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A diminution of Δ^9-tetrahydrocannabinol modulation of dynorphin A-(1–17) in conjunction with tolerance development

David J. Mason Jr. [a], John Lowe [b], Sandra P. Welch [a,*]

[a] *Department of Pharmacology and Toxicology, Virginia Commonwealth University / Medical College of Virginia, Box 980613, MCV Station, Richmond, VA 23298-0613, USA*

[b] *Pfizer Central Research, Groton, CT, USA*

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Abstract

Previous research in this laboratory concerning Δ^9-tetrahydrocannabinol-induced spinal antinociception indicated the critical role of dynorphin A-(1–17) in spinal antinociception following acute intrathecal (i.t.) administration. In the present study, tolerance development to Δ^9-tetrahydrocannabinol-induced spinal antinociception attenuated Δ^9-tetrahydrocannabinol-induced modulation of immunoreactive dynorphin A-(1–17). These data indicate that at lower doses of drug, desensitization of the cannabinoid receptor inhibits stimulation of downstream dynorphinergic neurons. However, at higher doses of drug, desensitization is overcome and spinal dynorphin A concentrations are increased by Δ^9-tetrahydrocannabinol Antinociception in the absence of elevated dynorphin A-(1–17) levels in the tolerant rat suggests that factors other than the attenuated dynorphin release are components of antinociception in the tolerant state. The shift from the critical role of dynorphin A in cannabinoid antinociception vs. that in the non-tolerant state may indicate tolerance also at the κ-opioid receptor, a role as yet undetermined.

Keywords: Antinociception; Δ^9-tetrahydrocannabinol; Dynorphin A; Opioid; Tail-flick latency

1. Introduction

The mechanism by which tolerance to Δ^9-tetrahydrocannabinol-induced tail-flick latency develops is poorly understood. The findings of previous studies, concerning cannabinoid tolerance development, are somewhat contradictory and most center on supraspinal components. Tolerance development is generally associated with pharmacokinetic or pharmacodynamic responses to repeated exposure to some pharmacologic agents. In the case of Δ^9-tetrahydrocannabinol, it is highly possible that tolerance development is a pharmacodynamic response. Martin et al. (1976) and Siemans and Kalant (1974) demonstrated in dog and rat models that Δ^9-tetrahydrocannabinol tolerance involves, at best, a minor pharmacokinetic component. That is to say tolerance development does not involve altered absorption, distribution, metabolism or excretion of Δ^9-tetrahydrocannabinol.

Other studies investigating the pharmacodynamic parameters concerning tolerance development have been conducted in several laboratories. A common pharmacodynamic response during tolerance development is modulation of the receptor protein. Cellular responses to exogenous agents maybe diminished or enhanced by decreasing or increasing receptor sensitivity, or availability. In the case of Δ^9-tetrahydrocannabinol, receptor down-regulation has been observed (Oveido et al., 1993). However, research concerning the relationship between cannabinoid receptor down-regulation and behavioral tolerance to Δ^9-tetrahydrocannabinol has yielded somewhat contradictory findings. Rodriguez de Fonseca et al. (1994) found that the development of behavioral tolerance to Δ^9-tetrahydrocannabinol occurred in conjunction with decreased cannabinoid binding in the striatal and limbic portions of the rat brain. Conversely, Romero et al. (1995) reported increased binding following chronic administration of Δ^9-tetrahydrocannabinol. Abood et al. (1993) found neither

* Corresponding author. Tel.: +1-804-828-8424; fax: +1-804-828-2117; e-mail: swelch@hsc.vcu.edu

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increased cannabinoid binding nor mRNA levels. It is also interesting to note that while the studies of Abood et al. (1993) assessed mRNA levels and binding in whole brain cells, previous studies utilized specific brain regions. Thus, localized receptor regulation may occur during the development of cannabinoid tolerance.

In keeping with a model previously developed in this laboratory, describing the mechanisms underlying Δ^9-tetrahydrocannabinol-induced spinal antinociception, the following mechanism of tolerance is proposed. In this model, repeated Δ^9-tetrahydrocannabinol exposure results in a decreased potency to enhance dynorphin A-(1–17) release. Such a compensatory response would result in decreased κ-opioid receptor stimulation and subsequent inhibition of substance P release. The net result of these actions would be normal transduction of the noxious stimuli. To investigate this putative mechanism of tolerance development, Δ^9-tetrahydrocannabinol modulation of dynorphin A-(1–17) and tail-flick latency were assessed using spinal perfusion and radioimmunoassay methodologies.

2. Methods and materials

2.1. Animal husbandry

These studies were conducted using male Sprague–Dawley rats, weighing between 450 and 500 g, obtained from Harlan Laboratories. Subjects were housed individually and maintained on a fixed 12-h light cycle at a temperature of 22 ± 2°C. Water and food (Harlan Rat Chow) were provided ad libitum.

2.2. Intrathecal (i.t.) administration of drugs and dynorphin collection

Intrathecal drug administration and dynorphin collection were performed using a modified version of techniques described by Yaksh (1981) and Tseng (1989). Subjects were selected for partitioning into experimental groups at random and anesthetized via intraperitoneal (i.p.) injection of sodium barbital (375 mg/kg) and a separate i.p. injection of 2 mg/kg atropine methyl nitrate. The anesthetized rats were placed in stereotaxis and an incision made on the atlanto–occipito membrane to expose the cisterna magna. A catheter of PE-10 polyethylene tubing was inserted through the exposed cisternal cavity, caudally, into the subarachnoid space of the spinal cord. The catheter contained an artificial cerebrospinal fluid, composed of 125 mM Na^+; 2.6 mM K^+; 0.9 mM Mg^{2+}; 1.3 mM Ca^{2+}; 122.7 mM Cl^-; 21.0 mM HOC^-; 2.4 mM HOP^{2-}; 0.5 mg/ml bovine serum albumen, bacitracin (30 mg/ml), 0.01% Triton X and effervesced with 95% O_2 and 5% CO_2. Positioned as such, the catheter extended caudally 8.5 cm passing through the thoracolumbar region to an area just above the sacral enlargement. Following catheter implantation, animals were allowed to acclimate approximately 30-min on a heating pad. Following acclimation, baseline tail-flick latency was assessed. Only animals exhibiting normal tail-flick response to noxious stimuli, greater than 1.5-s, but less than 4-s latency, were used. Test compounds were administered in a 20-ml bolus of vehicle, via spinal catheter, at a rate of 30 ml/min. Subjects were then segregated into groups for cerebrospinal fluid sampling and tail-flick latency assessment 10-min post-administration of the test compound. Cerebrospinal fluid collection entailed rapid perfusion of the spinal cavity with artificial cerebrospinal fluid culminating in the collection of 1.5 ml of the eluting artificial cerebrospinal fluid from the open cisternal space. This is an open system and the sampling technique is similar to the push–pull cannula technique commonly employed in the mouse. Collected fractions were boiled for 12-min and centrifuged at a rate of 10,000 rpm for 10 min. The supernatant was collected, frozen at −70°C and lyophilized. Samples were reconstituted in 250 μl radioimmunoassay buffer before dynorphin A-(1–17) peptide measurement.

2.3. Measurement of dynorphin A-(1–17) peptide

Measurement of dynorphin A-(1–17) (pg/ml) was accomplished using a dynorphin A-(1–17)-specific radioim-

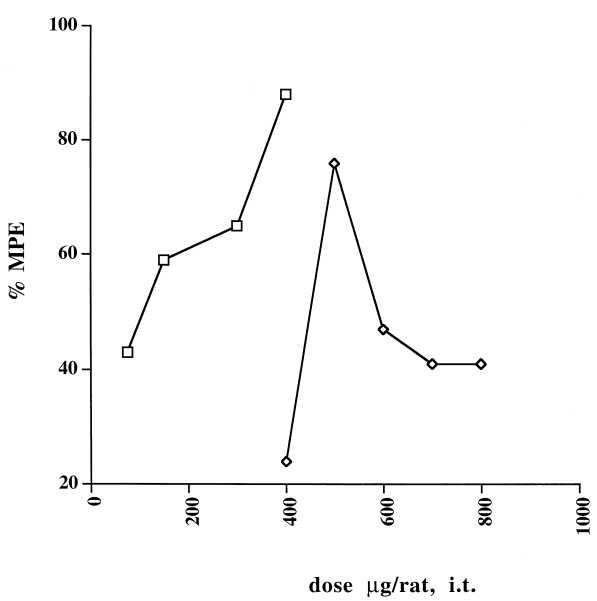

Fig. 1. Comparison of Δ^9-tetrahydrocannabinol-induced tail-flick latency dose–response relationships among animals repeatedly treated with vehicle or Δ^9-tetrahydrocannabinol. Rats were treated repeatedly with vehicle (open squares) and challenged with 20 μl DMSO, or Δ^9-tetrahydrocannabinol (small triangles) at doses shown (each dose is per rat in 20 μl vehicle) via catheter. The tail-flick test was used to quantitate antinociception using at least eight rats per dose as described in Section 2.

munoassay kit obtained from Peninsula Laboratories. The reconstituted samples were analyzed in duplicate. The manufacturer reported cross-reactivity of antibody as 100% vs. dynorphin-(1–24), a parent compound, and less than 2% vs. smaller peptide fragments. We found no cross-reactivity of the antibody to dynorphin A-(1–8), dynorphin A-(1–13), dimethyl sulfoxide or Δ^9-tetrahydrocannabinol. Only the linear portion of the radioimmunoassay standard curve, between 0.1 and 64 pg/ml of the standard dynorphin peptide, was used to calculate dynorphin concentration.

2.4. Assessment of tail-flick latency

Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith (1941). Each animal was acclimated in the laboratory 24 h prior to experimentation. Tail-flick latency was not found to be significantly increased by sodium barbital or catheterization in comparison to unanesthetized or non-catheterized animals. Base latencies were measured as 1.5–4 s with maximal post-drug latency set at 10 s, after which the noxious heat stimulus was terminated. Antinociception was measured in terms of percent maximal possible effect (%MPE) defined by Harris and Pierson (1964) and Dewey et al. (1970) as:

$$\%\text{MPE} = \frac{\text{test latency} - \text{control latency}}{10\,\text{s} - \text{control latency}} \times 100\%.$$

Each parameter (i.e., test or control tail-flick latency value) represents the mean of three recordings at 10-s intervals.

2.5. Statistical analysis

Using a randomized design, analysis of data concerning tail-flick latency or dynorphin peptide concentration was done using ANOVA (analysis of variance) followed by Dunnett's *t*-test (Dunnett, 1955).

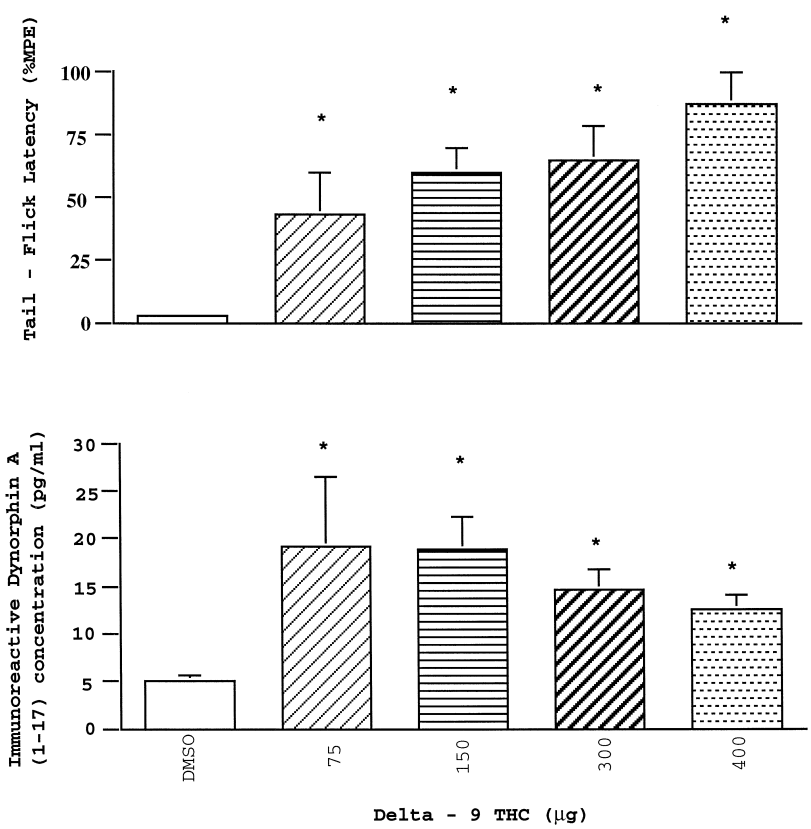

Fig. 2. Spinal immunoreactive dynorphin A-(1–17) concentrations and tail-flick latencies of subjects chronically administered 1:1:18 vehicle. Rats were treated repeatedly with vehicle and challenged with 20 μl DMSO, or Δ^9-tetrahydrocannabinol at doses of 75, 150, 300 or 400 μg (each dose is per rat in 20 μl vehicle) via catheter. Fractions of artificial cerebrospinal fluid were collected 10 min post-administration and dynorphin A-(1–17) concentrations were quantified via radioimmunoassay. Mean immunoreactive dynorphin A-(1–17) concentrations and tail-flick latencies (% MPE) + S.E. are presented. * $p < 0.05$ in comparison to control animals receiving DMSO vehicle i.t. The tail-flick test was used to quantitate antinociception using at least eight rats per dose as described in Section 2.

2.6. Drugs and vehicle

Δ^9-Tetrahydrocannabinol was obtained from the National Institute on Drug Abuse. For the repeated dosing regimen, Δ^9-tetrahydrocannabinol was administered in a vehicle composed of one part emulphor, one part ethanol, 18 parts saline (1:1:18). For i.t. challenges, Δ^9-tetrahydrocannabinol was administered using a 100% dimethyl sulfoxide vehicle (DMSO). This concentration of DMSO has been used in numerous studies and has no effect on the animals behavior (in awake, non-anesthetized animals) or on dynorphin release in our anesthetized rats.

2.7. Specifics of drug administration

2.7.1. Δ^9-Tetrahydrocannabinol tolerance development

Tolerance to Δ^9-tetrahydrocannabinol has been developed and reported by Tsou et al. (1995). In our studies, a similar regimen was employed. Rats were administered a dose of 15 mg/kg Δ^9-tetrahydrocannabinol, in a 1:1:18 vehicle, twice daily at 12 h intervals for 7 days via i.p. injection. Tolerance was verified by assessment of tail-flick latency, following an i.t. Δ^9-tetrahydrocannabinol challenge.

3. Results

To assess tolerance development to Δ^9-tetrahydrocannabinol-induced tail-flick latency, animals were administered vehicle or Δ^9-tetrahydrocannabinol in accordance with the regimen previously described. The subjects were subsequently challenged with varied concentrations of Δ^9-tetrahydrocannabinol (i.t.) and their respective tail-flick latencies, 10-min post-administration, were compared statistically. To quantify tolerance development, the dose–response relationship of the Δ^9-tetrahydrocannabinol-induced tail-flick latencies of these animals was plotted. A rightward shift in the dose–response curve was observed in animals repeatedly administered Δ^9-tetrahydrocannabinol

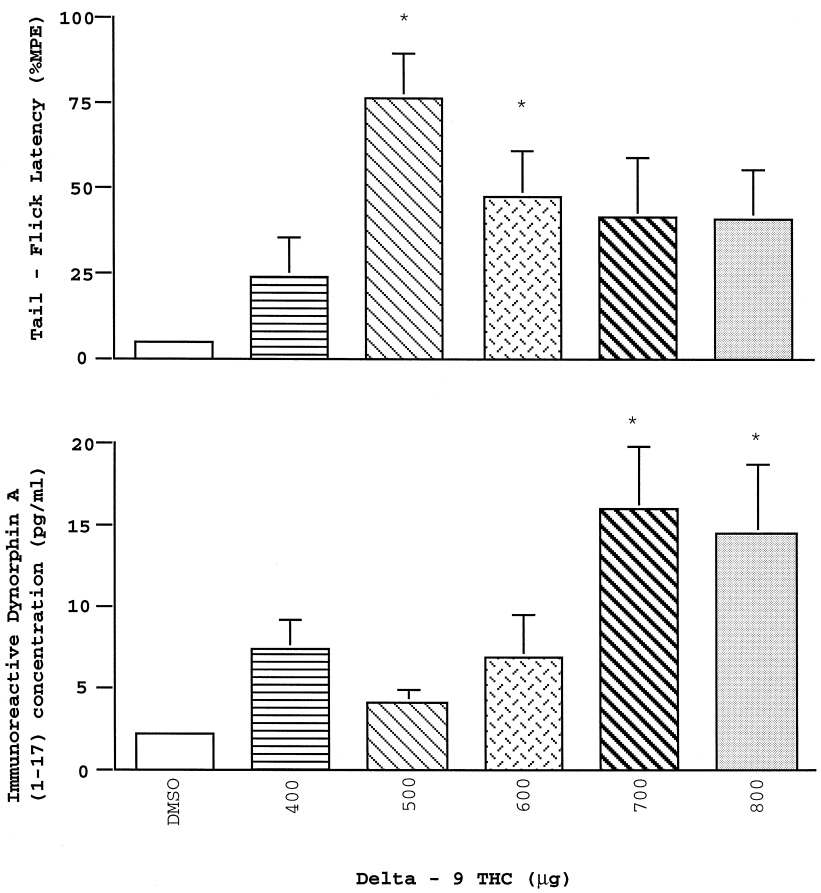

Fig. 3. Spinal immunoreactive dynorphin A-(1–17) concentrations and tail-flick latencies of subjects chronically administered Δ^9-tetrahydrocannabinol. Rats were treated repeatedly with Δ^9-tetrahydrocannabinol and challenged with 20 μl DMSO, or Δ^9-tetrahydrocannabinol at doses of 400, 500, 600, 700 or 800 μg via spinal catheter. Fractions of artificial cerebrospinal fluid were collected 10 min post-administration of Δ^9-tetrahydrocannabinol, and dynorphin A-(1–17) concentration was quantified via radioimmunoassay. Mean immunoreactive dynorphin A-(1–17) peptide concentrations and tail-flick latencies (% MPE) ± S.E. are presented. * $p < 0.05$ in comparison to control animals receiving DMSO vehicle i.t. The tail-flick test was used to quantitate antinociception using at least eight rats per dose as described in Section 2.

in comparison to those receiving vehicle. However, the non-linear nature of the dose–response curve generated from the Δ^9-tetrahydrocannabinol-treated group hampered an effective statistical comparison of ED_{50} values (Fig. 1).

Animals repeatedly administered vehicle and challenged with DMSO vehicle possessed a mean tail-flick latency of (3 ± 1%MPE) (Fig. 2)). Subjects challenged with various doses of Δ^9-tetrahydrocannabinol demonstrated significant spinal antinociception (doses in microgram per rat in 20 μl of artificial cerebrospinal fluid) (Fig. 2). Immunoreactive dynorphin A-(1–17) concentrations were also assessed 10 min post-Δ^9-tetrahydrocannabinol challenge. In animals receiving repeated vehicle treatment, mean immunoreactive dynorphin A-(1–17) levels were significantly higher following 75 (19 ± 7 pg/ml), 150 (19 ± 3 pg/ml), 300 (15 ± 2 pg/ml) and 400 μg (12 ± 1 pg/ml) Δ^9-tetrahydrocannabinol challenge vs. a DMSO challenge (5 ± 1 pg/ml) (Fig. 2). Thus, increased dynorphin release was associated with significant antinociceptive effects. However, the amount of dynorphin released did not increase in a dose-related manner and was not statistically different across the dose range tested. Thus, the degree of cannabinoid-induced antinociception did not parallel the amount of released dynorphin A-(1–17).

The dose–effect curve for Δ^9-tetrahydrocannabinol was bell-shaped in animals rendered tolerant to Δ^9-tetrahydrocannabinol (i.p.) (Fig. 3)). Only those animals challenged with 500 and 600 μg Δ^9-tetrahydrocannabinol demonstrated significant antinociception in comparison to vehicle-challenged subjects. Such bell-shaped curves for Δ^9-tetrahydrocannabinol have been previously noted by Lichtman and Martin (1991) and are indicative of non-specific, non-receptor-related effects of the drug at higher doses. In contrast to what was observed in the non-tolerant rats, fractions of cerebrospinal fluid collected from animals repeatedly exposed to Δ^9-tetrahydrocannabinol did not show enhanced levels of immunoreactive dynorphin A-(1–17) following Δ^9-tetrahydrocannabinol challenges of 400 (7 ± 2 pg/ml), 500 (4 ± 1 pg/ml) or 600 μg Δ^9-tetrahydrocannabinol (7 ± 3 pg/ml). However, i.t. challenges of 700 (16 ± 4 pg/ml) and 800 μg (14 ± 4 pg/ml) Δ^9-tetrahydrocannabinol enhanced immunoreactive dynorphin A-(1–17) concentrations significantly above those observed in the control group (Fig. 3). Thus, increased dynorphin release was not temporally associated with significant antinociceptive effects in rats that were tolerant to Δ^9-tetrahydrocannabinol .

4. Discussion

Our data may be interpreted as supportive of cannabinoid receptor down-regulation or inactivation as suggested in previous studies. Receptor down-regulation or inactivation would result in fewer active receptors available for interaction with Δ^9-tetrahydrocannabinol and initiation of subsequent receptor-mediated activities. Doses of Δ^9-tetrahydrocannabinol, which normally enhance immunoreactive dynorphin release, would be expected to yield less dynorphin release as fewer active receptors were available to initiate the response. Thus, the only method of increasing dynorphin release would be by increasing the dose of Δ^9-tetrahydrocannabinol. The observed diminution of Δ^9-tetrahydrocannabinol-induced immunoreactive dynorphin release may be indicative of such a pharmacodynamic response in tolerance development.

Whereas immunoreactive dynorphin A-(1–17) concentrations were significantly elevated following a 75-μg/rat dose of Δ^9-tetrahydrocannabinol challenge in control animals treated with vehicle, a dosage of 700 μg/rat (i.t.) was required to significantly elevate immunoreactive dynorphin A-(1–17) concentrations in animals administered Δ^9-tetrahydrocannabinol in the regimen demonstrated to produce tolerance in the tail-flick latency test. Such data may indicate that Δ^9-tetrahydrocannabinol exposure results in fewer active cannabinoid receptors available for stimulation. Alternatively, the diminished effect may result from repeated Δ^9-tetrahydrocannabinol-induced release of immunoreactive dynorphin and depletion of neuropeptide stores, changes in receptor coupling to intracellular messengers and G-proteins, or alterations in other neurotransmitters involved in the Δ^9-tetrahydrocannabinol-induced antinociceptive pathway.

A dissociation between immunoreactive dynorphin A-(1–17) concentration and antinociceptive effect was observed among animals repeatedly exposed to Δ^9-tetrahydrocannabinol. Although dynorphin A-(1–17) concentrations were not elevated by doses below 700 μg Δ^9-tetrahydrocannabinol, significant spinal antinociception was observed 10 min post-administration of 500 and 600 μg Δ^9-tetrahydrocannabinol. Similarly, the acute antinociceptive effects of Δ^9-tetrahydrocannabinol were dose-related, but the concurrent release of dynorphin was not dose-related. However, the release of dynorphin A or another κ-opioid receptor agonist appears to be an absolute requirement for Δ^9-tetrahydrocannabinol-induced antinociception since the κ-opioid receptor antagonist, nor-BNI, completely abolishes the antinociceptive effects of Δ^9-tetrahydrocannabinol. Thus, the release of dynorphin A appears to be necessary, but not the solitary component required for Δ^9-tetrahydrocannabinol-induced antinociception. These data indicate the existence of compensatory mechanisms by which factors other than dynorphin A-(1–17) mediate spinal transmission of nociceptive information following chronic Δ^9-tetrahydrocannabinol administration, whereas dynorphin release appears to be required, in part, for the antinociceptive effects of Δ^9-tetrahydrocannabinol in the non-tolerant rat. The other mechanisms which are critical in the tolerant rat may also act in the non-tolerant rat in conjunction with dynorphin to yield an antinocicep-

tive effect of the drug. Such mechanisms are yet to be elucidated, but clearly appear to be interacting in some way with the κ-opioid receptor.

In our hypothetical spinal model for Δ^9-tetrahydrocannabinol-induced spinal antinociception in the non-tolerant rat (Fig. 4)), the cannabinoid (CB1) receptor is located in the synaptic terminal of a descending GABAergic or noradrenergic neuron in the dorsal horn of the spinal cord. Δ^9-Tetrahydrocannabinol stimulation of the CB1 receptor inhibits GABA and norepinephrine reuptake. The increased presence of these neurotransmitters results in the inhibition of a tonically active inhibitory interneuron, via GABA and or α_2-adrenoceptor located on an inhibitory interneuron, and subsequent disinhibition of a dynorphinergic neuron. The resulting increase in dynorphin peptide leads to a κ-opioid receptor-mediated inhibition of substance P release. Such activity would diminish the amplification of the nociceptive stimulus and its perception (Fig. 4).

This model is highly hypothetical and supported by the findings of Romero et al. (1995) in brain, not cord, who found that cannabinoid stimulation of receptors in GABAergic neurons of the striatum and substantia nigra inhibit GABA reuptake and of Lichtman and Martin (1991) who demonstrated that antagonism of the a_2-adrenoceptor spinally attenuated cannabinoid-induced antinociception. The model does provide for an explanation of attenuation of both the Δ^9-tetrahydrocannabinol-induced dynorphin release and Δ^9-tetrahydrocannabinol-induced antinociception by the CB1 antagonist, SR 141716A, while only the antinociceptive effects of the drug, and not the drug-induced dynorphin release, are blocked by the κ-opioid receptor antagonist, norbinaltorphimine. Thus, dynorphin release requires CB1 receptor activation, while antinociception requires both CB1 receptor activation and κ-opioid receptor activation.

The development of tolerance in either system is likely a complex event. The data concerning immunoreactive

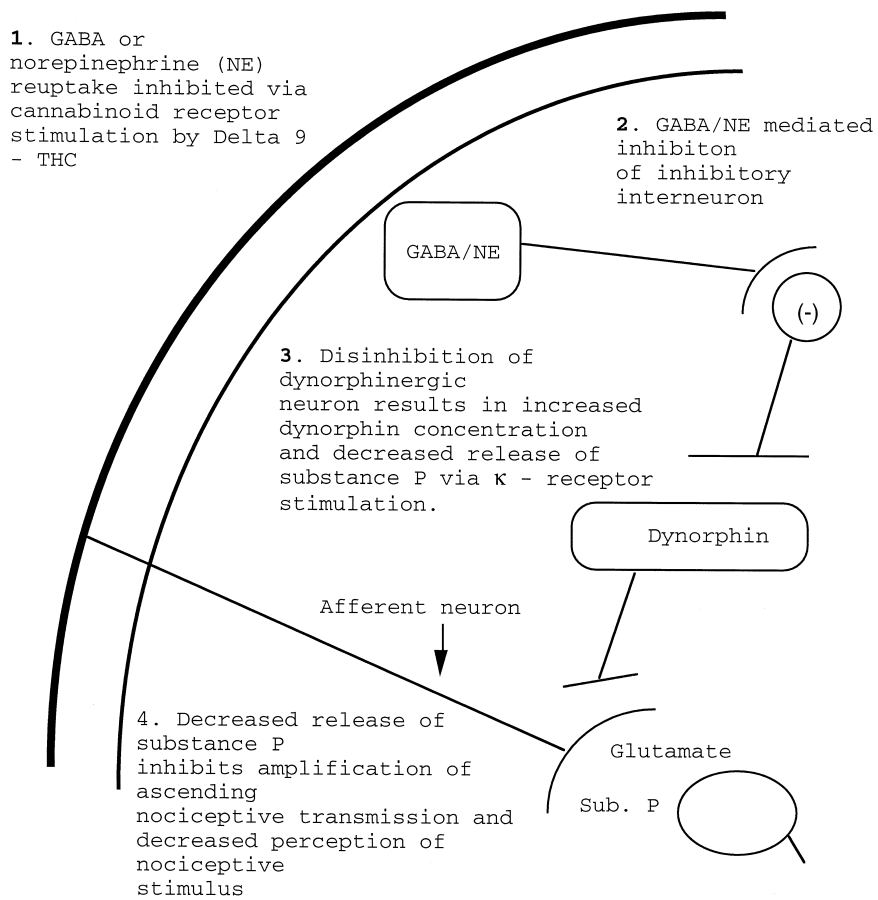

Fig. 4. Hypothesized model of Δ^9-tetrahydrocannabinol-induced spinal antinociception. Δ^9-Tetrahydrocannabinol stimulation of cannabinoid (CB1) receptors, located on the synaptic terminal of a descending spinal GABAergic neuron in the dorsal horn of the spinal cord, inhibits GABA reuptake (the dorsal horn of the spinal cord is represented by the outer circle). The increased presence of GABA results in the inhibition of a tonically active inhibitory interneuron, via GABA receptor stimulation, and disinhibition of a dynorphinergic neuron. The resulting increase in dynorphin peptides leads to a κ-opioid receptor-mediated inhibition of substance P release. Such activity would diminish the amplification of the nociceptive stimulus in small afferent A-delta and C-fibers (labeled ''afferent neuron'') and its perception. A separate noradrenergic component may also exist, in which, Δ^9-tetrahydrocannabinol inhibits the reuptake of norepinephrine in a separate descending neuron. The increased presence of norepinephrine would result in stimulation of α_2-adrenoceptors located on an inhibitory interneuron and subsequent disinhibition of the dynorphinergic neuron. The end result would be a κ-receptor-mediated attenuation of substance P release.

spinal dynorphin A-(1–17) concentrations are supportive of the proposed diminishing of Δ^9-tetrahydrocannabinol's ability to modulate spinal dynorphin A-(1–17) concentration after chronic Δ^9-tetrahydrocannabinol exposure. Significant dynorphin release at doses exceeding 700 μg also suggests some modulation resulting in desensitization of the cannabinoid receptor. In Δ^9-tetrahydrocannabinol-tolerant animals, lower doses of the drug do not affect spinal dynorphin concentrations, but higher doses will release dynorphin. These data indicate that at lower doses of drug, desensitization of the cannabinoid receptor inhibits stimulation of downstream dynorphinergic neurons. However, at higher doses of drug, desensitization is overcome and spinal dynorphin A concentrations are increased. The presence of antinociception in the absence of elevated dynorphin A-(1–17) levels in the tolerant rat suggests that factors other than the attenuated dynorphin release are critical components of cannabinoid-induced antinociception in the tolerant state. Such factors could include any number of ''players'' in the nociceptive pathway. The shift from the critical role of dynorphin A in cannabinoid antinociception in the non-tolerant state to the lack its critical role in the cannabinoid-tolerant state may also indicate that tolerance occurs at the κ-opioid receptor. The role of the κ-opioid receptor in tolerance development to cannabinoids is undetermined. Tolerance development might involve some undetected modulation of the κ-receptor protein such as internalization or inactivation.

5. Conclusion

In the dorsal horn of the spinal cord, we hypothesize that cannabinoid-induced release of dynorphin A-(1–17) is a critical factor in the production of antinociception. Evidence for such a critical role of dynorphin A-(1–17) comes from the lack of antinociception if the κ-opioid receptor is blocked with nor-BNI. In the cannabinoid-tolerant state, dynorphin release is clearly diminished, indicating some tolerance to the dynorphin release. However, there is not a clear temporal or dose–response relationship between the attenuation of dynorphin release and antinociception. Such data are an indication that cannabinoid tolerance alters the dynorphinergic system as well as other putative nociceptive modulators.

Acknowledgements

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