing the hypothermia and increased GABA activity facilitating the hypothermia. GABA is the principal inhibitory neurotransmitter in the mammalian brain, where it is exclusively synthesized from glutamate by glutamic acid decarboxylase. It acts through three GABA receptor subtypes, $GABA_A$, $GABA_B$, and $GABA_C$ (267). Ionotropic $GABA_A$ receptor activation produces an increased chloride conductance and membrane hyperpolarization (289) whereas metabotropic $GABA_B$ receptors mediate intracellular effects through a G-protein-coupled mechanism following activation (267).

In regard to thermoregulation, injection of GABA or the $GABA_A$ agonist muscimol induces hypothermia in rats (268, 269). Effects of $GABA_B$ receptor activation are mixed, with both hypothermic and hyperthermic responses reported following baclofen administration (268-270). Evidence indicates the hypothermia produced by GABA and CB_1 receptor activation is mediated by overlapping pathways. For example, GABA receptors are densely located in thermosensitive hypothalamic regions which express CB_1 receptor immunoreactivity (191, 271-273). Pharmacological studies have demonstrated that GABA agonists enhance cannabinoid-evoked hypothermia (274-276). $GABA_B$ receptors do not appear to play an important role because pretreatment of rats with the $GABA_B$ antagonist SCH 50911 (1-10 mg/kg i.p.) does not alter WIN 55212-2-induced hypothermia. The hypothermia is dependent on $GABA_A$ receptor activation because pretreatment with the $GABA_A$ antagonist bicuculline (2 mg/kg, i.p.) inhibits hypothermia produced by WIN 55212-2 (181). The converse is not true as cannabinoid CB_1 receptor antagonism with SR 141716A (2.5 mg/kg, i.m.) does not alter hypothermia produced by either $GABA_A$ receptor activation by muscimol (2.5 mg/kg, i.p.) or $GABA_B$ receptor activation by baclofen (5 mg/kg, i.p.). The combined data indicate cannabinoid agonists produce hypothermia which is dependent on downstream $GABA_A$ receptor activation but that CB_1 receptor signaling is not required for GABA agonists to induce hypothermia.

Similar to its effects on mu opioid responses, agmatine also modulates pharmacological effects of cannabinoids, including their hypothermic and analgesic actions (277-278). Both the hypothermic and analgesic effects of cannabinoid agonists are enhanced by exogenous agmatine. Changes in body temperature are not detected following administration of agmatine (10, 25 and 50 mg/kg, i.p.) by itself. Co-administration of agmatine (50 mg/kg, i.p.) and WIN 55212-2 (1, 2.5, 5 and 10 mg/kg, i.m.) results in an enhancement of the hypothermic response. Joint-action analysis indicates the relative potency of WIN 55212-2 is increased 2.7-fold in the presence of agmatine, indicating the drug-drug interaction between agmatine and WIN 55212-2 is synergistic. Agmatine injected directly into the lateral ventricle (25 and 50 micrograms/rat, i.c.v.) produces an enhancement of WIN 55212-2-induced

hypothermia which is remarkably similar to that produced by systemically injected agmatine. The congruent effect of agmatine following systemic and ventricular administration identifies the brain as the site of action of agmatine. It is not yet clear whether or not agmatine administration controls effects produced by long-term exposure to cannabinoids. As discussed earlier, agmatine administration blocks the physical dependence, analgesic tolerance, and rewarding effects produced by repeated morphine exposure. Unpublished observations from our laboratory suggest that agmatine administration is capable of blocking the development, but not the expression of hypothermic tolerance, produced by repeated WIN 55212-2 administration.

A novel finding is that cannabinoids modulate LPS-induced fever (279). Cannabinoids and LPS both affect the immune and thermoregulatory systems. Non-hypothermic doses of WIN 55,212-2 significantly reduce LPS-induced fever in rats (279). This inhibitory effect is not due to a nonspecific interaction with hydrophobic regions of functional proteins or their lipid surroundings in the cell membrane because WIN 55,212-3, an enantiomer of WIN 55,212-2, does not affect the LPS-induced fever, indicating that the effect of WIN 55,212-2 on LPS-induced fever is stereoselective. Delta[9]-THC injected at doses of 0.5 or 1 mg/kg also attenuates LPS-induced fever in a manner similar to WIN 55212-2. Cytokines (e.g. IL-6) act as endogenous pyrogens and play an important role in the mechanisms responsible for the development of the febrile response during infection and inflammation (280). Because cannabinoids display immunosuppressive effects, the inhibition of LPS-induced fever by WIN 55,212-2 may be due to a reduction of IL-6 produced during the LPS-induced fever and a reduction of the fever. SR141716A prevents the WIN 55,212-2 effects on LPS-induced fever, indicating that a CB_1 receptor mechanism mediated the response (279).

Because cytokines are released in response to LPS, and the CB_2 receptor has a modulatory role in the immune system, including the cytokine network (281), it is tempting to speculate that CB_2 receptors also contribute to the LPS-induced fever as well, but SR144528 does not alter the inhibitory effect of WIN 55,212-2 on LPS-induced fever (279). Benamar and colleagues (2009) have recently provided the first *in vivo* evidence of a physiological interaction between cannabinoid and chemokine systems. One of the chemokine receptors thought to have important functions in the brain is CXCR4. CXCR4 has also been identified as one of co-receptors for the human immunodeficiency virus-1 (HIV-1) (282). Pretreatment with stromal cell-derived factor-la (SDF-1alpha/CXCL12) significantly attenuates the hypothermic effect of WIN 55,212-2, indicating that this chemokine interferes with the pathway involved in mechanisms that control the development of hypothermia induced by a cannabinoid (242). To establish that the SDF-1alpha/CXCL12 effect was mediated through its receptor, the SDF-1alpha/CXCL12 antagonist, AMD 3100, was given directly into the POAH. In rats pretreated with AMD 3100, SDF-1alpha/CXCL12 is unable to alter WIN 55,212-2-induced

hypothermia (242). This finding indicates that the inhibitory effect of SDF-1alpha/CXCL12 on WIN 55,212-2-induced hypothermia is mediated by CXCR4. Because both chemokines and cannabinoids are found in the POAH (189-191, 283), and their receptors are members of the G protein-coupled receptor family, one likely explanation for the antagonistic effect of SDF-1alpha/CXCL12 on WIN 55,212-2 is interference with CB_1 receptor function in the POAH through CXCR4 receptor activation. A heterologous desensitization mechanism may occur at the G protein-coupled receptor level. These data support the idea of a functional interaction between chemokine and cannabinoid systems in the brain and show that a thermoregulatory action of the cannabinoid agonist WIN 55,212-2 can be antagonized by elevated levels of SDF-1alpha/CXCL12.

The temptation to assume that all neurotransmitters and messengers modulate the hypothermic effect of cannabinoids and opioids should be resisted. In fact, two of our own hypotheses – that cannabinoids produce hypothermia which is dependent on a downstream increase in $TRPV_1$ receptor signaling and vice versa (i.e., that capsaicin and other vanilloid agonists produce hypothermia which is dependent on downstream cannabinoid CB_1 receptor activation) and that acetaminophen-induced hypothermia was dependent on cannabinoid CB_1 receptor activation were disproven (220, 284). Furthermore, acetaminophen-induced hypothermia was not altered by mu, kappa, or delta opioid receptor antagonists (284). The ineffectiveness of opioid and cannabinoid CB_1 receptor antagonists is entirely different from their effects on APAP-evoked analgesia in rats (285-287). Pharmacological antagonism of mu, kappa, or delta opioid receptors inhibits acetaminophen-evoked analgesia in the paw withdrawal test and cannabinoid CB_1 receptor antagonism prevents the analgesic effect of acetaminophen in the hot-plate assay (286-287). The interpretation from these combined results is that two of acetaminophen's most noteworthy effects - hypothermia and analgesia - are mediated by mechanisms that are not entirely identical, with the analgesic effect dependent on the downstream activation of opioid and cannabinoid signaling pathways and the hypothermic response produced by pathways that are independent of opioid and cannabinoid receptor activation.

6. CONCLUSIONS

A number of discoveries over the past two decades have enhanced our understanding of the roles of cannabinoid, opioid, and TRP systems in thermoregulation. One of the more noteworthy findings is the impact of delta opioid receptors on body temperature. Contrary to earlier beliefs that delta opioid receptors exerted only minor effects on body temperature, it has now been shown through the use of selective agonists and antagonists that delta opioid receptor activation produces pronounced hypothermia in a wide variety of species. The roles of the endogenous opioid and cannabinoid systems in the control of body temperature have also been more clearly defined. Mu and kappa opioid receptors are thought to be in tonic balance, with mu receptor activation producing

hyperthermia and kappa receptor activation producing hypothermia, but a major role for delta opioid receptors in the tonic regulation of body temperature is not supported by the current literature. When injected exogenously, the endogenous cannabinoid anandamide produces hypothermia through cannabinoid CB_1 receptor activation. More work is needed to determine if endogenous anandamide exerts a hypothermic tone at CB_1 receptors, primarily because due to the low concentration and short-half life of endogenously produced anandamide and the inability to detect significant body temperature changes following administration of CB_1 receptor antagonists. Recent development of neutral, selective CB_1 receptor antagonists which lack the inverse agonist properties associated with SR 141716A and AM 251 should provide an opportunity to clearly elucidate the impact of CB_1 receptor tone on body temperature.

In addition to the effects of cannabinoid and opioid receptor systems on body temperature, related receptor systems such as sigma and TRP also modulate body temperature. Development of more selective sigma site antagonists has now led to the belief that activation of these sites can produce hypothermia. A number of receptor systems which contribute to the thermoregulatory actions of opioids and cannabinoids have been identified. Nitric oxide and glutamatergic signaling are particularly important. The hyperthermia produced by mu opioid receptor and the hypothermia produced by kappa or delta opioid receptor activation are highly dependent on nitric oxide production. Characterization of TRP channels at the behavioral, neurochemical, and molecular levels continues to be an intense focus of many research groups. It is now clear that warm channels (i.e., $TRPV_1$) produce hypothermia upon activation and that cold channels (i.e., $TRPM_8$, $TRPA_1$) produce hyperthermia upon activation.

Clinical evidence citing the thermoregulatory roles of opioid, cannabinoid, and TRP receptors is sparse, but it should be noted that agonists and antagonists acting at some of these receptors can alter body temperature in humans. One recent example relates to $TRPV_1$ antagonists, which produce marked hyperthermia in a wide variety of species, including humans. The hyperthermia detected following administration of $TRPV_1$ antagonists indicates that tone exerted at $TRPV_1$ channels reduces body temperature and that additional preclinical and clinical studies are needed to determine whether or not the hyperthermia will limit the clinical utility of these antagonists (224). In regard to the cannabinoid system, delta^9-THC produces acute, dose-dependent hypothermia in animals, but its relevance for human conditions remains unclear. Correlating thermoregulatory effects of delta^9-THC in animals and humans is challenging because distinct administration routes are frequently used for different species. Rats and mice are usually administered delta^9-THC intraperitoneally (i.p.) or intravenously (i.v.) whereas humans normally receive delta^9-THC through smoke inhalation or ingestion of infused oils. Furthermore, animals are often given much higher doses of delta^9-THC to enable the detection and quantification of physiological and behavioral responses. Nonetheless, patients smoking

marijuana have reported a sense of cold that is accompanied by brief shivering. With the continued identification and characterization of molecular targets within the endogenous cannabinoid system, it is quite possible that the development of compounds that enhance endocannabinoid signaling, by either increasing synthesis or slowing degradation, will lead to medications that produce therapeutically useful hypothermia. For example, animal models of ischemia have shown that the neuroprotective effects of cannabinoids are due in part to their hypothermic effects (288). While speculative, indirect cannabinoid agonists may be helpful in countering malignant hyperthermia, a rare genetic condition caused by common anesthetic agents and a reaction to the paralytic agent succinylcholine; countering hyperthermia resulting from psychostimulant exposure, in particular that caused by acute methamphetamine intoxication; and in producing a long-term reduction in metabolic rate, an effect which could theoretically enhance longevity.

In summary, a number of important findings have led to a clearer understanding of the roles that cannabinoid, opioid, and TRP systems play in thermoregulation. Additional research directed at clarifying the role that endogenous components of these systems exert on body temperature under physiological and pathophysiological conditions may have broad clinical implications and provide insights into interactions among neurotransmitter systems involved in thermoregulation.

7. ACKNOWLEDGEMENTS

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