Endocannabinoid modulation of sympathetic and cardiovascular responses to acute stress in the periaqueductal gray of the rat

C. Dean
Department of Anesthesiology, Medical College of Wisconsin, and Department of Veterans Affairs Medical Center, Milwaukee, Wisconsin

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Dean C. Endocannabinoid modulation of sympathetic and cardiovascular responses to acute stress in the periaqueductal gray of the rat. Am J Physiol Regul Integr Comp Physiol 300: R771–R779, 2011. First published January 12, 2011; doi:10.1152/ajpregu.00391.2010.—Activation of the sympathetic nervous system is fundamental to the coordinated response to stress or danger. The midbrain periaqueductal gray (PAG) contains the neural substrate required to recruit the sympathetic nervous system and organize the physiological and behavioral responses required to respond to imposed challenges. Endocannabinoids have been shown to influence associated behavioral responses. The defense response was used in this study as a working model to examine endocannabinoid modulation of the sympathetic response to acute stress in the anesthetized rat. Microinjection of the cannabinoid 1 (CB1) receptor agonist anandamide into the defense pathway of the dorsal PAG could elicit an increase in renal sympathetic nerve activity and blood pressure, twitching of the whiskers, and movement of the limbs. The response was attenuated by prior microinjection of the CB1 receptor antagonist AM-281 at the same site. Electrical stimulation of the hypothalamic defense area could evoke similar sympathoexcitatory and pressor responses, which were significantly attenuated by microinjection of AM-281 into the dorsal PAG. These data indicate that endocannabinoids can modulate the sympathetic and cardiovascular components of the acute stress response via CB1 receptors at the level of the PAG.

STRESS OR FEAR INITIATES SUBTLE OR OVERT PHYSIOLOGICAL CHANGES PROPORTIONAL TO THE ASSOCIATED DEMAND, THE BASIS OF WHICH IS ESTABLISHED IN THE NEURAL NETWORKS THAT ORGANIZE DEFENSE RESPONSES. FUNDAMENTAL TO THESE CHANGES, THE SYMPATHETIC NERVOUS SYSTEM IS EFFECTIVELY MOBILIZED TO PRODUCE THE CHARACTERISTIC PHYSIOLOGICAL ADJUSTMENTS ASSOCIATED WITH FIGHT OR FLIGHT, INCLUDING SYMPATHOEXCITATION, HYPERTENSION, TACHYCARDIA, AND INCREASED BLOOD FLOW TO SKELETAL MUSCLE. THE CHANGES IN SYMPATHETIC NERVE ACTIVITY REPRESENT A HALLMARK OF THE RESPONSE TO ACUTE STRESS, WHICH THREATENS THE HOMEOSTASIS OF THE ORGANISM, AND FEAR, WHICH IS AN ADAPTIVE COMPONENT EVOKED IN RESPONSE TO MORE INTENSE AND DANGEROUS STIMULI. ACTIVATION OF THE DEFENSE PATHWAY CAN EVOKE THE FULL VISCERAL AND SOMATIC RESPONSES. THE DEFENSE PATHWAY DESCENDS THROUGH THE MIDBRAIN PAG, WHICH IS HIGHLY ORGANIZED TO COORDINATE THE AUTONOMIC AND BEHAVIORAL RESPONSES TO STRESS, FEAR, AND PAIN. THE DORSAL AND LATERAL COLUMNS OF THE PAG INTEGRATE KEY COMPONENTS OF THE DEFENSE PATHWAY AND COORDINATE REACTIONS TO PHYSICAL AND PSYCHOLOGICAL STRESSORS.

Cannabis is a popular recreational drug because of its acute effects of euphoria and relaxation. However, unpleasant side effects of anxiety and panic reactions are most common in new users and occur also in experienced users, especially after a higher than usual dose (9), and they are more common in adults than adolescents (29). Paralleling the effects of cannabis, cannabinoid agonists have been reported to have biphasic effects, with low doses being anxiolytic and higher doses being anxiogenic (10). Much research has been directed toward stress-related behavioral responses to endocannabinoids, with conflicting results, and has established many variables that could contribute to the diversity of responses. The route of drug administration, the extensive central distribution of the cannabinoid 1 (CB1) receptor, the efficacy of the agonist, the species, the type of behavioral test employed, and stress exposure could determine the evoked responses (35).

CB1 receptors are expressed throughout the brain, including the amygdala, hypothalamus, and PAG, which are located along the defense pathway (33). The endocannabinoid system is neuromodulatory, influencing excitatory and inhibitory neurotransmission throughout the brain (5). Unlike classical neurotransmitters, endocannabinoids are synthesized on demand and released from postsynaptic neurons to retrogradely activate CB1 receptors located in presynaptic terminals (36, 37). Activation of Gi proteins and inhibition of adenylate cyclase and calcium currents with potentiation of potassium currents lead to a reduction of neuronal firing and inhibition of transmitter release, resulting in a reduction of synaptic input to the postsynaptic neuron (37). Cannabinoid agonists have been shown to be integral to the modulation of behavioral components of anxiety-like responses (6, 22, 27) and stress-induced analgesia (12) in the dorsal PAG. Sympathoexcitation is also a fundamental component of the responses to stress, anxiety, and fear coordinated in the PAG, leading to the hypothesis that endocannabinoids can modulate sympathetic nerve activity during the response to acute stress. The defense response was used as an experimental model of imposed stress in the design of this study to examine the role of CB1 receptors in the dorsal PAG (dPAG) in this autonomic component. While it is accepted that electrical stimulation can activate cell bodies and fibers of passage, chemical stimulation of the hypothalamus, unlike the midbrain PAG, does not consistently evoke a full defense response, a
finding that has been debated for decades (11). Therefore, in
the present study, the defense pathway in the hypothalamus
was activated by electrical stimulation, and activation of the
pathway in the dPAG was achieved by chemical stimulation.

EXPERIMENTAL PROCEDURES

The protocol for this study was approved by the Animal Care and
Use Committees at the Medical College of Wisconsin and Zablocki
Department of Veterans Affairs Medical Center. Sprague-Dawley rats
(250–380 g body wt) were anesthetized with pentobarbital sodium (50
mg/kg ip), and a catheter was inserted into a femoral vein for
supplemental administration of anesthetic. Arterial blood pressure
was monitored continuously from a cannula inserted into a tail artery,
connected via a pressure transducer to a computer-based data acqui-
sition-and-storage system (Apple Macintosh G4 computer, AD Instu-
mements PowerLab/8Sp with Chart software). The trachea was can-
nulated through a midline cervical incision for ventilation with room air
(Harvard 683 respirator) when the animal was paralyzed for defense
area stimulation. A heating pad was used to maintain body tempera-
ture at 37°C. The head of the animal was fixed in a stereotaxic frame
(Kopf), and a renal nerve was exposed retroperitoneally. Renal symp-
pathetic nerve activity was recorded using flexible silver wire elec-

trodes positioned on a renal nerve. The electrodes were fixed in
position with silica gel, which allows adjustment of the body of the
animal without disturbing the recording. The electrophysiological
signals were directed to high-impedance differential amplifiers
(gain = 1,000, band pass = 0.1–10 kHz) and then to filter/amplifiers
(gain ≤400; high- and low-pass filtering 10 Hz–3 kHz). The
amplifier output was displayed online and also directed to precision
full-wave rectifiers and averaged using Bessel linear averaging filters
(averaging interval = 100 ms) to obtain an online moving time
average. Arterial blood pressure, renal sympathetic nerve activity, and
a moving time average of renal sympathetic nerve activity were
monitored and recorded on the computer-based data acquisition
system. Raw signals were also recorded on tape (Vetter PCM recor-
ding adaptor model 3000A) for backup and subsequent data analy-
sis.

The test agents microinjected in these studies were the synaptic
agonist and antagonist (Tocris Cookson, St. Louis, MO) were diluted in
H2O, the CB1 receptor agonist anandamide, and the CB1 receptor
antagonist 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-
linyl-1H-pyrazole-3-carboxamide (AM-281). The CB1 receptor ago-
nist and antagonist (Tocris Cookson, St. Louis, MO) were diluted in
0.01% Tocrisolve 100 (Tocris Cookson). At the end of each trial, 1% pon-
tamine sky blue dye (35 nl) was microinjected to mark the location of
the microinjection site.

Hypothalamic brain sites were stimulated electrically via a mono-
polar tungsten carbide electrode that was insulated, except at the tip
(20–50 μm); the anode was attached to the retracted skin. For
hypothalamic defense area stimulation, 1- to 2-ms trains of rectangu-
lar constant-current cathodal pulses at 70 Hz were delivered for 10 s
at 50–150 μA. Stimulation sites were marked at the conclusion of
each experiment by passage of a 50-μA direct positive current
through the stimulating electrode for 30 s. Brains were removed
postmortem and frozen (~80°C) for subsequent histological analysis.

In all animals, a dorsal craniotomy was performed, and the dura
was reflected to allow the insertion of a four-barreled glass micropi-
pette (20 μm total tip diameter) attached to a pressure ejection system
to microinject agents into the brain. The barrels contained DLH (4
mM), anandamide (50 μM), AM-281 (1 μM), and pontamine sky blue
dye. Limited to four barrels, a series of control studies were performed
in a separate group of animals with three barrels of a micropipette
filled with DLH, anandamide, and vehicle. Microinjections of Toc-
risolve 100 vehicle were made as controls for volume and pressure
effects of ejected solution and reproducibility of anandamide re-
sponses. The micropipette was advanced into the midbrain slowly via
a microdrive, and initial coordinates with respect to bregma, midline,
dorsal surface were ~7.2, 0.5, and 4.0 mm, respectively, targeting
the dPAG at a site identified functionally as described below. The
volume of injectate (14–50 nl) was measured by observation of the
level of the fluid meniscus through a graduated monoculus microscope
eye piece (7 nl/div). Identification of a site from which the components
of a defense response could be evoked was accomplished by micro-
injection of the synaptic excitant DLH (56 pmol, 14 nl). With fine
adjustment of the micropipette as necessary, a site was located at
which a >10-mmHg increase in the pressor response and an accom-
panying increase in renal sympathetic nerve activity were evoked.
The cardiovascular effects were accompanied by twitching of the whiskers
and hyperventilation and, occasionally, movement of the tail and
limbs. This protocol was used to indicate tip placement in the defense
pathway of the dPAG (18).
Anandamide microinjection protocol. After recovery from the DLH protocol, the endocannabinoid anandamide (1.75 pmol, 35 nl) was microinjected at the dPAG pressor site, and sympathetic nerve and blood pressure responses were monitored. After ≥45 min of recovery, the CB1 receptor antagonist AM-281 (0.05 pmol, 50 nl) (16, 17) was microinjected at the same site, and 1 min later the anandamide microinjection was repeated. Recovery responses were assessed at 45 min after microinjection of AM-281.

Control protocol. A control study to demonstrate reproducibility of the anandamide response and lack of response to vehicle was performed in eight animals. The DLH and anandamide microinjection protocol described above was followed, but a Tocrisolve 100 vehicle microinjection replaced microinjection of the CB1 receptor antagonist prior to the second anandamide microinjection.

Hypothalamic defense area stimulation protocol. In a separate study, the DLH and anandamide microinjection protocols described above were followed to locate a site in the dPAG at which microinjection of anandamide could produce an increase in sympathetic nerve activity and blood pressure. Once located, the dPAG micropipette remained in place. In preparation for hypothalamic defense area stimulation, the animals were paralyzed with pancuronium bromide (0.1 mg/kg iv) to prevent movement and artificially ventilated. Electrical stimulation was used, because, unlike chemical stimulation with synaptic excitants, it consistently produces the full defense response from the medial hypothalamus (11). A tungsten carbide electrode inserted into the brain targeted the dorsomedial hypothalamus using initial coordinates with respect to bregma, midline, and dorsal surface of −3.0, 0.9, and 9.0 mm, respectively. The electrode was lowered into the brain in 0.25-mm increments, with electrical stimulation at each depth to locate a site at which an increase in renal sympathetic nerve activity and blood pressure was elicited. Once identified, the electrode remained in place, and the renal sympathetic nerve and blood pressure responses to a 10-s control stimulus were recorded. After recovery of baseline parameters, AM-281, the CB1 receptor antagonist (0.05 pmol), was microinjected into the site previously identified in the dPAG. Electrical stimulation in the hypothalamus was repeated at 1, 3, 5, 10, and 20 min following antagonist administration. In a control study to examine reproducibility of the response, repeated electrical stimulation of the hypothalamic defense area was performed at 1-min intervals over a 10-min time frame without pharmacological intervention in the midbrain.

Data analysis. For analysis of recorded data, blood pressure and averaged renal sympathetic nerve activity were sampled at a rate of 50

Table 1. Response to vehicle microinjection

<table>
<thead>
<tr>
<th>Time After Microinjection</th>
<th>10 s</th>
<th>20 s</th>
<th>30 s</th>
<th>40 s</th>
<th>50 s</th>
<th>60 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in RSNA, %baseline</td>
<td>0.3 ± 1.7</td>
<td>−0.6 ± 1.2</td>
<td>0.7 ± 1.6</td>
<td>−2.7 ± 1.2</td>
<td>−1.3 ± 1.3</td>
<td>−2.5 ± 1.7</td>
</tr>
<tr>
<td>Change in BP, mmHg</td>
<td>−0.4 ± 0.4</td>
<td>−0.3 ± 0.4</td>
<td>−0.2 ± 0.4</td>
<td>0.1 ± 0.3</td>
<td>0.2 ± 0.4</td>
<td>0.4 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 13). BP, blood pressure; RSNA, renal sympathetic nerve activity.
Hz using a personal computer built in-house with a 12-bit analog-to-
digital converter running programs written in HTBasic. Blood pres-
sure and averaged renal sympathetic nerve activity were displayed
along with a movable cursor. The cursor was set at the onset of the
microinjection or the electrical stimulus and acted as a time 0
marker for the analysis. For the microinjection study, data were collected and
averaged in sequential 10-s periods from 1 min prior to microinjection
(baseline) to 1 min after baseline levels were regained, usually a total
of 4 min. To eliminate noise, zero renal sympathetic nerve activity
was obtained at the end of the experiment by crushing the nerve
proximal to the recording electrodes, averaging the remaining noise
level, and subtracting it from the averaged activity. Nerve activity was
subsequently expressed as percent change from mean 10-s baseline
periods. The percent changes in renal sympathetic nerve activity and
the absolute changes in blood pressure for each 10-s postmicroinjec-
tion or stimulus period were compared using one-way ANOVA.

Responses to microinjection of anandamide were compared before
and after microinjection of AM-281 and with recovery responses. For
the electrical stimulation study, data were collected in 10-s periods for
1 min prior to stimulation and for the 10-s stimulus period. Nerve
activity during the 10-s electrical stimulus was subsequently ex-
pressed as percent change from mean 10-s baseline periods preceding
the stimulus. Responses to hypothalamic defense area stimulation at 1,
3, 5, 10, and 30 min after microinjection of AM-281 were compared
with the control stimulation prior to administration of AM-281 in the
PAG using one-way ANOVA. All significantly different means were
located using Duncan’s post hoc test, with significance set at $P < 0.05$.

To identify the location of central microinjection and stimulating
sites, sequential 25-μm frozen, transverse sections were cut, stained
with neutral red, and examined microscopically to identify marked
sites. Diagrammatic representations of the location of microinjection
and stimulus sites were compiled. Photomicrographs were taken using
a Macrofire digital color camera (Optronics, Goleta, CA). Photomicro-
graphic images were acquired at a resolution of 2,048 × 2,048 pixels,
captured using Optronics picture frame software on a Mac G4 1.25-
GHz computer, and stored in TIFF format. Images were imported into
Adobe Photoshop 8.0 (Adobe, San Jose, CA) and adjusted for optimal
visual quality.

RESULTS

The synaptic excitant DLH was microinjected into the dPAG
to identify sites from which an abrupt increase in renal sym-
pathetic nerve activity and an increase in arterial blood pres-
sure were elicited (Fig. 1). The response included twitching of
the whiskers, hyperventilation, and, occasionally, movement of
the tail and limbs. The responses are characteristic components
of a defense response. The volume of dPAG injectate was
minimized to prevent limbs and movement of the recording
electrode. The sympathoexcitatory response to DLH peaked
between 20 and 30 s at 39.2 ± 10.6% ($n = 19$) above baseline

Fig. 4. Transverse sections through the midbrain indicating sites of microin-
jection of DLH and anandamide into the PAG (6.8 and 7.8 mm caudal to
bregma). ●, sites from which anandamide could elicit a sympa-thoexcitatory
and pressor response; ○, sites from which anandamide elicited no response.
DR, dorsal raphe.

Fig. 5. Response to repeated electrical stimula-
tion of the hypothalamic defense area (solid
horizontal bar) on arterial blood pressure (BP),
renal sympathetic nerve activity, and averaged
renal sympathetic nerve activity at 1, 5, and 10
min without pharmacological intervention in the
PAG.

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levels, and the response lasted ~1 min. In these animals, baseline blood pressure was 86 ± 2 mmHg (n = 19), and at the peak of the response to DLH the mean blood pressure rose 7 mmHg, with peak increases of 2–23 mmHg. The effect of microinjection of anandamide was tested at these midbrain sites on the defense pathway. Microinjection of anandamide could evoke renal sympathetic and blood pressure responses similar to those elicited by DLH (Fig. 1). The sympathoexcitatory response to anandamide peaked at 30 s at 44.6 ± 8.1% (n = 19) above baseline levels, with the response lasting ~80 s (Fig. 2). At the peak of the response, mean blood pressure rose 7 mmHg, with peak increases of 0–19 mmHg. After recovery, the CB1 receptor antagonist AM-281 was administered at the same site, and baseline parameters were unchanged (−3.0 ± 2.3%, P < 0.05) prior to a second anandamide microinjection (n = 11). The response to anandamide was significantly attenuated (P < 0.05), reaching 9.1 ± 4.0% (n = 12) above baseline levels. There was a partial recovery of the renal sympathoexcitatory response to anandamide at 45 min after antagonist administration, reaching 18.3 ± 2.2% (n = 9) above baseline levels. The accompanying pressor response was also significantly attenuated at 20–40 s (Fig. 2).

Over the course of the study, baseline sympathetic nerve activity and blood pressure remained unchanged from that prior to drug intervention (P < 0.05). Compared with baseline prior to DLH intervention, baseline nerve activity was +6.0 ± 1.8% prior to anandamide administration, −2.0 ± 2.1% prior to AM-281 administration, and −2.0 ± 2.9% prior to the recovery response to anandamide. Blood pressure also remained stable at +2 ± 1 mmHg prior to anandamide, +1 ± 1 mmHg prior to AM-281, and +2 ± 2 mmHg prior to the recovery response. At sites at which DLH and anandamide could elicit increases in renal sympathetic nerve activity and blood pressure, administration of the vehicle Tocrisolve 100 (35 nl) had no effect on baseline sympathetic nerve activity or blood pressure (Table 1) or the anandamide-evoked response.

Fig. 6. Response to electrical stimulation of the hypothalamic defense area (HDA, solid horizontal bar) on arterial blood pressure, renal sympathetic nerve activity, and averaged renal sympathetic nerve activity prior to (HDA) and 3, 10, and 30 min after microinjection of the CB1 receptor antagonist AM-281 into the dPAG.
To confirm reproducibility of anandamide responses, the anandamide microinjection was repeated in the absence of the CB1 receptor antagonist over the same time frame in eight animals. The magnitude of the renal sympathetic nerve and blood pressure responses was not significantly different following the two microinjections (Fig. 3).

At some sites, DLH microinjection could produce a quantitatively similar renal sympathoexcitatory response (40.7% above baseline, n = 10), but microinjection of anandamide at these sites had no effect on renal sympathetic nerve activity (3.8% above baseline, n = 10).

Histological examination of stimulus sites showed that all sites from which a sympathoexcitation could be evoked by DLH and by anandamide were localized to the dPAG, in the dorsolateral and dorsal quadrants (Fig. 4). These sites were located between 6.0 and 7.6 mm caudal to bregma. The majority of sites from which a sympathoexcitatory response could be elicited by DLH (Fig. 4), but not anandamide, microinjection were more rostral or more caudal to the anandamide-sensitive sites. Those that were at the same rostrocaudal level were located adjacent to the aqueduct in the dorsal column of the PAG.

In a separate series of experiments, the DLH and anandamide microinjection protocol was used to identify sites in the dPAG for cannabinoid receptor blockade during hypothalamic defense area stimulation. At these dPAG sites, the mean increase in renal sympathetic nerve activity elicited by DLH and anandamide microinjections were 39.8 ± 7.0% and 24.7 ± 5.7%, respectively, above baseline. Electrical stimulation of the hypothalamic defense area evoked an abrupt increase in renal sympathetic nerve activity and a rise in arterial blood pressure (Figs. 5 and 6) lasting the duration of the stimulus. Stimulus duration was limited to 10 s to allow for reproducibility of responses over the experimental period. Stimulation of the hypothalamic defense area elicited an increase in sympathetic nerve activity of 34.7 ± 3.8% (n = 15) over baseline levels and a mean increase in blood pressure of 11 ± 2 mmHg (n = 15; Fig. 7, Control). Microinjection of the CB1 receptor antagonist AM-281 in the dPAG attenuated the renal sympathoexcitatory and pressor responses evoked by hypothalamic defense area stimulation, with recovery of the response over time (Figs. 6 and 7). Renal sympathoexcitation was significantly reduced to 21.3% (P < 0.05) at 3 min after AM-281 microinjection, while the pressor response to hypothalamic defense area stimulation was significantly decreased at 3 and 5 min after AM-281 microinjection (Fig. 7). By 10 min after AM-281 microinjection, the autonomic and cardiovascular responses to electrical stimulation were recovering toward control levels (Figs. 6 and 7).

Repeated electrical stimulation of the hypothalamic defense area over the same 10-min time frame in the absence of pharmacological intervention in the midbrain produced reproducible increases in renal sympathetic nerve activity and blood pressure (Figs. 5 and 8).

Stimulation sites in the hypothalamus were identified adjacent to the third ventricle and ventral and medial to the fornix in the dorsomedial and ventromedial hypothalamic nuclei (Fig. 9) from 1.2 to 3.4 mm caudal to bregma. Midbrain microinjection sites in the same animals were located in the dorsal and dorsolateral PAG (Fig. 9) from 6.0 to −7.8 mm caudal to bregma.

![Fig. 7](image-url) Increase in renal sympathetic nerve activity and blood pressure in response to electrical stimulation of the hypothalamic defense area prior to (control) and at 1, 3, 5, 10, and 30 min after microinjection of the CB1 receptor antagonist AM-281 into the dPAG (n = 15). *Significant difference from control (P < 0.05).

![Fig. 8](image-url) Reproducible increase in renal sympathetic nerve activity and blood pressure in response to electrical stimulation of the hypothalamic defense area at 0, 1, 3, 5, and 10 min without pharmacological intervention in the dPAG (n = 16).
DISCUSSION

The central defense pathway connects through the amygdala, hypothalamus, midbrain, and rostroventrolateral medulla and, when activated at sites along its axis, can elicit an integrated autonomic, cardiovascular, and behavioral response to prepare an animal for fight or flight. This response includes a characteristic abrupt increase in discharge of the renal sympathetic nerve and increase in blood pressure (4). The physiological response to acute stress, of which sympathoexcitation is a fundamental component, is based in the activation of the defense pathway, justifying the use of the defense response as an experimental model in the present study.

In the present study, microinjection of the CB1 receptor antagonist AM-281 at sites on the defense pathway in the dPAG attenuated the renal sympathoexcitation induced by activation of the hypothalamic defense area, indicating a role for the CB1 receptor in modulation of the defense-like response at this level of the neuroaxis. Microinjection of the CB1 receptor agonist anandamide at similar sites in the dPAG elicited renal sympathoexcitation and an increase in blood pressure. Cannabinoid receptor agonist microinjections into the dPAG could also induce twitching of the whiskers, hyperventilation, and movements of the tail and limbs, typical of a defense response. The responses to the CB1 receptor agonist were attenuated by prior microinjection of a CB1 receptor antagonist at the same site. Repeated microinjections of anandamide in the absence of the CB1 receptor antagonist evoked comparable responses of the same magnitude, indicating no desensitization of the anandamide response. These data indicate that CB1 receptors are components of the defense pathway in the dPAG involved in the modulation or mediation of the autonomic response to acute stress.

The extensive central distribution of CB1 receptors and the diverse effects of endocannabinoid agonists promoted interest in the cardiovascular effects of cannabinoids. There is evidence that cannabinoids affect central pathways controlling sympathetic nerve activity and blood pressure, but, as with behavioral studies, the data are conflicting. A study of the cardiovascular effects of cannabinoid agonists following systemic administration reports hypotension and bradycardia mediated by CB1 receptors (14). A subsequent study of the cardiovascular effects of systemic anandamide reported a pressor response followed by a prolonged depressor response, with the latter component considered to be mediated by CB1 receptors (15). One of the first studies of autonomic responses to intracisternally administered cannabinoids demonstrated a sympathoexcitation and bradycardia in the conscious rabbit (24). A few studies have localized cannabinoid administration to cardiovascular centers. When microinjected into the rostroventrolateral medulla of the anesthetized rat, cannabinoids evoked a slight decrease in blood pressure (23) or a sympathoexcitation and increase in arterial blood pressure (25). The discrepancies in cardiovascular effects could be related to many factors, including central location, cannabinoid agonist, cannabinoid...
concentration, mechanism of action, and species. Endocannabinoid microinjection into the nucleus tractus solitarius did not affect baseline blood pressure (23, 30) or sympathetic nerve activity but prolonged baroreflex sympathoinhibition in the anesthetized rat (30). The latter effect has been shown to be mediated by attenuation of GABAergic inhibition (2) of second-order baroreceptor neurons (3). Recent evidence supports a role for similar endocannabinoid processing of cardiovascular information in the PAG. Blockade of CB1 receptors in the ventrolateral PAG reversed the electroacupuncture attenuation of blood pressure changes during visceral afferent stimulation. Furthermore, the effect was reversed by blockade of GABA receptors, indicating endocannabinoid inhibition of GABA release (7), leading to modulation of arterial blood pressure (32). These studies suggest that disinhibition of GABAergic neurons plays a key role in endocannabinoid modulation of sympathetic nerve activity and blood pressure. With regard to the defense response, the autonomic and cardiovascular components are considered to be under excitatory glutamatergic and inhibitory GABAergic influences in the dPAG (31). The former act to augment, while the latter to suppress, defensive responses, providing a variable level of activity in the tonically active alerting system. Endocannabinoids are mobilized in response to appropriate stimuli, and the lack of effect of the CB1 receptor antagonist on baseline parameters in the present study is consistent with the concept of endocannabinoid release on demand from depolarized neurons (30). Stress-dependent endocannabinoid release could lead to short-term modulation of GABA release in the dPAG, contributing to the sympathetic and pressor responses evident in the present study.

The PAG is a heterogeneous structure involved in the coordination of autonomic and cardiovascular control, antinoceception, and behavior. While much research regarding cannabinoids and the PAG has centered around the antinociceptive and behavioral effects, the present study indicates a role for endocannabinoids in sympathoexcitation in the context of acute stress. This finding complements data from a recent study by Hohmann et al. (12), who suggest that endocannabinoids mediate stress-induced analgesia in the PAG. Antinociception and sympathoexcitation are common components of the defense response (18, 19), and these findings suggest that both are integrated by endocannabinoid neurotransmission at the level of the dPAG. Studies aimed at disassociating the two components at this level of the neuroaxis would be key to determining if synaptic processing in the dPAG is a point of divergence for antinociceptive and cardiovascular pathways en route to regions of the ventromedial and ventrolateral medulla.

The present study does not identify the endocannabinoid involved in the stress response, but Hohmann et al. (12) demonstrated an increase in anandamide and 2-arachidonyl glycerol in response to acute stress. While the present study did not examine the effects of 2-arachidonyl glycerol, anandamide could elicit a sympathoexcitation mediated via CB1 receptors when microinjected into the dPAG. In addition to activation of CB1 receptors, anandamide can act as an agonist at vanilloid transient receptor potential vanilloid type 1 (TRPV1) receptors (34). Activation of TRPV1 receptors in the dPAG by anandamide in the present study has not been ruled out, but prior microinjection of a CB1 receptor antagonist reduced the sympathoexcitatory response to anandamide by 78%. There may be a dose-dependent recruitment of TRPV1 receptors contributing to the conflicting behavioral outcomes reported in the literature for anandamide. Studies demonstrating that TRPV1 receptors in the dPAG contribute to descending modulation of nociception (21, 26) also raise the possibility that these receptors may contribute to the integration of the full defense response of which antinociception is a component.

The actions of endocannabinoids may be due to anatomically separate populations of CB1 receptors. The columnar organization and the heterogeneity of the PAG allow for different responses, depending on stimulus site. In addition to anandamide-sensitive sites, the present study also identified sites at which DLH microinjection elicited an increase in sympathetic nerve activity and blood pressure, while anandamide had no effect on these parameters. In general, these sites were located more rostral to those from which the fight-or-flight response is elicited and may have been sites coordinating distal danger or freezing behavior typically localized at a more rostral level of the neuroaxis (13). Cannabinoids may not be engaged in the latter responses, or they may be acting to suppress autonomic and behavioral responses. The effects of the cannabinoid receptor agonist appear to be site-specific and suggest that large volumes of agents injected into the PAG encompass multiple subsets of neurons and could produce conflicting data.

**Perspectives and Significance**

The dPAG possesses a common substrate to coordinate behavioral and physiological responses to acute stress. The physiological component is driven by activation of the sympathetic nervous system, and data presented here indicate that endocannabinoids act at CB1 receptors in the dPAG to modulate sympathetic nerve activity and blood pressure during acute stress. These findings complement behavioral studies and suggest that endocannabinoids are integral to the acute stress response. The level of ongoing activity in the defense pathway may determine the extent of involvement of endocannabinoids, while their location of action in the PAG may influence the response.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

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Cannabinoid Modulation of the Acute Stress Response


